Effects of Treatments on the Structure of *Camellia oleifera* Cake Protein and the Properties of Adhesives

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Although the preparation of wood adhesives from protein in oilcake has significant advantages in terms of environmental protection and cost control, it still faces many challenges that need to be addressed in practical applications. This study used *Camellia oleifera* cake protein extracted from *C. oleifera* seeds as raw material to prepare adhesives through thermal, alkaline, acidic, and enzymatic treatments, which were then applied in the production of plywood. The results showed that thermal treatment increased the viscosity of the protein adhesive and improved the bonding strength to 1.12 MPa. Fourier transform infrared spectroscopy analysis indicated partial transformation of the secondary structure of protein molecules, while differential scanning calorimetric analysis showed an increase in curing temperature. Alkaline treatment slightly reduced the viscosity but achieved a bonding strength of 1.14 MPa, disrupting some of the crystalline structures of the protein and lowering the curing temperature. Acidic treatment greatly reduced the viscosity but resulted in the highest bonding strength of 1.36 MPa, partially hydrolyzing peptide bonds and reducing both the curing temperature and crystallinity. Enzymatic treatment decreased the viscosity of the protein adhesive but lowered the bonding strength to 0.82 MPa, with extensive hydrolysis of peptide bonds. Different treatment methods altered the rheological properties and bonding properties of the adhesives by affecting the molecular structure, aggregation state, and chemical reactivity of *C. oleifera* protein.

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Keywords: Camellia oleifera protein; Heat treatment; Alkali treatment; Acid treatment; Enzymatic treatment; Bonding properties

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INTRODUCTION

Conventional adhesives are predominantly composed of petroleum-derived synthetic resins. Their production not only consumes a large amount of non-renewable resources, but it also potentially causes environmental pollution during use and after disposal (Mirski *et al*. 2020; Zheng *et al.* 2022; Staciwa *et al*. 2024; Zhang *et al*. 2024). Therefore, it is of great practical significance to seek renewable, biodegradable, and high-performance natural adhesive raw materials. In recent years, protein-based adhesives have gradually attracted the attention of researchers due to their wide availability, low cost, good biocompatibility, and suitable degradability (Chen *et al*. 2024a; Zheng *et al.* 2024).

The research on protein-based adhesives has a long history, but their application still faces many challenges. For example, although soy protein adhesives have good

biocompatibility and renewability, their complex molecular structure, with active groups buried inside the molecules, leads to high viscosity, poor flowability, and insufficient water resistance, which has limited their widespread industrial application. To address these issues, researchers have attempted various modification methods, including physical modifications (such as thermal and microwave treatments), chemical modifications (such as cross-linking and grafting), and biological modifications (such as enzymatic hydrolysis) (Hand *et al*. 2018; Lydia Alexandra 2019; Xu *et al*. 2020; Zhao *et al*. 2021; Huang *et al.* 2023; Zhang *et al*. 2023; Bai *et al*. 2023, 2024; Ye *et al*. 2024). These methods improve the bonding performance and water resistance by altering the molecular structure and aggregation state of proteins and exposing more active groups. In recent years, biomimetic modification methods have made significant progress in the field of protein adhesives (Zhang *et al*. 2022; Li *et al*. 2023; Chen *et al.* 2024b). For example, inspired by mussel adhesive proteins, researchers have significantly improved the water resistance and bonding strength of soy protein adhesives by introducing catechol structures such as dopamine. Additionally, introducing nanomaterials or biomineralization systems into soy protein can effectively enhance its antibacterial and mechanical properties. These studies have provided new ideas for the development of high-performance protein-based adhesives.

Camellia oleifera cake is a by-product of oil extraction from camellia seeds, rich in protein with a diverse amino acid composition and good biocompatibility. Currently, the utilization of camellia in China is almost limited to oil extraction, and the remaining oil cake, which is bitter and toxic, is mostly discarded. A small portion is used directly as pond cleaning agents or fuel. These irrational uses of oil cake not only lead to significant resource waste but also affect the balance and stability of the ecological environment (Lan *et al*. 2021; Zhang 2021; Li *et al*. 2022; Quan *et al*. 2022; Gao *et al*. 2024).

How to improve the utilization of camellia cake resources and generate economic benefits has become an urgent issue for the camellia industry. Developing an adhesive-grade protein from this by-product not only can improve resource efficiency but also promote sustainable industrial practices in wood-based panel production. This study focused on the development and performance optimization of camellia protein adhesives. The novelty of this study lies in employing thermal, alkaline, acidic, and enzymatic treatments to investigate the effects of different treatments on the molecular structure, rheological properties, and bonding performance of camellia protein and applying the findings to the production of plywood, aiming to provide theoretical basis and technical support for the development of high-performance, environmentally friendly natural adhesives.

