Dual-Pathway Glyoxal–Peptide Reaction Mechanisms under Acidic and Alkaline Conditions for *Camellia oleifera* Protein-based Adhesive Performance Optimization

Jiankun Liang,[a,#] Yuqi Yang,[b,#] Ningyuan Zuo,[b] Qiuli Li,[b] Zhixian Song,[b] Caihong Long,[b] Yulan Jian,[a,*] and Zhigang Wu,[b,*]

The pH-mediated regulation of the glyoxal-dipeptide reaction pathway was systematically investigated *via* electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance (^{13}C-NMR) spectroscopy. The resulting mechanistic insights were then applied to optimize *Camellia oleifera* protein-based adhesive performance. Under alkaline conditions, glyoxal undergoes an intramolecular Cannizzaro reaction, where one aldehyde group is reduced to an alcohol hydroxyl group, and the other aldehyde group is oxidized to a carboxyl group, resulting in the salt form of glycolic acid ($HOCH_2COO^-$). Glycolic acid enables extensive cross-linking *via* bifunctional, cooperative condensation with peptide amino and amide groups. A critical pH threshold of 11 was established for this process. In contrast, under acidic conditions, intramolecular cyclization of glyoxal to cyclic ether structures was observed. Simultaneously, the dipeptide's aliphatic amino groups were protonated and inactivated, leaving only weakly nucleophilic amide groups available for reaction, which led to a significant reduction in overall efficiency. When applied to these adhesives, bond strength was shown to exhibit a distinct pH dependency. In the glyoxal-only system, a bond strength of 0.76 MPa was attained at pH 11, corresponding to a ~65% increase relative to acidic conditions. For the melamine-glyoxal modification, this value was further optimized to 0.95 MPa at pH 13, a result ascribed to the synergistic cross-linking effect of the triazine ring.

DOI: 10.15376/biores.21.1.1207-1223

Keywords: Glyoxal; pH-mediated control; Cross-linking reaction; Reaction mechanism; Camellia oleifera; Protein-based adhesive

*Contact information: a: College of Civil Engineering, Kaili University, Qiandongnan 556011, China; b: College of Forestry, Guizhou University, Guiyang 550025, China; #: These two authors contributed equally to this work; *Corresponding authors: Jianyulan1992@163.com; wzhigang9@163.com*

INTRODUCTION

Escalating global petroleum scarcity and heightened environmental consciousness have positioned renewable biomass exploitation as a pivotal research direction in wood adhesive development. Biobased adhesives derived from natural polymers—including carbohydrates (Watcharakitti *et al*. 2022; Li *et al*. 2023, 2024a; Cai *et al*. 2024; Xu *et al*. 2024), lignin (Singh *et al*. 2022; Yang *et al*. 2023; Li *et al*. 2024b; Ghahri and Park 2025; Zheng *et al*. 2025), tannins (Arias *et al*. 2021; Aristri *et al*. 2022; Borah and Karak 2022; Xu *et al*. 2023; Zhou *et al*. 2024; Zheng *et al*. 2024), and soy protein—have been extensively investigated, with soy protein-based formulations yielding particularly fruitful outcomes (Aladejana *et al*. 2023; Hao *et al*. 2023; Liu *et al*. 2023; Jiang *et al*. 2024; Chen

et al. 2024; Kan *et al*. 2024; Hu *et al*. 2024; Hou *et al*. 2025; Li *et al*. 2025; Liu *et al*. 2025). However, large-scale industrial utilization of soy—a staple food crop—for adhesive production poses threats to food security and contravenes the sustainable development principles advocated by the Food and Agriculture Organization. Consequently, alternative protein sources that avoid competition with human food supplies and arable land have emerged as an urgent necessity.

Oilseed meals, the abundant byproducts of edible oil and biodiesel extraction, are rich in protein and carbohydrates, making them promising feedstocks for wood adhesive synthesis (Frihart *et al*. 2019; Wu *et al*. 2020). Current investigations have predominantly adopted crosslinking modification strategies adapted from soy protein adhesive successes. Through crosslinking reactions between synthetic resins and degraded protein residues, the vulnerable secondary structures of soy protein can be supplemented and reinforced, thereby enhancing cohesive strength, bond strength, and water resistance. Among these approaches, formaldehyde-based resins—including urea-formaldehyde (UF), melamine-urea-formaldehyde (MUF), melamine-formaldehyde (MF), and phenol-formaldehyde (PF)—have been most commonly employed as crosslinking agents (Gao *et al*. 2012; Zhang *et al*. 2018; Wu *et al*. 2017, 2019; Xu *et al*. 2020). Although notable improvements in bond strength and water durability can be achieved, the incorporation of formaldehyde-based resins severely compromises the green credentials of protein adhesives, creating a dilemma in which performance gains are offset by environmental sacrifices.

In contrast to formaldehyde, glyoxal exhibits low volatility and toxicity, establishing its credentials as an environmentally benign crosslinker (Deng *et al*. 2014a,b). Its application in wood adhesives encompasses two primary aspects: (1) partial or complete replacement of formaldehyde in reactions with urea, melamine, and other precursors to produce novel amino resins; and (2) direct employment as a crosslinking or curing agent in biomass adhesives, where it has been successfully utilized in tannin and lignin formulations. As the simplest saturated C2-dialdehyde, glyoxal combines high reactivity, low toxicity, and economic viability, attributes that have attracted considerable attention in recent years (Park *et al*. 2023; Müller *et al*. 2024).

In this study, the Ala-Gly dipeptide was employed as a model compound to systematically investigate the pH-dependent regulation of glyoxal–peptide reaction pathways through electrospray ionization mass spectrometry (ESI-MS) and ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectroscopy. The optimized process parameters were subsequently applied to enhance the performance of *Camellia oleifera* protein-based adhesives, thereby elucidating structure–activity relationships within this crosslinking system and providing a theoretical foundation for developing high-performance, eco-friendly protein-based adhesives.

EXPERIMENTAL

Materials

Camellia oleifera protein (protein content 36 wt.%) was processed from camellia seed meal supplied by Rongjiang Industrial Park, Guizhou, China. Ala-Gly dipeptide (98 wt%, analytical reagent grade) was sourced from Amresco. Melamine (analytical grade) and glyoxal (40 wt%) were obtained from Guangzhou Fuyang Chemical Technology Co., Ltd. and Chengdu Kelong Chemical Reagent Factory, respectively. All other reagents, including formic acid, were of analytical purity.

