Development and Performance of a Wood Adhesive with *Camellia oleifera* Protein Doped with Soy Oligopeptide

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A high-performance wood adhesive was successfully developed by incorporating soybean oligopeptides into *Camellia oleifera* protein through a combination of degradation and epoxy resin crosslinking techniques. The results showed that the presence of oligopeptides boosted the creation of more active functional groups in the degradation liquid system. This process enhanced the adhesive's initial viscosity and overall stability. As a result, the adhesive's performance was significantly improved, making the bond more robust and long-lasting. However, a notable drawback was observed: the storage duration of the adhesive was shortened from 10 h to 3.5 h. Despite this limitation, the adhesive exhibited several advantageous properties, including a high curing reaction rate, a relatively low curing temperature, and excellent thermal stability. Additionally, the prepared adhesive demonstrated superior bonding strength and outstanding water resistance, making it a promising alternative for various wood-based applications.

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INTRODUCTION

With the popularization of philosophy of green health food, people have begun to change their concepts and habits related to consumption of edible plant oil. China's *Camellia oleifera* industry has enters into a rapid development stage (Li *et al*. 2016; Liu *et al*. 2020; Yang *et al*. 2020; Lan *et al*. 2021). The *C. oleifera* industrial planting area in China will reach 90 million mu (6 million hectares) and the *C. oleifera* yield may exceed 2 million tons by 2025 (Zhang 2021; Li *et al*. 2022). Therefore, the oil production is accompanied with great production of oilcake by-products rich in proteins and carbohydrates in the process of oil-tea extraction. At present, the use of *C. oleifera* is limited to oil squeezing in China, but the residual oil cakes after oil squeezing are mainly wasted due to bitter taste and toxicity. The disposal of some oil cakes involves landfilling or open-field burning. Such wasteful handling of oil-cake resources is unsustainable, and it also may destabilize the ecological environment. How to increase utilization of oil-tea-cake resources and generate economic profits has become a problem that has to be solved urgently in the current *Camellia oleifera* industry (Quan *et al.* 2022; Gao *et al*. 2024).

The oilcake by-products, which are rich in proteins and carbohydrates and generated during edible oil and biodiesel extraction, have the conditions and potential to be developed into wood adhesives. Some scholars have prepared wood adhesives using oilcake by-products, such as rapeseed cake (Bandara and Wu 2018), sesame-seed cake (Wei *et al*. 2021), *Jatropha curcas* cake (Wu *et al*. 2018), peanut cake (Chen *et al*. 2021a), walnut cake (Wang *et al*. 2018), soybean cake (Chang *et al*. 2020; Gu *et al*. 2020; Huang *et al*. 2022; Bai *et al*. 2023), and so on. Similarly, *C. oleifera* cake can also be used to prepare wood adhesive. This approach is not only environmentally friendly by reducing the pollution caused by *C. oleifera* cake residues, but it also enhances the added value of the *C. oleifera* industry and helps to lower production costs. In previous work (Chen *et al*. 2021b; Deng *et al*. 2021, 2022, 2023), the authors found that preparation of wood adhesive based on *C. oleifera* protein was challenged by poor initial viscosity and poor storage stability. In this study, soybean oligopeptide was mixed into *C. oleifera* protein and adhesives were prepared through degradation and crosslinking, aiming to improve initial viscosity and storage stability of adhesives and improve the bonding strength.

EXPERIMENTAL

Materials

Camellia oleifera protein (protein content 36%) was obtained from Guizhou University, China. Soybean oligopeptide was bought from Shandong Gushen Biotechnology Group Co., Ltd., China. Sodium dodecylbenzene sulfonate, analytically pure and was purchased from Tianjin Zhiyuan Chemical Reagent Co., LTD. The E-44 epoxy resin (crosslinking agent) was used, which has an epoxide number (0.41 to 0.47) and a softening point of 12 to 20 ºC; it was obtained from Shanghai Mijiazhan Adhesive Products Co., LTD. Poplar veneer with the dimensions 400 mm× 400 mm × 2 mm and 8% to 10% moisture content was purchased from Qunyou Wood Co., Ltd. (Linyi, China) for plywood preparation. Other chemical reagents, such as NaOH and urea, were analytically pure and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of *Camellia oleifera* Protein Degradation Solution

A total of 100 g water was added into the round-bottom three-mouth flask with a mechanical stirring rod, a thermometer, and a condenser pipe. The mechanical stirring rod was started, and then 40 g *Camellia oleifera* proteins and 0.6 g sodium dodecyl benzene sulfonate (SDBS) were added. The mixture was heated to 65 ℃ and then 2.4 g NaOH was added to react for 90 min. Next, 4 g urea was added and it was further stirred for 20 min. After cooling and emptying, the *C. oleifera* protein degradation solution was obtained. Similarly, 36 g of *C. oleifer*a protein and 4 g of oligopeptide were mixed and subjected to degradation, thereby producing the *C. oleifera* protein complex degradation solution.