EXPERIMENTAL

Materials

Camellia oleifera protein (protein content 36%), obtained from camellia seed cake processed in Forestry Science Research Institute Guizhou, China was used. Hydrochloric acid (38 wt%) and sodium hydroxide (96 wt%), from Chengdu Jingshan Chemical Reagent Co., Ltd. were used. Alkaline protease, analytical grade, from Yuanye Biotechnology Co., Ltd. was also used in the study. Poplar (*Populus* spp.) veneers, with dimensions of 400 mm × 400 mm and thickness of 2 mm, moisture content 8% to 10%, were purchased from Jiangsu, China.

Preparation of *C. oleifera* Protein Adhesive

At room temperature, 80 g of water and 20 g of *C. oleifera* protein were added to a flask and stirred evenly at a speed of 300 rpm for 50 minutes using a magnetic stirrer. The protein was then subjected to heat, alkali, acid, and enzymatic treatments according to the methods listed in Table 1 to prepare the adhesives. The viscosity was measured according to the method specified in the national standard GB/T 14074-2006.

Plywood Preparation and Performance Testing

The prepared adhesives were evenly applied to the surface of poplar veneers (170 mm × 110 mm) using a flat brush to ensure a single-sided application rate of 180 g/m^2. The coated veneers were then stacked to form a three-layer structure and left to stand for 15 minutes before being hot-pressed at 140 °C and 1.5 MPa for 3 minutes to produce the plywood. The plywood was left at room temperature (25 °C, 50% relative humidity) for 24 h before being cut into standard test specimens of 100 mm × 25 mm. The dry bonding strength of the plywood was tested in accordance with the national standard GB/T 17657 (2022). The final strength value was the average of 10 specimens, with the standard deviation recorded as an indicator of data reliability.

Fourier Transform Infrared Spectroscopy (FTIR) Testing

The freeze-dried adhesives sample powder was mixed with potassium bromide at a mass ratio of 1:200, ground evenly, and then pressed into pellets. The pellets were placed in a desiccator to remove moisture before infrared spectroscopy measurement. A Thermo Fisher Scientific Nicolet iS20 spectrometer (USA) was used to record the spectra in the range of 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans.

Differential Scanning Calorimetry (DSC) Testing

The freeze-dried adhesives sample powder was tested using a Netzsch DSC 204 F1 differential scanning calorimeter (Germany) after freeze-drying. The tests were conducted under nitrogen protection with a heating rate of 10 °C/min and a temperature range of 30 to 220 °C.

X-ray Diffraction (XRD) Testing

The cured adhesive sample powder was tested using a Rigaku Ultima IV X-ray diffractometer (Japan) with a Cu-Kα radiation source (40 kV). The scanning range was from 5 to 80°, with a step size of 0.02° and a scanning rate of 5°/min.

Table 1. Sample Processing Method

No.	Processing Method
Control Group	Stirred at room temperature for 30 min
Heat Treatment	Heated to 80 °C and reacted for 30 min
Alkali Treatment	Added 7.5 g of 15% NaOH solution and reacted for 30 min
Acid Treatment	Added 7.5 g of 15% HCl solution and reacted for 30 min
Enzymatic Treatment	Added 1.125 g of alkaline protease, adjusted pH to 9 to 11, and reacted for 30 min

RESULTS AND DISCUSSION

Effects of Different Treatments on the Viscosity of Adhesive

Thermal treatment induced denaturation in *C. oleifera* protein, increasing the viscosity of the protein solution from 585 to 708 mPa·s. Under heating conditions, the protein molecules, initially in a tightly coiled structure, began to unfold (Ma *et al*. 2020). This denaturation process exposed the hydrophobic groups within the molecules to the exterior, thereby relatively reducing the number of hydrophilic groups on the exterior. As a result, the solubility of the protein decreased. Additionally, the denatured protein molecules may have undergone association after being heated, which led to an increase in viscosity.