The Reaction between Glyoxal and Ala-Gly Dipeptide

A 30 wt% aqueous solution of Ala-Gly dipeptide and glyoxal at 1:3 molar ratio was prepared. The pH values were systematically varied to 1, 3, 5, 7, 9, 11, and 13 using 10% HCl or NaOH. Each mixture was reacted for 1 h at 75 to 80 °C under magnetic stirring while maintaining stable pH, generating products across the pH spectrum.

Synthesis of Melamine–Glyoxal Crosslinker

Glyoxal was placed in a three-neck flask equipped with mechanical stirring, temperature control, and reflux condensation, then adjusted to the pH range 4.0 to 4.5. After heating to 70 °C, melamine was gradually introduced and reacted for 90 min at this temperature.

Preparation of Modified *Camellia oleifera* Protein-based Adhesives

A protein pre-treatment solution was first prepared by dissolving 4 g sodium hydroxide in 300 g water, then slowly incorporating 80 g protein powder in a 500 mL three-neck flask with stirring at 50 °C for 60 min. Either glyoxal or the melamine–glyoxal crosslinker was subsequently added, and the pH was tuned to 1, 3, 5, 7, 9, 11, or 13, producing the respective modified adhesives after thorough mixing.

Plywood Preparation and Performance Testing

Three-layer poplar plywood was laboratory-fabricated by applying adhesive at 300 g/m^2 (single-side spread) to veneer surfaces, followed by hot pressing at 160 °C and 1.5 MPa for 4 min. After 24-h conditioning at ambient temperature, wet bond strength was evaluated per national standard GB/T 17657 (2022).

Electrospray Ionization Mass Spectrometry (ESI-MS) Testing

Chloroform solutions of the samples were configured at 10 μL/mL concentration for direct infusion electrospray ionization mass spectrometry. A Waters Xevo TQ-S instrument was employed, with sample introduction at 5 μg/s flow rate, positive ion detection, and ion energy maintained at 0.3 eV.

Nuclear Magnetic Resonance (^{13}C-NMR) Testing

For ^{13}C-NMR analysis, liquid samples were blended with DMSO-d6 at a 3:1 volume ratio. Spectra were acquired on a Bruker Avance 600 MHz spectrometer using inverse-gated proton decoupling (zgig) to optimize signal-to-noise performance. All measurements employed a 6 s relaxation delay, with chemical shift accuracy within 1 ppm.

RESULTS AND DISCUSSION

ESI-MS Analysis of the Reaction Products of Glyoxal and Alanyl-Glutamine Dipeptide

The electrospray ionization mass spectrometry (ESI-MS) spectra of the Ala-Gly (AG) dipeptide-glyoxal reaction system under pH 1, 3, and 5 conditions are presented in Fig. 1. Under electrospray ionization conditions, nitrogen-containing moieties readily undergo protonation to yield $[M+H]^+$ adducts, whereas oxygen-rich functional groups preferentially form sodium adducts ($[M+Na]^+$), reflecting the differential ionization affinities of these heteroatoms.

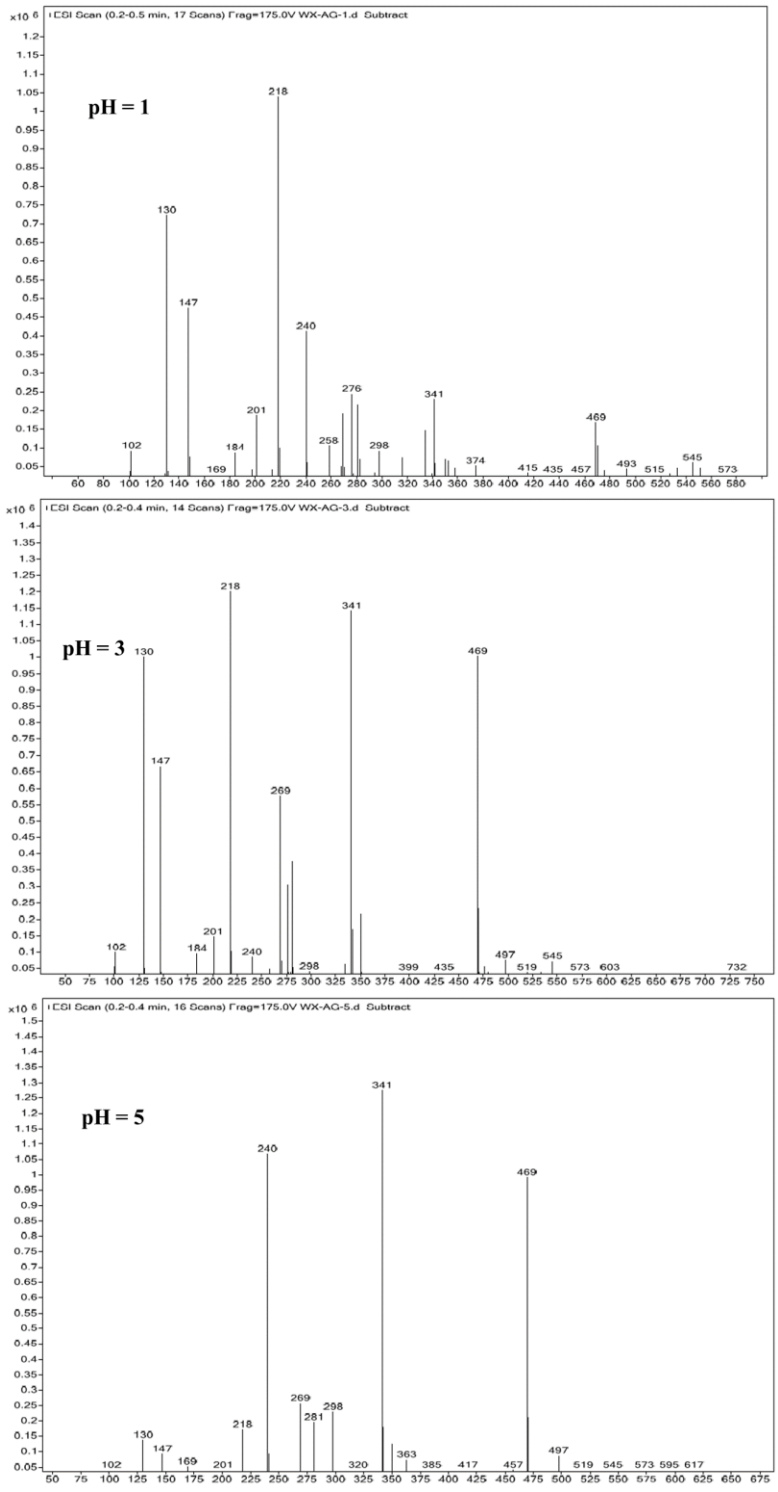
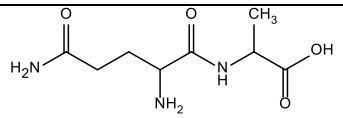
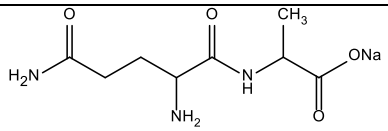
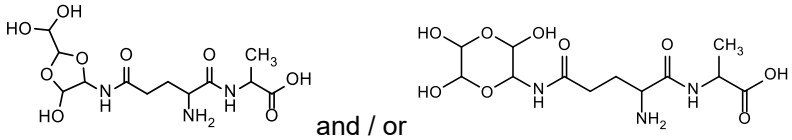
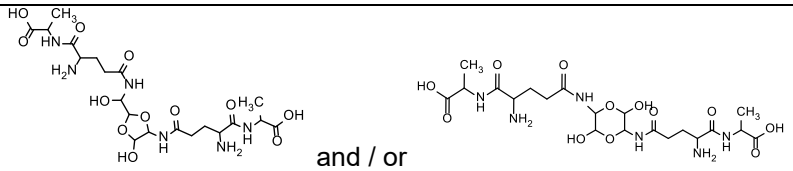
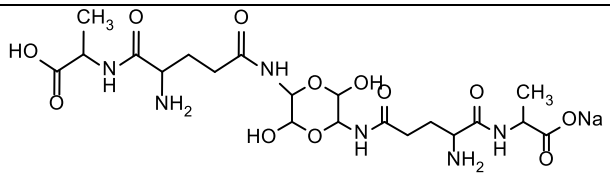
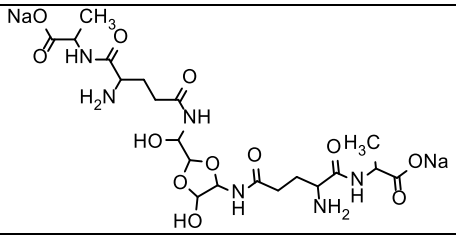