Preparation of *Camellia oleifera* Protein Adhesives

Before preparation of plywood, 12% epoxy resin cross-linking agent based on the mass of *C. oleifer*a protein and oligopeptide was added into the above *C. oleifera* protein degradation liquid and composite degradation liquid, respectively. The mixture was thoroughly stirred for 10 minutes and then directly applied as the adhesive for plywood. With reference to Deng *et al.* (2021, 2022), the viscosity, storage time, and insolubilization rate of the cured adhesive was tested.

Preparation of Plywood and the Test of Bonding Strength

A piece of three-layer poplar plywood was prepared in laboratory, with a surface of 400 mm × 400 mm. The hot-pressing technology (XLB type, single-layer hot press unit, Shanghai, China) with a hot-pressing temperature of 160 °C, pressing pressure of 1.0 MPa, pressing time of 5 min, and double-face adhesive dosage of 220 g/m^2 was used. With reference to the national standard GB/T 17657 (2013), the shear strength of plywood in warm water was tested.

Characterization

A Varian Fourier Transform infrared spectrometer (Varian 1000; Varian, Palo Alto, CA, USA) was used for testing. A DSC 204 F1 differential scanning calorimeter produced by NETZSCH (Rodgau, Germany) was used for the curing test. Approximately 5 to 6 mg of sample was heated for DSC analysis in N_2 atmosphere with the temperature range of 30 to 180 °C and a heating rate of 10 °C/min. Thermogravimetric analysis of resin was conducted using a TG 209 F3, NETZSCH device (Germany), with the test conditions of N_2 protection, temperature range of 30 to 800 °C, and the heating rate of 10 °C/min. The section of cured resin was observed with a Hitachi S–3400N scanning electron microscope (Tokyo, Japan).

Statistical Analysis

The data were processed in Excel (Microsoft Corp., Excel 2024, Redmond, WA, USA) and Origin software (OriginLab Corporation, Origin 2024, Northampton, MA, USA), and the significance of differences was judged *via* the one-way analysis of variance (ANOVA) ($P < 0.05$).

RESULTS AND DISCUSSION

Bonding Performances of *Camellia oleifera* Protein Adhesives

Results of viscosity, storage time, insoluble rate, and bonding strength of *Camellia oleifera* protein adhesives are listed in Fig. 1. Viscosities of *C. oleifera* protein degradation liquid and adhesive prepared by *C. oleifera* protein degradation liquid were small. In the test process, both exhibited layering phenomena. This reflected that *C. oleifera* protein alone cannot degrade effectively. It influenced the subsequent cross-linking reaction degree and storage stability of the adhesive (Chen *et al*. 2021b; Deng *et al*. 2021, 2022). The *C. oleifera* protein adhesive solution prepared by doping of oligopeptide was uniform (unstratified), accompanied with significant increase of viscosity of 74.2 mPa·s and significant shortening of storage time of 3.5 h. This demonstrated that cross-linking density of adhesive was further increased and the cross-linking structure was further strengthened.

The degree of insolubilization in curing products of adhesive can be used to qualitatively characterize cross-linking density and water tolerance of the adhesive (Deng *et al*. 2021; Song *et al*. 2023). The insolubilization levels in curing products of adhesives prepared by *C. oleifera* protein degradation liquid and adhesive prepared by *C. oleifera* protein degradation liquid were 69% and 75%, respectively. Although there was no cross-linking reaction in the former one, there were involvement of protein molecules in the curing process, and it had some water tolerance within the experimental range. Due to the loose and porous structures of the curing layer, water molecules were easy to enter in and the water resistance was poor (Deng *et al*. 2021, 2022). For the adhesive prepared with *C.*