Alkali treatment resulted in a modest increase in the viscosity of *C. oleifera* protein solution to 681 mPa·s. This change can be attributed to several structural and conformational alterations induced by the alkaline environment (Zhang *et al*. 2017). The alkaline conditions likely caused the molecular structure of *C. oleifera* protein to undergo significant changes. Specifically, the protein molecules may have stretched or aggregated, leading to enhanced intermolecular forces and increased entanglement. These changes in molecular interactions and conformation directly contributed to the observed increase in viscosity. In an alkaline environment, the disruption of hydrogen bonds and disulfide bonds within and between protein molecules is a common occurrence. This disruption alters the native conformation of the protein, causing it to adopt a more extended or aggregated state. The increased intermolecular interactions and entanglement result in greater resistance to flow, thereby raising the viscosity of the solution. Moreover, the alkaline treatment may have also led to the deprotonation of certain amino acid residues, further modifying the charge distribution on the protein surface. This change in charge can enhance electrostatic interactions between protein molecules, contributing to the increased viscosity.

Acid treatment had a profound impact on the viscosity of *C. oleifera* protein solution, reducing it significantly to 312 mPa·s. This substantial reduction can be attributed to several key chemical changes that occurred within the protein molecules under acidic conditions. In an acidic environment, the amino groups present in the *C. oleifera* protein molecules underwent protonation, transforming into positively charged ammonium ions (Liu *et al*. 2016). This protonation event is a critical factor in altering the electrostatic properties of the protein. Conversely, the carboxyl groups within the protein molecules did not dissociate under these conditions. This differential behavior of the amino and carboxyl groups led to a significant change in the overall charge distribution across the protein molecules. The protonation of amino groups and the undissociated state of carboxyl groups resulted in a decrease in electrostatic repulsion between the protein chains. This reduction in repulsive forces allowed the protein chains to come closer together, leading to aggregation or partial folding due to electrostatic attraction. This conformational change reduced the extent of intermolecular entanglement, thereby lowering the flow resistance of the solution. Essentially, the protein chains became more streamlined and less entangled, facilitating easier flow and thus reducing viscosity. Moreover, the acidic conditions likely facilitated the hydrolysis of peptide bonds within the protein molecules. This hydrolysis process broke down larger protein molecules into smaller peptides. The presence of smaller peptides in the solution further contributed to the reduction in viscosity, as smaller molecules generally exhibit lower resistance to flow compared to larger, more complex structures.

The viscosity after enzymatic treatment was 324 mPa·s. Enzymatic treatment is a more specific method of protein degradation. Enzymes can recognize and cleave specific peptide bonds in protein molecules, breaking down large protein molecules into smaller peptides, thereby reducing the viscosity of the solution (Liang *et al*. 2020). Similar to acid treatment, the peptides resulting from enzymatic hydrolysis have lower molecular weights and weaker intermolecular interactions, enhancing the fluidity of the solution and reducing viscosity.

The results of the viscosity measurements indicate that different treatments have distinct mechanisms of action on the molecular structure and aggregation state of *C. oleifera* protein, leading to obvious differences in viscosity. Thermal and alkaline treatments tend to cause protein molecules to aggregate or stretch, increasing viscosity, while acid and enzymatic treatments primarily degrade protein molecules, reducing molecular weight and intermolecular forces, thereby lowering viscosity.

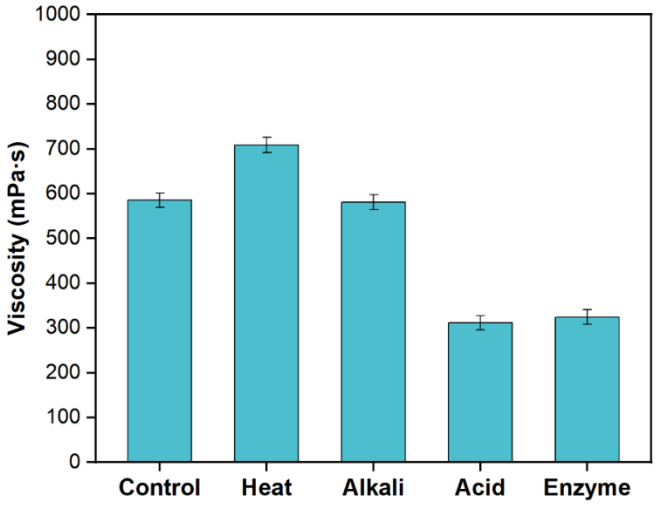

Fig. 1. Effects of treatments on the viscosity of *C. oleifera* protein adhesive

Effects of Treatment Methods on the Bonding Performance of Adhesive

The bonding strength after thermal treatment increased to 1.12 MPa. Appropriate thermal treatment can promote cross-linking reactions between *C. oleifera* protein molecules. The elevated temperature intensifies molecular motion, increasing the frequency of collisions between molecules, which is conducive to the formation of new chemical bonds (such as disulfide bonds and covalent bonds). These cross-linked structures enhance the cohesive strength of the adhesive, making it more resistant to being pulled apart by external forces, thereby improving the bonding strength. Additionally, thermal treatment may facilitate reactions between the active groups of *C. oleifera* protein molecules and the surface of wood or other adherends, thereby forming tighter interfacial bonds and further enhancing the bonding strength.