Fig. 1. ESI-MS curves of the reaction products between AG and glyoxal under pH 1, 3, and 5

The molecular structural assignments of each mass spectrometric peak under acidic conditions are summarized in Table 1. Among them, *m*/*z* 218 and 240 Da are assigned to the $[M+H]^+$ and $[M+Na]^+$ protonated ions of free alanyl-glutamine dipeptide, which is the typical ionization mode for peptide compounds in positive ion mode. The *m*/*z* 262 Da is

assigned to its sodium carboxylate adduct ion; this sodiation phenomenon is attributed to the coordination of trace sodium ions from the mobile phase during the ionization process in the mass spectrometer, while the substrate in the actual reaction system is still present in its free form—a phenomenon that is widely observed in ESI-MS analysis. Upon comparison of the spectra at pH 1, 3, and 5, significant signals of free alanyl-glutamine dipeptide were observed in each system, with no apparent decrease in relative intensity as pH increased, indicating that a substantial portion of the substrate did not participate in reactions with glyoxal and its cyclization products, the overall reaction extent was limited, and it is suggested that acidic conditions are unfavorable for the nucleophilic addition-condensation reaction.

Table 1. Main Ion Peaks of ESI-MS and Their Assignment the Reaction Products Between AG and Glyoxal under pH 1, 3, and 5

Experimental		Structures
[M+H]+	[M+Na]+	
218	240	 (AG)
	262	
	374	 and / or
435	457	2AG
	573	 and / or
	595	
	617	

The *m*/*z* 374 Da signal is assigned to the product of dehydration condensation between the free hydroxyl group of tetrahydroxyethylene cyclic ether (formed by cyclization of glyoxal) and the amino group of alanyl-glutamine dipeptide; the structure is speculated to be an N,O-acetal formed between the N-terminal or side-chain amino group of the dipeptide molecule and the hydroxyl group of the cyclic ether, and the formation of this product suggests that intramolecular cyclization and rearrangement of glyoxal may be induced under acidic conditions.

Three peaks at *m*/*z* 573, 595, and 617 Da, which exhibit a regular mass increment of 22 Da, are assigned to the dehydration polycondensation products of two alanyl-glutamine dipeptide molecules with one tetrahydroxyethylene five-membered or six-membered ring structure. The formation of such polymers indicates the presence of complex intermolecular condensation processes in the reaction system, possibly leading to the generation of cyclic or linear oligomers, suggesting that glyoxal, as a bifunctional cross-linking agent, can bridge peptide molecules. However, the low abundance of these polymers further corroborates the conclusion that the reaction conversion was limited at pH 1, 3, and 5.

The ESI-MS spectra of the alanyl-glutamine dipeptide-glyoxal system under pH 7, 9, 11, and 13 conditions are presented in Fig. 2, with product assignments summarized in Table 2.