oleifera protein degradation liquid, some water-resistant products were produced because some cross-linking reactions occurred. However, the water resistance only increased 9% due to the low cross-linking density. The insolubilization in curing products of *C. oleifera* protein adhesive prepared by doping of oligopeptide was 82%, which increased 19%. The variation trend of insolubilization was consistent with that of plywood failure percentage, which also further demonstrated that the cross-linking density of curing products of adhesive was improved and the water resistance was enhanced significantly. *C. oleifera* proteins were present at low levels. Impurities like cellulose can affect protein degradation and subsequent cross-linking reactions by influencing the availability and solubility of protein contact bases during degradation. As a result, the initial viscosity and uniformity of the prepared adhesive were poor, and it was easy to be layered up. After oligopeptide was introduced, more reaction groups were produced in the system, which was conducive to bonding of adhesive in chemical forms and constructing a compact cross-linking structure. Secondly, oligopeptide was degraded into peptide chains with lower molecular weight. These peptide chains ran through the system, thus increasing the overall flexibility of adhesives and strengthening the acting force between cross-linking agent and degradation liquid. Moreover, it brought better elastic contact and regular arrangement among cross-linking products. The cross-linking structure and cross-linking density were further strengthened.

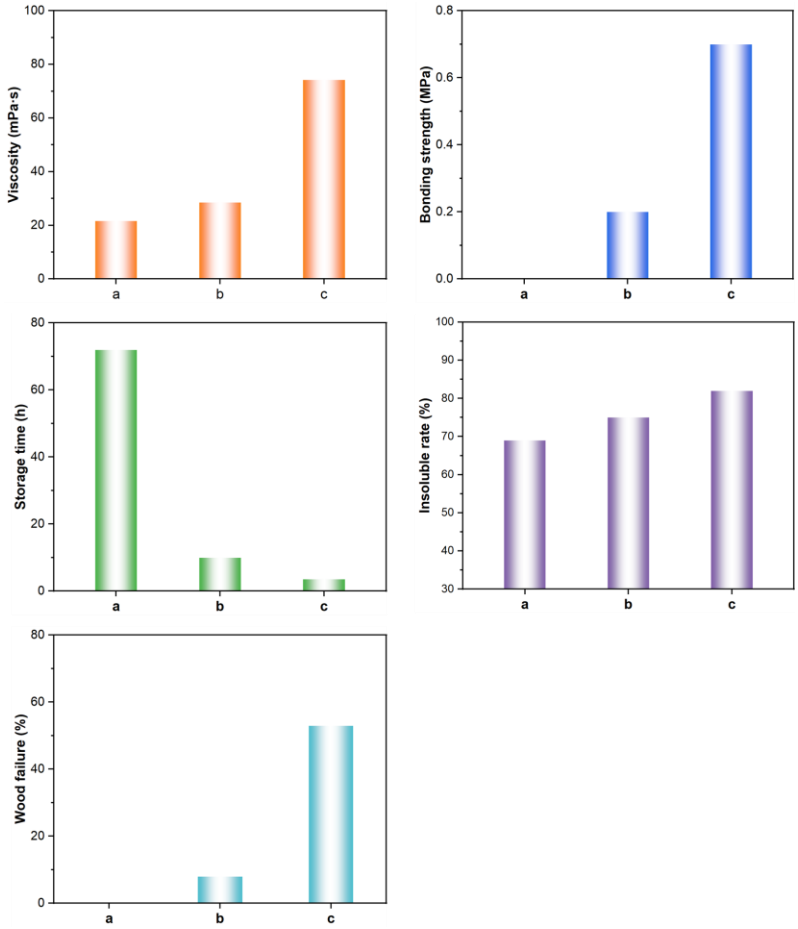

Fig. 1. Bonding performances of *Camellia oleifera* protein-based adhesives: a. *Camellia oleifera* protein degradation liquid; b. adhesive prepared by *Camellia oleifera* protein degradation liquid; c. adhesive prepared by *Camellia oleifera* protein compound degradation liquid

The adhesive after curing formed a compact network structure, which was beneficial to prevent wedging by water molecules, thus increasing the water resistance of the adhesives (Deng *et al*. 2022; Song *et al*. 2023). Moreover, the toughening effect of oligopeptide relieved excessive cross-linking of the adhesive system and increased brittleness caused by hot pressing (Wang *et al*. 2018; Chang *et al*. 2020), which avoided degradation of bonding performances and improved bonding strength of adhesives.