The bonding strength after alkali treatment reached 1.14 MPa. The condition may promote chemical reactions between the amino groups in *C. oleifera* protein molecules and the hydroxyl groups on the wood surface, forming hydrogen bonds, ionic bonds, and other interactions that improve the interfacial bonding between the adhesive and wood. Moreover, alkali treatment may cause a certain degree of aggregation or cross-linking of *C. oleifera* protein molecules, forming a more stable network structure that enhances the cohesive strength of the adhesive, thereby increasing the bonding strength.

The bonding strength after acid treatment was the highest of 1.36 MPa. The acidic environment makes the active groups in *C. oleifera* protein molecules more reactive with the components of wood. For example, the protonation of amino groups in protein molecules under acidic conditions may facilitate hydrogen bonding or electrostatic interactions with the hydroxyl groups in wood components such as cellulose and hemicellulose. Additionally, acid treatment may increase the number of active groups on the wood surface, such as partially hydrolyzing cellulose to expose more hydroxyl groups, which can form more binding sites with adhesive, thereby enhancing the bonding strength. The bonding strength after enzymatic treatment was 0.82 MPa. Although enzymatic treatment reduced the viscosity of the *C. oleifera* protein solution, it may have disrupted some structures or groups that are beneficial for bonding. The enzymatic hydrolysis of protein molecules into smaller peptides may result in these peptides lacking sufficient length and active groups to form strong bonds with the wood surface.

The results of bonding strength indicate that different treatments have distinct effects on the chemical reactivity of *C. oleifera* protein and its ability to interact with the adherend materials.

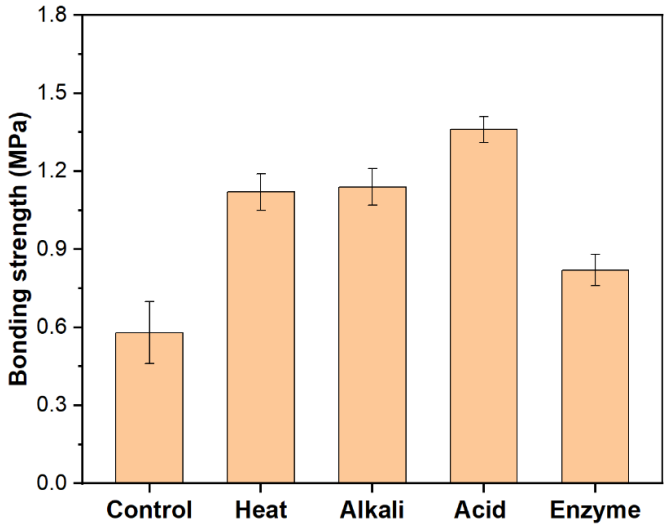

Fig. 2. Effects of treatments on the bonding strength of *C. oleifera* protein adhesive

FTIR Analysis

Figure 3 shows the infrared spectrum of *C. oleifera* protein. The characteristic peaks in the infrared spectrum of *C. oleifera* protein are mainly related to the peptide bonds. The peak at a wavenumber of 1749.4 cm^{-1} corresponds to the C=O stretching vibration in the amide I region of the amide bond. The peak at 1511.7 cm^{-1} is a combination peak of the N-H bending vibration and C-N stretching vibration in the amide II region of the amide bond. The peak at 1243.9 cm^{-1} is the C-N stretching vibration peak in the amide III region of the amide bond. The peak at 1376.3 cm^{-1} is the characteristic peak of the carboxyl group. The peaks at 1086.0 and 1054.4 cm^{-1} correspond to the absorption peaks of primary alcohols.