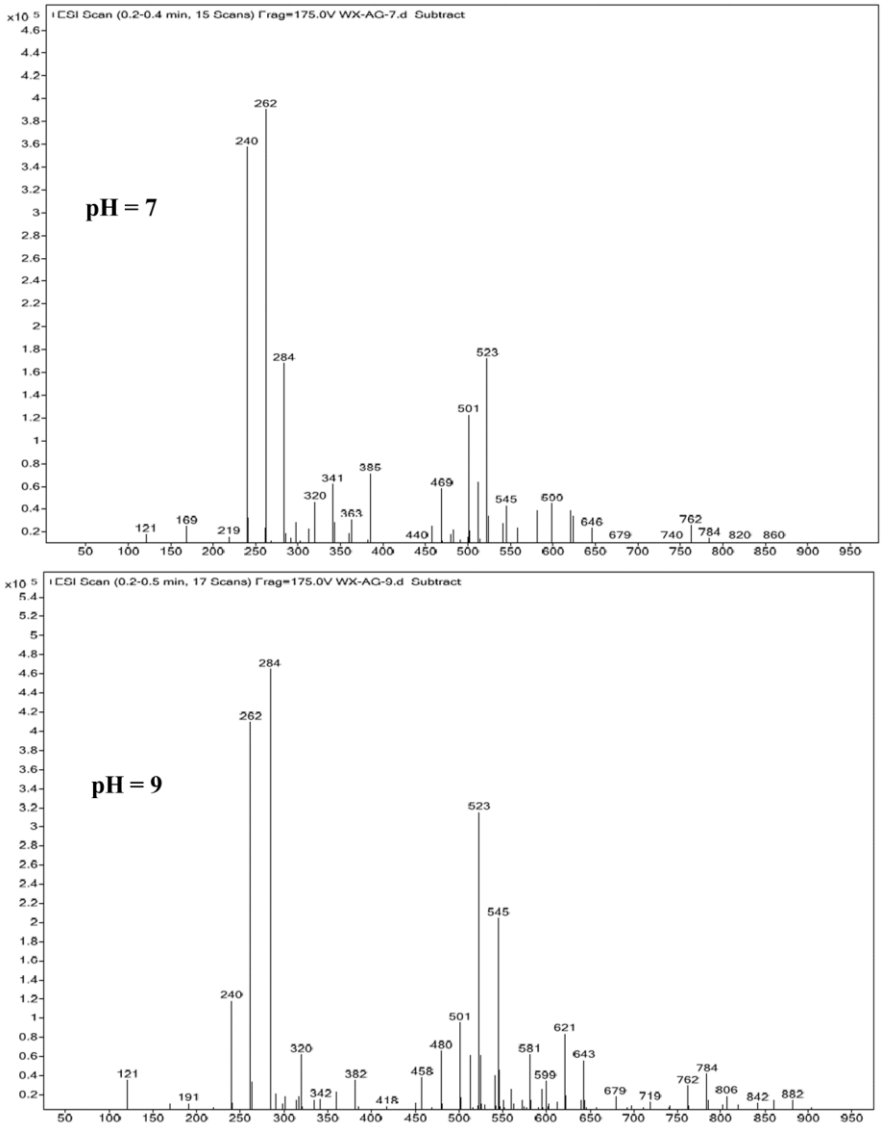
The *m*/*z* values 240 and 262 Da are assigned to the $[M+Na]^+$ ion of free alanyl-glutamine dipeptide and its double sodium carboxylate adduct $[M-2H+2Na]^+$, respectively. Their abundances are observed to increase with rising pH, consistent with the promotion of carboxylate deprotonation and sodium coordination under alkaline conditions. However, this phenomenon is attributed to the characteristics of ESI ionization and does not affect the actual speciation of substrates in solution. The *m*/*z* 501 Da peak is assigned to the double sodium carboxylate adduct of the dipeptide dimer ($[2M-2H+3Na]^+$), which further reveals the tendency for sodiation at carboxyl sites of polypeptides during mass spectrometric detection.

The mass difference between *m*/*z* 320 Da and 262 Da is equivalent to the molecular weight of glycolic acid, suggesting that glyoxal predominantly participates in the polycondensation reaction in its glycolic acid form. The reactive sites in the alanyl-glutamine dipeptide molecule capable of reacting with glycolic acid are identified as the nucleophilic amino group and the hydroxyl oxygen of the carboxyl group. The amino group is considered to possess significantly greater nucleophilicity than the carboxyl hydroxyl oxygen due to its available lone pair electrons, and thus it is predominantly involved in the dehydration condensation reaction with high selectivity.

In contrast to acidic systems, an order-of-magnitude enhancement in reactivity is exhibited under alkaline conditions. A dramatic increase in the abundance of condensation products is observed: the signal intensities of the *m*/*z* 320, 458, 545, and 599 Da series are significantly higher than those of the *m*/*z* 374, 573-617 series observed under acidic conditions, peaking at pH 9 to 11, which clearly demonstrates that the alkaline environment effectively facilitates the kinetics of nucleophilic addition-condensation reactions. Extension of product molecular weight is evidenced by the detection of high-molecular-weight oligomers at *m*/*z* 740 and 820 Da (despite their weak signals), suggesting that glyoxal, as a bifunctional cross-linking agent, can bridge three or more peptide molecules under alkaline conditions to form oligomeric network structures. This demonstrates that the reaction is promoted by alkaline conditions through the following proposed mechanisms: (1) elevation of pH shifts the deprotonation equilibrium of amino groups to

the right, enhancing nucleophilicity and significantly increasing their attack frequency on carbonyl groups; (2) the alkaline environment facilitates the enolization of glyoxal and aldol condensation, resulting in the formation of more reactive dimers/oligomers; and (3) alkaline conditions lower the activation energy of the dehydration step, driving the condensation equilibrium toward the product side.

In conclusion, under alkaline conditions, glyoxal predominantly reacts with the amino groups of alanyl-glutamine dipeptide in its glycolic acid form, and the extent of polycondensation becomes more complete with increasing alkalinity, leading to the formation of more stable cross-linked products.