FTIR Analysis

As illustrated in Fig. 2, the FTIR spectral variation trends of *C. oleifera* protein degradation liquid and compound degradation liquid were generally consistent, with the primary difference being the variation in peak intensities within the amide region I and region II. Specifically, the characteristic peak intensities corresponding to *C. oleifera* protein degradation liquid in these amide regions were relatively low. This could be attributed to the relatively low concentration of *C. oleifera* protein in the degradation liquid, as well as the presence of impurities such as fiber residues (Deng *et al*. 2021, 2022, 2023). These impurities may interfere with the accessibility and solubility of reactive functional groups in the protein structure during the degradation process, thereby hindering the efficiency of *C. oleifera* protein degradation. The limited availability of active protein sites in the degradation liquid could subsequently reduce the extent of protein breakdown, leading to weaker absorption intensities in the FTIR spectra.

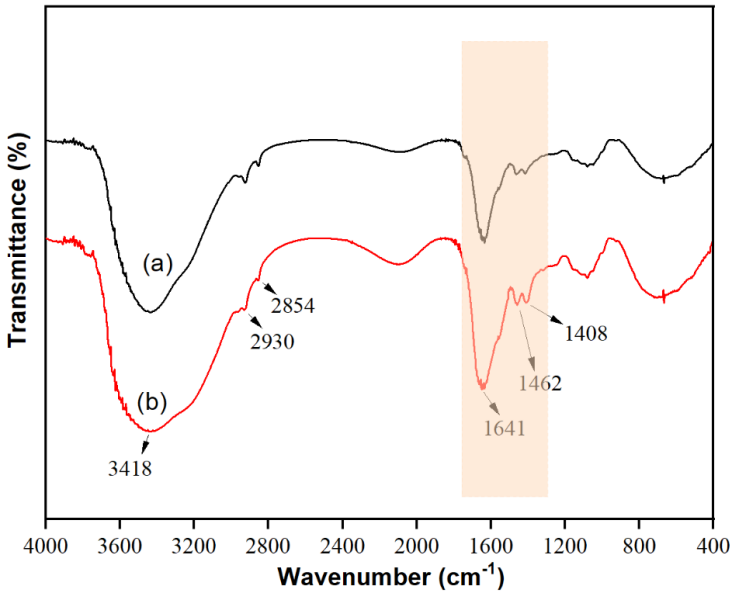

Fig. 2. FTIR curves of *C. oleifera* protein degradation liquid: (a). *Camellia oleifera* protein degradation liquid; (b). *Camellia oleifera* compound degradation liquid

However, after doping with oligopeptides, enhanced peak intensities of the compound degradation liquid in amide region I and region II was observed (Deng *et al*. 2022, 2023). This enhancement suggests an increase in the number of active functional groups within the system, which could be attributed to the structural modifications induced by oligopeptide integration. The introduction of oligopeptides likely contributed to improved molecular interactions, better solubility, and enhanced exposure of reactive sites within the protein matrix.

The presence of more active functional groups in the compound degradation liquid is particularly beneficial for subsequent cross-linking reactions in adhesive formulations. The stronger amide peaks indicate improved reactivity, facilitating more effective bonding interactions during the adhesive curing process. This, in turn, enhances the structural stability and performance of the final adhesive product, making it more suitable for applications requiring strong and durable adhesion properties.

Curing Performance of *Camellia oleifera* Protein Adhesive

The DSC test results of *C. oleifera* protein adhesives are shown in Fig. 3. Clearly, *C. oleifera* protein degradation liquid has no obvious cross-linking reaction exothermic peak above 100 °C. This is because *C. oleifera* protein degradation liquid alone cannot execute the cross-linking reaction and it mainly involved twining and degradation of protein molecules as well as formation of hydrogen bonds in the curing process. The peak at 85 °C was mainly caused by breakage of disulfide bonds in protein molecules (Deng *et al*. 2021, 2022; Zhu *et al*. 2024).