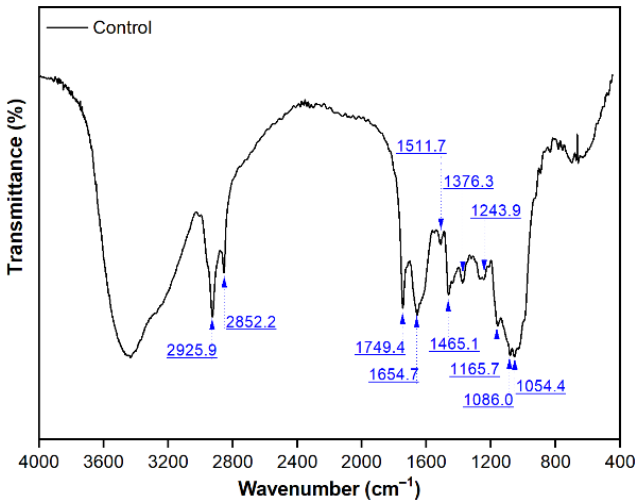

Fig. 3. FTIR spectrum of *C. oleifera* protein

Figure 4 shows the FTIR spectrum of heat-treated *C. oleifera* protein. In the infrared spectrum of *C. oleifera* protein after thermal treatment, the peak at 1749.4 cm^{-1} was shifted to 1743.4 cm^{-1}. This indicates that thermal treatment altered the C=O stretching vibration frequency of some peptide bonds in the *C. oleifera* protein molecules. This shift is likely due to partial changes in the secondary structure of the protein molecules caused by heat. The orderly arranged β-sheet structures may have undergone structural relaxation or reorganization in some areas due to thermal interference, altering the chemical and surrounding electric field environments of some C=O bonds and thus creating new absorption peak positions.

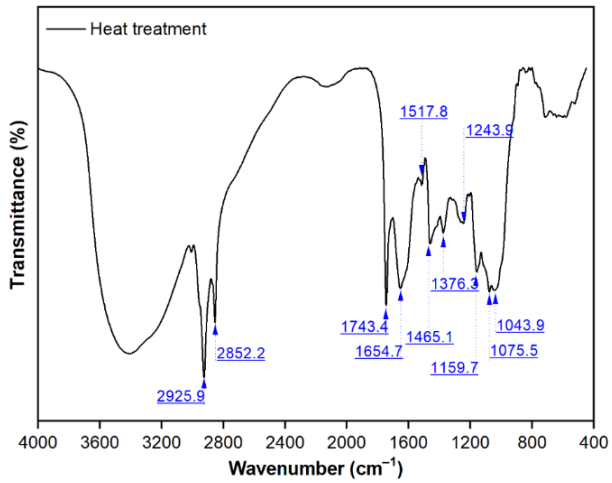

Fig. 4. FTIR spectrum of heat-treated *C. oleifera* protein

The peak at 1511.7 cm^{-1} was shifted to 1517.8 cm^{-1}, indicating that heat treatment has also affected the coupling mode of amide bond vibrations. Some of the hydrogen bond networks within the *C. oleifera* protein molecules were disrupted, altering the coupling of N-H and C-N vibrations. This resulted in splitting or changes in the intensity of the peaks in this region, reflecting that the internal structural order of the protein molecules had been disturbed by heat treatment and that local conformational changes had occurred.

Therefore, heat treatment caused the *C. oleifera* protein molecules, which were originally in a tightly coiled structure, to unfold. This exposed the hydrophobic groups within the molecules to the exterior, thereby relatively reducing the number of hydrophilic groups on the exterior. As a result, the solubility of the protein decreased. Additionally, the protein molecules may have undergone association after being heated, which led to an increase in viscosity.

Figure 5 shows the FTIR spectrum of *C. oleifera* protein treated with acid. In the acid-modified *C. oleifera* protein, the main changes in the peaks were observed in the amide II and amide III regions. The decrease in the intensity of the C-N stretching absorption indicates that some peptide bonds had been hydrolyzed under the influence of strong acid. The increase in the intensity of the N-H absorption in the amide II band suggests that the spatial globular structure of *C. oleifera* protein had changed considerably under acidic conditions. The polypeptide chains had been separated, exposing more -NH_2 groups.

Additionally, both of these bands have experienced a blue shift to some extent, which may be due to the inductive effect of the acid. Meanwhile, the characteristic peak in the amide I region remained largely unchanged. This indicates that although some peptide bonds or amide bonds had been hydrolyzed under the influence of acid, the overall impact on the peptide chain was minimal. There was also no indication of intramolecular or intermolecular cross-linking at the sites where the peptide bonds had been broken.