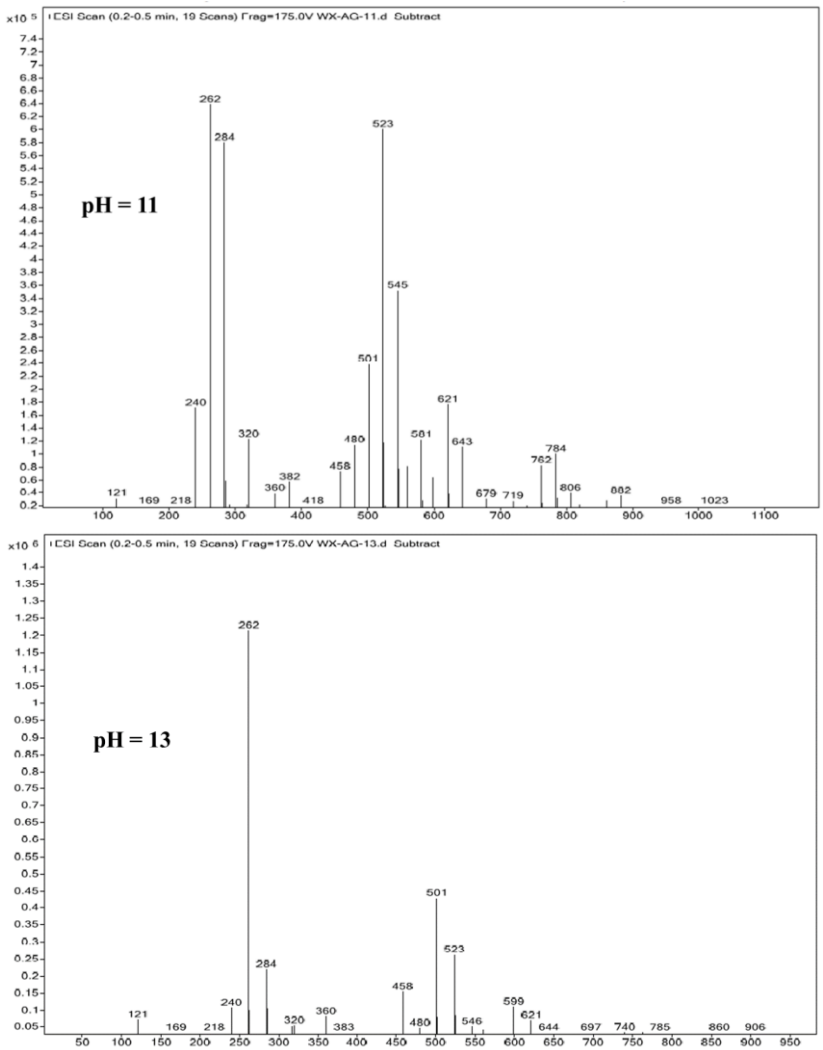
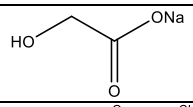
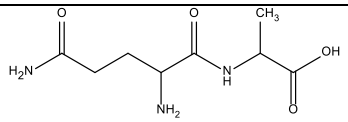
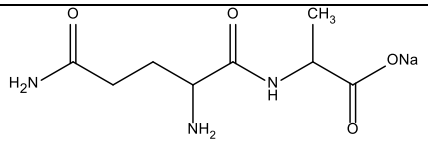
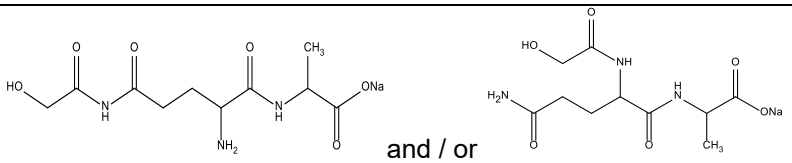
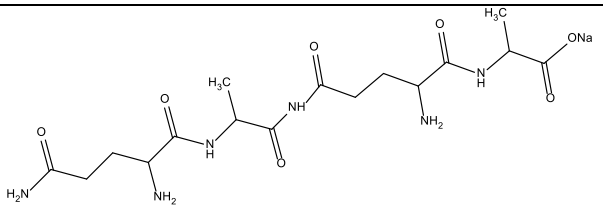
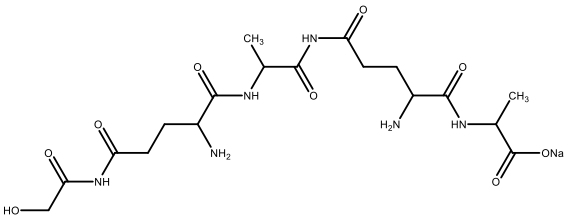
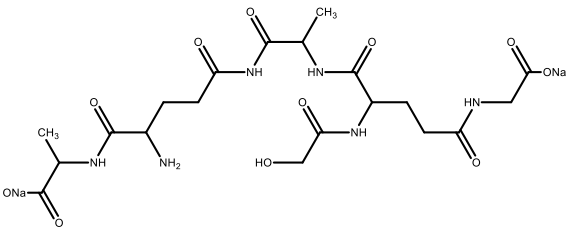
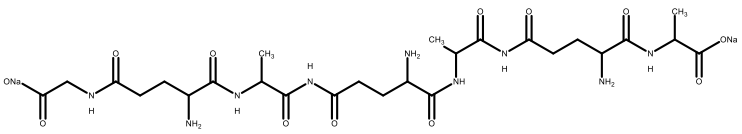
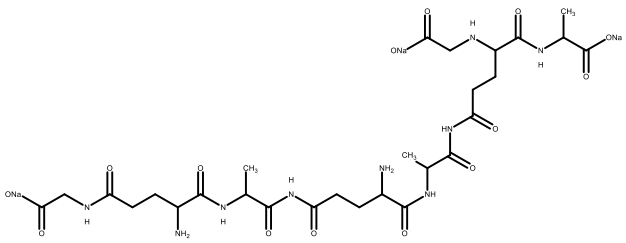


Fig. 2. ESI-MS curves of the reaction products between AG and glyoxal under pH 7, 9, 11, and 13

Table 2. Main Ion Peaks of ESI-MS and Their Assignment the Reaction Products between AG and Glyoxal under pH 7, 9, 11, and 13

Experimental (Da)		Structures
[M+H]+	[M+Na]+	
	121	
219	240	
	262	

	320	 and / or
	458	
	545	
	599	
	740	
	820	

^{13}C-NMR Analysis of the Reaction of Glyoxal and Ala-Gly Dipeptide

Based on the phenomenon revealed by ESI-MS that alkaline conditions promote cross-linking reactions, NMR can be further employed to verify reaction sites and structural evolution patterns at the solution level. The ^{13}C-NMR spectra of the alanyl-glutamine dipeptide-glyoxal system under pH 7, 9, 11, and 13 conditions are presented in Fig. 3.

The signal peaks at chemical shifts of 180.40 to 180.83 ppm and 62.67 to 62.77 ppm are assigned to the carbonyl carbon and alcoholic carbon in CH_2OH-COONa, respectively. This assignment is in good agreement with literature data on the hydration-disproportionation equilibrium of glyoxal under alkaline conditions, confirming that glyoxal predominantly exists as sodium glycolate in alkaline environments and undergoes nucleophilic addition with peptide chains as the actual reactive unit. Strong mutual verification is provided with the *m*/*z* 320 Da (peptide-glyoxal adduct) observed in ESI-MS, compensating for the limitation of mass spectrometry in directly reflecting solution-phase species.