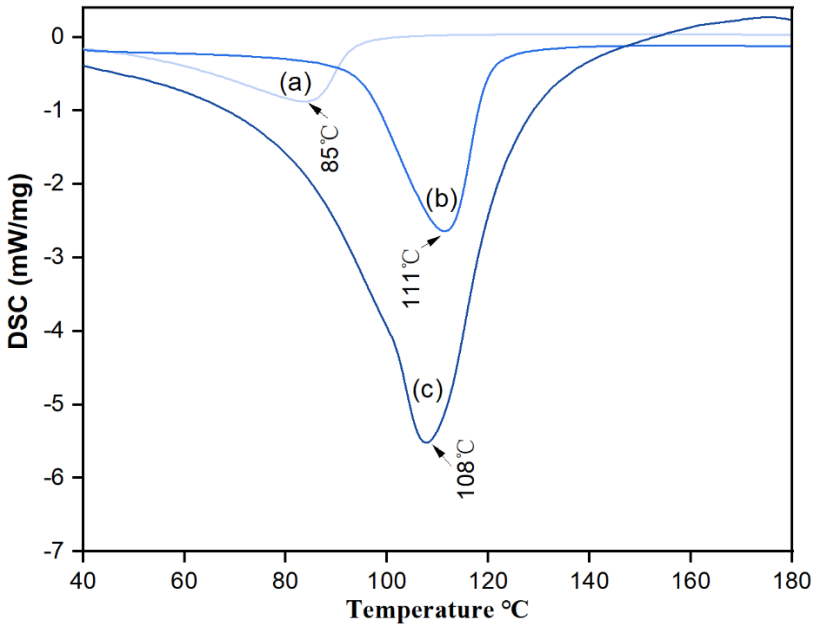

Fig. 3. DSC results of *C. oleifera* protein-based adhesives: a. *C. oleifera* protein degradation liquid; b. Adhesive prepared by *C. oleifera* protein degradation liquid; c. Adhesive prepared by *C. oleifera* protein compound degradation liquid

The adhesive that was prepared by *C. oleifera* protein degradation liquid had an obvious cross-linking reaction exothermic peak at 111 °C. The adhesive prepared by *C. oleifera* protein compound degradation liquid had obvious cross-linking reaction exothermic peak at 108 °C. This revealed that the cross-linking reaction temperature between the pure *C. oleifera* protein degradation liquid and cross-linking agent was high, and the heat release was low. In other words, the pure *C. oleifera* protein degradation liquid had low reaction activity and its reaction with cross-linking agent was not ideal. The crossing-linking reaction degree was low. The cross-linking reaction temperature between the *C. oleifera* protein compound degradation liquid and cross-linking agent was lower and the heat release increased. This showed that the introduction of oligopeptide increased the reaction activity of degradation liquid significantly, and its reaction with cross-linking

agent was complete, resulting in a high degree of cross-linking reaction. The DSC results further supported the results of FTIR. In other words, doping of oligopeptide increased the active groups per unit volume of degradation liquid.

Heat Resistance of *Camellia oleifera* Protein Adhesive

The TG and DTG test results of *C. oleifera* protein adhesives are presented in Fig. 4, providing insights into their thermal decomposition behavior. The analysis revealed that the temperatures corresponding to the maximum weight loss rate for *C. oleifera* protein degradation liquid and the adhesive derived from it were 283 ℃ and 284 ℃, respectively. This minimal difference suggests that the adhesive retains a thermal stability similar to that of the degradation liquid itself (Deng *et al*. 2021, 2022, 2023).