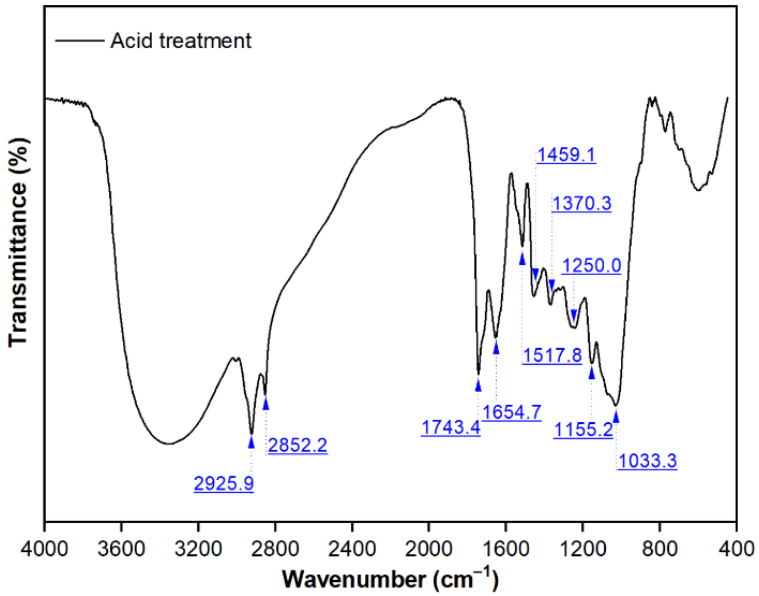

Fig. 5. FTIR spectrum of acid treated *C. oleifera* protein

Figure 6 shows the FTIR spectrum of the *C. oleifera* protein after alkali treatment. In the infrared spectrum of alkali-treated *C. oleifera* protein, the main changes in the peaks were observed in the N-H in-plane bending vibration in the amide II region and the C-N stretching vibration in the amide III band. These two bands had also experienced blue shifts to varying degrees, which may have been due to the inductive effect of the alkaline environment on the amphoteric protein molecules. Compared to acid treatment, alkaline treatment had a stronger ability to hydrolyze peptide bonds or amide bonds. The C-N stretching vibration at 1243.9 cm^{-1} was almost completely absent after alkaline treatment. Additionally, the peak at 1517.8 cm^{-1} was also nearly gone, indicating that alkaline

treatment may have catalyzed some active groups to participate in intramolecular or intermolecular cross-linking reactions.

Therefore, the mechanisms by which acid and alkali treatments affect the molecular structure of *C. oleifera* protein are different. Acid treatment alters the protein structure through protonation and partial hydrolysis of peptide bonds. In contrast, alkali treatment mainly changes the protein structure by breaking peptide bonds and altering the charge distribution.

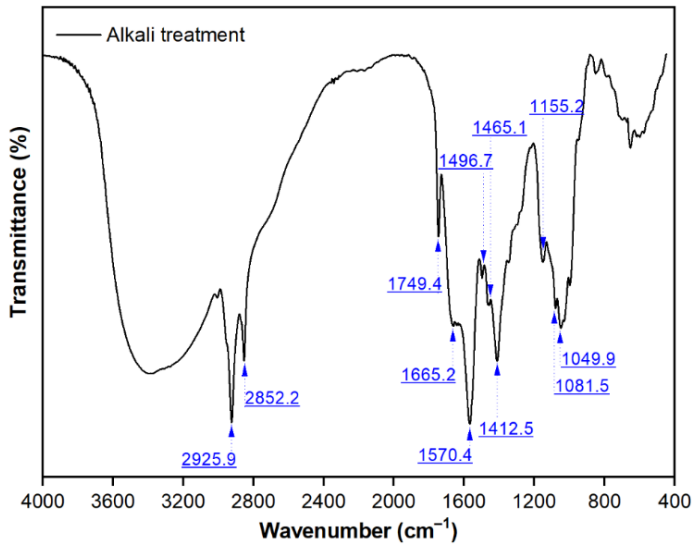

Fig. 6. FTIR spectrum of alkali treated *C. oleifera* protein

Figure 7 shows the infrared spectrum of *C. oleifera* protein treated with enzymes. The infrared spectra of *C. oleifera* protein treated with protease show that the peaks at 1749.4, 1654.7, and 1511.7 cm^{-1} almost disappeared, indicating that the peptide bonds of the protein underwent intense hydrolysis. At this time, the viscosity of the adhesive was also the lowest, with low cohesive strength and poor bonding strength.