The two new peaks in the spectrum at 64.00 to 64.05 ppm and 69 to 70 ppm are assigned to aliphatic amino hydroxymethylation products (N-terminal amino group attacking the -CH_2OH of glycolic acid) and amide hydroxymethylation products (side-chain –$CONH_2$ of alanyl-glutamine dipeptide participating in the reaction), respectively. It is indicated that both the amino group and side-chain amide group of alanyl-glutamine dipeptide are effective nucleophilic sites, with higher reactivity observed for the amino group (consistent with ESI-MS analysis). The reversibility of such hydroxymethyl adducts in solution should be noted; they are identified as key intermediates prior to imine/acetal formation, and the initial reaction rate is directly reflected by their signal intensities.

The characteristic peak at 74.73 ppm is observed only in the pH 11 system. A deviation from the conventional range for hydroxymethyl carbon is exhibited by this chemical shift, and correspondence to ether or acetal carbon structures formed through secondary polycondensation (such as –NH–CH_2–O–CH_2–NH–) is speculated. Based on the uniqueness of this peak, pH 11 is suggested to be an inflection point in reaction kinetics, where optimal matching between the nucleophilicity of the amino group and the activity of glycolic acid is achieved, and the conversion of mono-addition products to deep cross-linking is promoted. However, the disappearance of this peak is observed at strongly alkaline pH 13, possibly caused by over-disproportionation of glyoxal into oxalate or occurrence of side reactions under extreme alkaline conditions, and the cross-linking pathways are blocked. This observation is consistent with the phenomenon of weakened signals for high-molecular-weight products at pH 13 in ESI-MS.

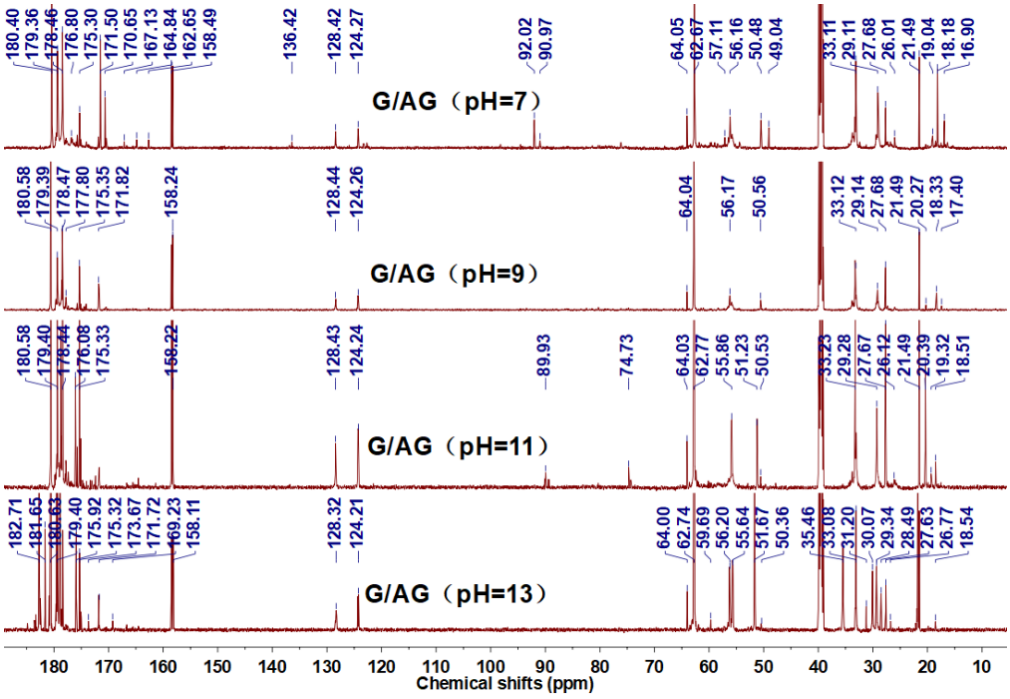


Fig. 3. ^{13}C-NMR spectra of the reaction between AG and glyoxal under pH 7, 9, 11, and 13

The ^{13}C-NMR spectra of the alanyl-glutamine dipeptide-glyoxal acidic system (pH 1, 3, 5) are shown in Fig. 4. All carbon signals were observed as clear, sharp singlets. Compared with the carbon spectrum of free alanyl-glutamine dipeptide (Wu *et al*. 2019), a new peak was detected only at 169.83 ppm, which was assigned to the amide carbonyl carbon that had shifted upfield after participating in the reaction, indicating that its

chemical environment had been altered by nucleophilic attack.

From the perspective of reaction sites, two potential nucleophilic sites (aliphatic amino group and amide group) were identified in the alanyl-glutamine dipeptide molecule. In strongly acidic media, the aliphatic primary amino group was completely deactivated by the facile protonation of its lone pair electrons, leading to complete loss of nucleophilic activity. In contrast, the amide nitrogen atom, due to p-π conjugation-induced electron delocalization, possessed extremely weak basicity and was difficult to protonate, thus becoming the sole effective nucleophilic site. The carbonyl/hydroxyl oxygen atoms in glyoxal or its cyclization product (tetrahydroxyethylene cyclic ether) could be readily protonated, significantly enhancing their electrophilicity. Therefore, the reaction under acidic conditions was essentially identified as a selective nucleophilic addition-dehydration condensation between protonated glyoxal/cyclic ether and the amide nitrogen of alanyl-glutamine dipeptide, rather than involving the deactivated aliphatic amino group.

Under alkaline conditions, glyoxal was established to function as a C1 building block in its glycolic acid form; both amino and amide groups were found to be capable of undergoing hydroxymethylation (with the former being the primary reaction site), and pH 11 was determined to be the critical condition for deep polycondensation. The high complementarity of NMR and ESI-MS data collectively established a complete reaction pathway of "hydroxymethylation, dehydration polycondensation, cross-linked network". In the acidic system, precise regulation of reaction sites was achieved through the protonation effect: the amide group, as the sole nucleophilic site, dominated the condensation reaction with glyoxal, while the aliphatic amino group was blocked by protonation, resulting in overall reaction activity that was far lower than that under alkaline conditions.