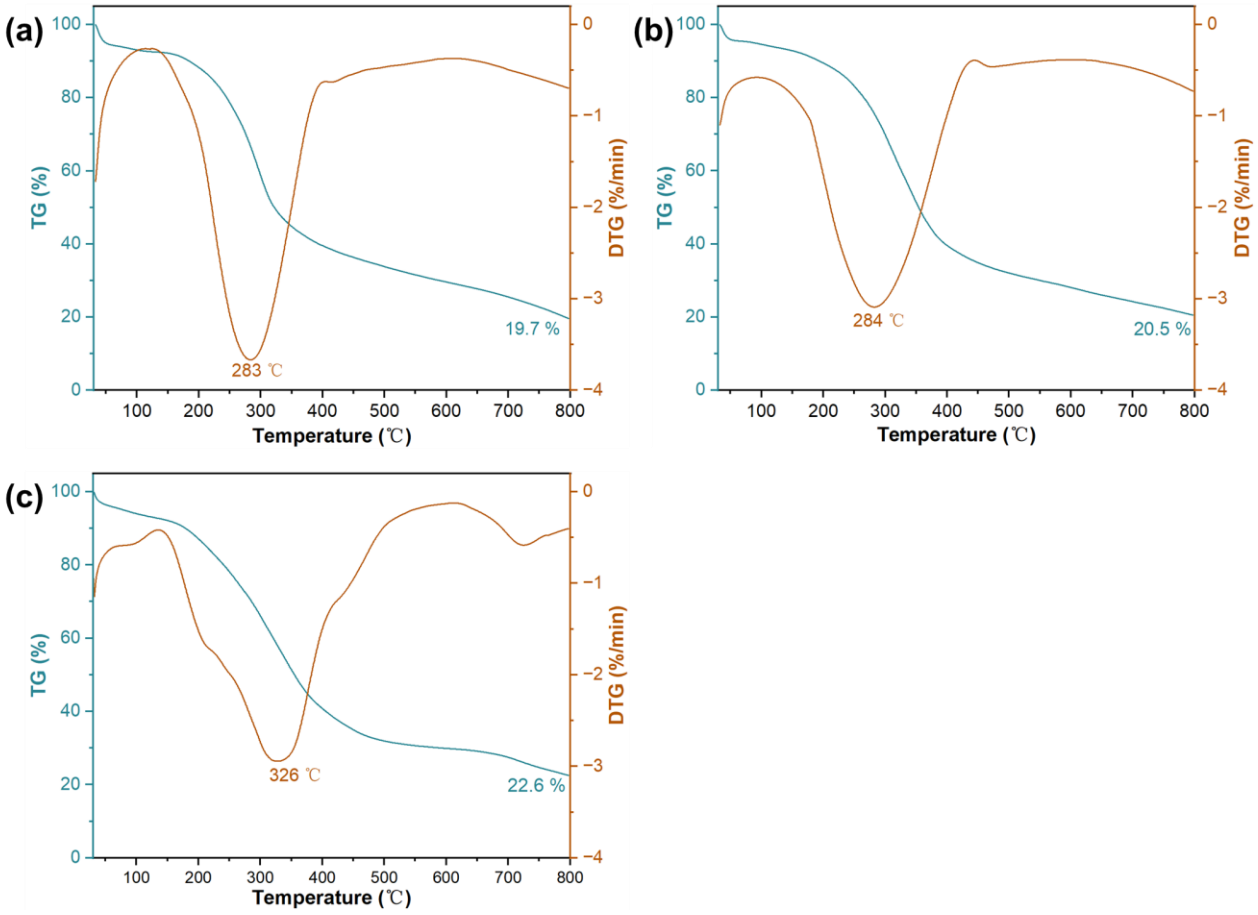

Fig. 4. TG/DTG results of the cured *Camellia oleifera* protein-based adhesives: a. *Camellia oleifera* protein degradation liquid; b. Adhesive prepared by *C. oleifera* protein degradation liquid; c. Adhesive prepared by *C. oleifera* protein compound degradation liquid

The observed thermal behavior supports the conclusion that a cross-linking reaction took place between the *C. oleifera* protein degradation liquid and the cross-linking agent. This reaction resulted in the formation of a more stable cross-linked structure, which enhanced the adhesive's thermal properties. Furthermore, when the *C. oleifera* protein degradation liquid was compounded with additional components, forming an adhesive prepared by *C. oleifera* protein compound degradation liquid, the maximum weight loss temperature increased significantly to 326 ℃. This remarkable improvement indicates that

the incorporation of oligopeptides played a crucial role in enhancing the thermal stability of the adhesive (Deng *et al*. 2022; Song *et al*. 2023; Zhu *et al*. 2024). The doping with oligopeptides likely facilitated stronger intermolecular interactions and improved the overall structural integrity of the adhesive matrix, making it more resistant to thermal degradation. These findings suggest that modifying *C. oleifera* protein adhesives with oligopeptides is a promising strategy for developing thermally stable bio-based adhesives, which could potentially expand their applications in industries requiring high-performance adhesive materials.

SEM Analysis

The SEM images of the fractured cross-sections of cured adhesives are presented in Fig. 5, providing valuable insights into the microstructural characteristics of the adhesive systems. Combined with the DSC results, it can be inferred that *C. oleifera* protein degradation liquid alone lacked the ability to undergo an independent cross-linking reaction. During the curing process, the volatilization of water and other small molecules led to the formation of a loose and porous microstructure in the cured layer. This porous morphology is indicative of weak intermolecular cohesion, increased brittleness, and poor water resistance, making the adhesive structurally fragile and less durable for practical applications (Deng *et al*. 2022).