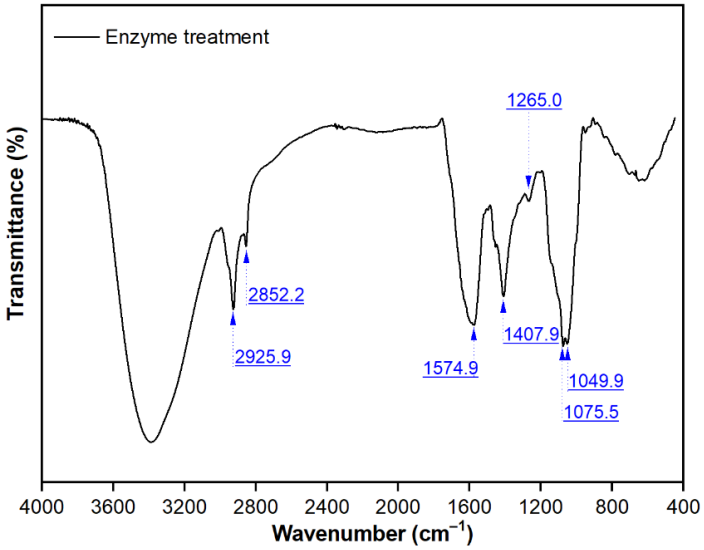

Fig. 7. FTIR spectrum of enzyme treated *C. oleifera* protein

In summary, thermal treatment caused *C. oleifera* protein to undergo denaturation and association, resulting in increased viscosity. Acid treatment partially hydrolyzed peptide bonds but did not affect the peptide chains, resulting in the best bonding performance. Alkaline treatment greatly hydrolyzed peptide bonds or amide bonds, exposing a large number of polar groups. However, these groups underwent cross-linking reactions under the catalysis of alkali, resulting in good bonding strength. Enzymatic treatment also greatly hydrolyzed peptide bonds or amide bonds, but it led to reduced viscosity, low cohesive strength, and poor bonding performance.

Curing Performance Analysis

The DSC curves of *C. oleifera* protein adhesives subjected to different treatments exhibited a distinct exothermic peak temperatures (Fig. 8). The adhesives treated by thermal, alkaline, acid, and enzymatic methods each showed a single prominent exothermic peak. The peaks were centered at 119.2, 113.2, 113.9, and 113.3 °C, respectively.

Thermal treatment relaxes the compacted structure of protein molecules, enhancing the freedom of movement between them. Consequently, a higher curing temperature is needed to prompt the molecules to reorganize and form a stable network structure. In contrast, alkaline, acid, and enzymatic treatments stretch the protein molecules, breaking some of the hydrogen and disulfide bonds. This change transforms the molecules from a compact shape to a more extended linear form. The extended structure increases intermolecular entanglement and contact area, making it easier for the molecules to aggregate during curing. As a result, lower curing temperatures are required.

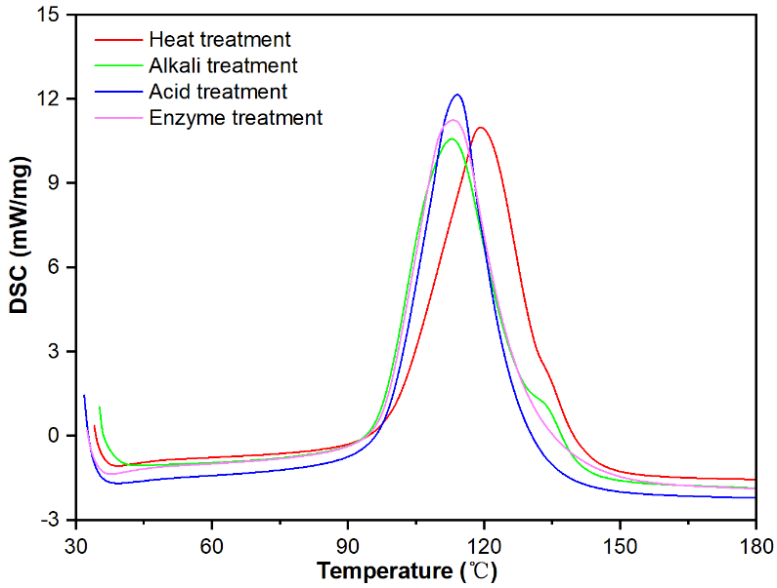

Fig. 8. Effects of different treatments on DSC results of *C. oleifera* protein adhesive

XRD Analysis

Figure 9 presents the XRD patterns of *C. oleifera* protein adhesives subjected to various treatments. The untreated protein exhibits a well-preserved natural crystalline region, with distinct diffraction peaks near $2\theta = 10°$, 20°, and 30°. These peaks reveal the inherent crystalline structural features of the protein.