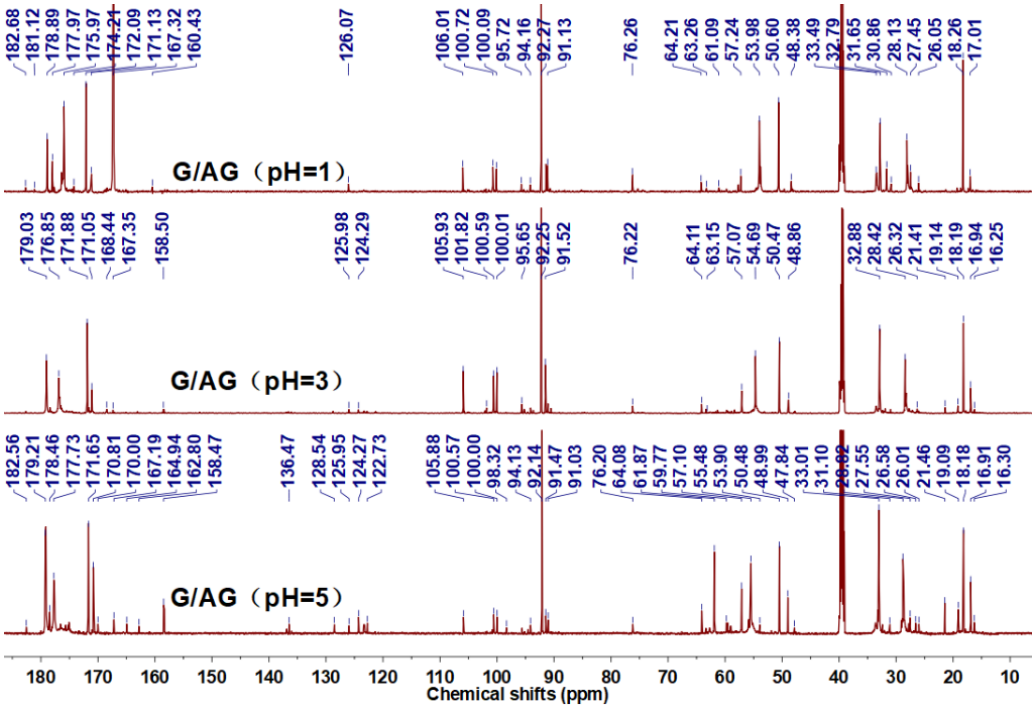


Fig. 4. ^{13}C-NMR spectra of the reaction between AG and glyoxal under pH 1, 3, and 5

Bonding Performance of *Camellia oleifera* Protein-based Adhesive

The molecular chains of protein are enriched with active groups such as -NH_2, -$CONH_2$, -COOH, and -OH. Although the terminal free amino and carboxyl groups are highly active, their direct reaction extent with polar groups on the wood surface is limited. The introduction of glyoxal can significantly increase the hydroxymethyl content within the protein structure, thereby enabling effective chemical bonding with the wood surface. It has been elucidated by previous ESI-MS and ^{13}C-NMR studies that the existing forms of glyoxal vary in different media, which inevitably results in pH-dependent modification mechanisms and bonding performance.

As shown in Fig. 5, over the pH range of 1 to 13, a significant alkaline advantage in wet strength was demonstrated for glyoxal- or melamine-glyoxal-modified *Camellia oleifera* protein-based adhesives. For glyoxal modification, a peak value of 0.76 MPa was reached at pH 11, representing an approximately 65% improvement compared with acidic conditions. This is attributed to the fact that under acidic conditions, glyoxal is primarily present in the form of cyclic ether compounds, aliphatic amino groups are deactivated by protonation, and only weakly nucleophilic amide groups can participate in reversible reactions. This combination results in low crosslinking density and products susceptible to hydrolysis. Under alkaline conditions, glyoxal is transformed into glycolic acid possessing dual reactivity of both carboxylic acid and alcohol functionalities. Dehydration condensation can be induced between its carboxyl group and the amino/amide groups of the protein, while ether bonds can be formed between its hydroxyl group and the carboxyl groups of the protein as well as the hydroxyl groups of wood cellulose. Through its advantages as a small molecule with low steric hindrance, a "protein-crosslinker-wood" three-dimensional network is efficiently constructed, bisite synergistic crosslinking is achieved, and cohesive strength and water resistance are significantly enhanced (Du *et al*. 2024; Yang *et al*. 2025). For melamine-glyoxal modification, a maximum strength of 0.95 MPa was obtained at pH 13, and superior performance was demonstrated under all pH conditions compared with glyoxal alone. In addition to the aforementioned reasons, extra triazine rings and -NH_2 groups are introduced by melamine, which can form three-dimensional bulk crosslinking networks with glyoxal under alkaline conditions, demonstrating a synergistic enhancement effect and further enhancing the cohesive strength of the adhesive.

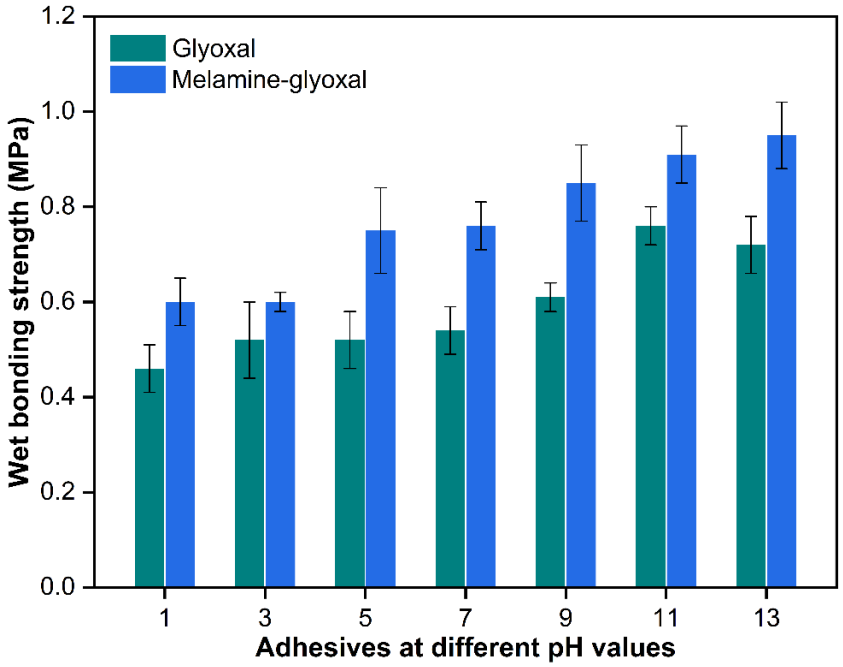


Fig. 5. Bonding performance of *Camellia oleifera* protein-based adhesives crosslinked by glyoxal and melamine-glyoxal under different pHs