In contrast, the cured layer of the adhesive prepared using *C. oleifera* protein degradation liquid exhibited a denser structure, although with noticeable non-uniformity. This structural improvement suggests that cross-linking reactions did occur to some extent during the curing process, contributing to an increase in cohesion. However, the uneven degradation of *C. oleifera* protein limited the extent of the cross-linking reaction, leading to an irregular and inconsistent adhesive network. The presence of structurally heterogeneous regions within the cured layer may have resulted in mechanical weaknesses, reducing the overall performance of the adhesive.

A significant transformation was observed in the microstructure of the cured layer when using the adhesive formulated with *C. oleifera* protein compound degradation liquid. The loosening characteristics that were previously present were markedly improved, and the cured adhesive layer became notably more compact and smooth. This structural refinement indicates a substantial enhancement in cohesion strength and cross-linking density. The key factor behind this improvement is the incorporation of oligopeptides, which facilitated the generation of a higher number of reactive functional groups within the system. These active groups promoted more extensive and efficient cross-linking reactions, resulting in the formation of a well-integrated adhesive network.

Upon curing, the adhesive layer evolved into a complex three-dimensional spatial network system, which contributed to its improved cohesion strength and water resistance. The increased cross-linking density strengthened intermolecular interactions, enhancing the adhesive's mechanical integrity and durability. The improved water resistance further suggests that the adhesive formulation with *C. oleifera* protein compound degradation liquid has greater potential for applications requiring high-performance, moisture-resistant bonding. This advancement highlights the effectiveness of oligopeptide doping in optimizing the adhesive’s structural and functional properties, making it a promising candidate for bio-based adhesive development.

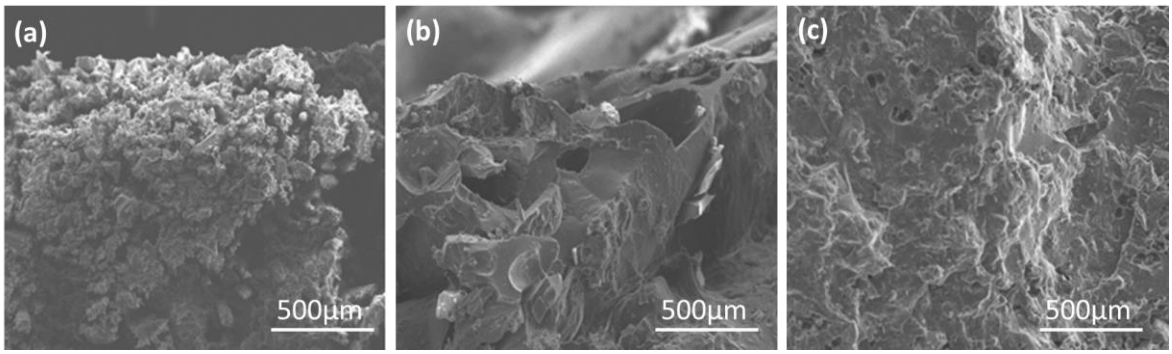

Fig. 5. SEM images of fractured cross-sections of cured *Camellia oleifera* protein-based adhesives: a. *Camellia oleifera* protein degradation liquid; b. Adhesive prepared by *Camellia oleifera* protein degradation liquid; c. Adhesive prepared by *Camellia oleifera* protein compound degradation liquid

CONCLUSIONS

To improve initial viscosity, stability and bonding strength of *Camellia oleifera* protein-based adhesive, soybean oligopeptide was mixed in. Meanwhile, adhesives were prepared through degradation and cross-linking methods. Results demonstrated that:

1. The oligopeptide enabled the formation of more active groups in the degradation system, which increased initial viscosity and stability of the adhesive. However, the storage time of the adhesive was shortened from 10 to 3.5 h.
2. The *Camellia oleifera* protein adhesive prepared by doping with oligopeptide had a high curing reaction rate, lower curing temperature, higher thermostability, and water resistance. Bonding strength increased from 0.20 to 0.70 MPa
3. Adding of oligopeptide to the formulation increased the overall flexibility of the prepared adhesive, which brought better elastic contact and regular arrangement among cross-linking products. Moreover, the cross-linking density was further increased and the cross-linking structure was further strengthened.

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