Thermal treatment had minimal influence on the crystalline structure of *C. oleifera* protein. It likely only induced slight thermal motion of the molecules without disrupting the original crystalline regions. In contrast, alkali treated protein showed reduced diffraction peak intensities near 2θ = 10°, 20°, and 30°, with broadened peaks. This suggests that alkali treatment disrupted the protein's crystalline structure and decreased its crystallinity. This was probably due to the breaking of hydrogen bonds and hydrophobic interactions between protein molecules, leading to partial disintegration of the crystalline structure and increased molecular disorder. Acid treated protein exhibited markedly reduced diffraction peak intensities near 2θ = 10°, 20°, and 30°. Acid treatment likely hydrolyzed the protein macromolecules into smaller peptide fragments, destroying the original crystalline structure and rendering the sample more amorphous. Enzyme-treated protein also showed low diffraction peak intensities near 2θ = 10°, 20°, and 30°, with broadened peaks. Enzymatic treatment reduced the crystallinity of *C. oleifera* protein, but to a lesser extent than acid treatment. Enzymatic treatment selectively hydrolyzed specific peptide bonds in the protein molecules, breaking down the macromolecules into smaller peptides, disrupting the integrity of the crystalline structure, and weakening the crystalline characteristics of the sample.

The low crystallinity and weaker intermolecular interactions in *C. oleifera* protein treated with alkali, acid, and enzymes mean that a smaller energy barrier needed to be overcome during the curing process. As a result, lower DSC exothermic peak temperatures was observed.

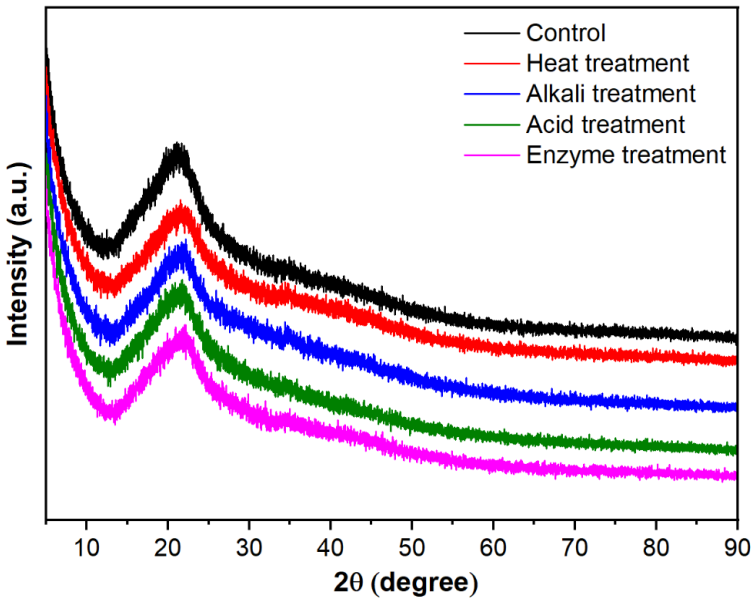

Fig. 9. Effects of different treatments on XRD results of *C. oleifera* protein adhesive

CONCLUSIONS

1. Heat treatment induced partial alteration in the secondary structure of *Camellia oleifera* protein molecules, leading to the exposure of hydrophobic groups and subsequent molecular association. The differential scanning calorimetry (DSC) analysis revealed an elevated curing temperature of 119.2 °C. This suggests that thermal treatment

relaxed the aggregated structure of the protein molecules, thereby necessitating a higher curing temperature to facilitate the formation of a stable network.

2. Alkali treatment induced partial disruption of the crystalline structure of *C. oleifera* protein, leading to a reduction in its crystallinity. DSC analysis revealed a curing temperature of 113.2 °C. The treatment caused the protein molecules to adopt an extended conformation by cleaving some hydrogen bonds and disulfide bonds. This structural alteration enhanced intermolecular interactions, thereby facilitating aggregation and promoting the formation of a stable network during the curing process.
3. Acid treatment partially hydrolyzed the peptide bonds in *C. oleifera* protein molecules without significantly altering the entire peptide chain. The curing temperature was 113.9 °C, and the crystallinity of the protein was weakened. This treatment modified the protein structure by protonating amino groups and partially hydrolyzing peptide bonds, which increased the reactivity of the protein's active groups with wood components. Additionally, acid treatment enhanced the reactivity of functional groups on the wood surface, thereby improving the bonding performance of the protein adhesive.
4. Enzymatic treatment led to extensive hydrolysis of peptide bonds in *C. oleifera* protein, significantly reducing its crystallinity. DSC indicated a curing temperature of 113.3 °C. The specific enzymatic hydrolysis targeted key structural elements of the protein, which disrupted its native conformation. This alteration hindered the ability of the protein molecules to form robust bonds with wood components, thereby affecting the adhesive properties.
5. *C. oleifera* protein, as a renewable natural resource, has significant advantages of low cost and environmental friendliness. This research not only provides a theoretical basis and technical support for the development of *C. oleifera* protein adhesives but also offers new directions for future research.

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