The performance of glyoxal- or melamine-glyoxal-modified *Camellia oleifera* protein-based adhesives is essentially the result of dual regulation by the alkaline environment on both the existing form of glyoxal and the activity of protein nucleophilic sites. The pH range of 11 to 13 is identified as the optimal window for crosslinking reactions, at which point the nucleophilicity of amino groups is strongest due to deprotonation, and both the activity of glyoxal and the reaction equilibrium are brought to optimal states. However, at pH 13, performance is slightly inferior to that at pH 11 due to side reactions that are easily induced by excessive alkalinity.

CONCLUSIONS

1. The regulatory effects of pH on the reaction pathway of glyoxal with alanyl-glutamine dipeptide and the performance of *Camellia oleifera* protein-based adhesive were examined. The reaction pathway was dominated by pH through its determination of the form of glyoxal that was present and the activity of nucleophilic sites on the alanyl-glutamine dipeptide. Under acidic conditions, glyoxal was cyclized to form cyclic ethers, aliphatic amino groups were completely deactivated by protonation, and only amide groups were involved in inefficient and reversible nucleophilic addition. Under alkaline conditions, glyoxal was converted into the salt form of glycolic acid, whose dual reactivity (carboxyl and hydroxyl groups) enabled simultaneous bridging of peptide amino and amide groups.
2. High complementarity was observed between ESI-MS and ^{13}C-NMR data, which jointly confirmed that the pH range of 9 to 11 represents the optimal kinetic window. During this range, the nucleophilicity of amino groups was maximized through deprotonation, glyoxal was stably present as sodium glycolate, and the highest abundance of polycondensation products was observed. At pH 11, the appearance of

ether/acetal structures formed through secondary polycondensation was indicative of deep cross-linking initiation. However, at pH 13, excessive disproportionation of glyoxal and side reactions were induced by strong alkalinity, resulting in a decline in cross-linking efficiency, thereby confirming the importance of moderate alkalinity for equilibrium regulation.

3. A significant positive correlation was observed between the wet strength of camellia cake protein adhesive and the reaction activity of the model peptide. For the glyoxal-modified adhesive, a peak bonding strength of 0.76 MPa was achieved at pH 11, and cohesive strength and water resistance were synergistically enhanced by alkaline conditions through the construction of a "protein-crosslinker-wood" three-dimensional network. The strength was further increased to 0.95 MPa at pH 13 by the melamine-glyoxal system; the potential synergistic effect of multi-component crosslinkers is demonstrated by its multifunctional structure.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (32160348), Science-technology Support Foundation of Qiandongnan of China ([2025]0009), Science and Technology Achievement Project of Qiandongnan of China ([2024]2), the Specialized Fund for the Doctoral Development of Kaili University (BSFZ202505), Outstanding Youth Science and Technology Talent Project of Guizhou Province of China (YQK[2023]003).

REFERENCES CITED

Arias, A., Gonzalez-Garcia, S, Feijoo, G, and Moreira, M. (2021). "Tannin-based bio-adhesives for the wood panel industry as sustainable alternatives to petrochemical resins," *Journal of Industrial Ecology* 26(2), 627-642. https://doi.org/10.1111/jiec.13210

Aladejana, J., Zeng, G., Zhang, F., Li, K., Dong, Y., Li, X., and Li, J. (2023). "Tough, waterproof, and mildew-resistant fully biobased soybean protein adhesives enhanced by furfuryl alcohol with dynamic covalent linkages," *Ind. Crops Prod.* 198, article 116759. https://doi.org/10.1016/j.indcrop.2023.116759

Aristri, M.A., Lubis, M.A.R., Laksana, R.P.B., Sari, R.K., Iswanto, A.H., Kristak, L., Antov, P., and Pizzi, A. (2022). "Thermal and mechanical performance of ramie fibers modified with polyurethane resins derived from acacia mangium bark tannin," *J. Mater. Res. Technol.* 18, 2413-2427. https://doi.org/10.1016/j.jmrt.2022.03.131

Borah, N., and Karak, N. (2022). "Tannic acid based bio-based epoxy thermosets: Evaluation of thermal, mechanical, and biodegradable behaviors," *J. Appl. Polym. Sci.* 139, article 51792. https://doi.org/10.1002/app.51792

Cai, L., Chen, Y., Lu, Z., Wei, M., Zhao, X., Xie, Y., Li, J., and Xiao, S. (2024). "Citric acid/chitosan adhesive with viscosity-controlled for wood bonding through supramolecular self-assembly," *Carbohyd. Polym.* 329, article 121765. https://doi.org/10.1016/j.carbpol.2023.121765

Chen, S., Li, X., Bai, M., Shi, S., Aladejana, J., Cao, J., and Li, J. (2024). "A strong and tough supramolecular assembled β-cyclodextrin and chitin nanocrystals protein

adhesive: Synthesis, characterization, bonding performance on three-layer plywood," *Carbohydr. Polym.* 333, article 121971. https://doi.org/10.1016/j.carbpol.2024.121971

Deng, S., Pizzi, A., Du, G., Zhang, J., and Zhang, J. (2014a). "Synthesis, structure, and characterization of glyoxal-urea-formaldehyde co-condensed resins," *J. Appl. Polym. Sci.* 131, 41009-41018. https://doi.org/10.1002/app.41009

Deng, S., Du, G., Li, X., and Pizzi, A. (2014b). "Performance and reaction mechanism of zero formaldehyde-emission urea-glyoxal (UG) resin," *J. Taiwan Inst. Chem. E.* 45(4), 2029-2038. https://doi.org/10.1016/j.jtice.2014.02.007

Du, X., Li, Z., Zhang, J., Li, X., Du, G., and Deng, S. (2024). "Development of environmentally friendly glyoxal-based adhesives with outstanding water repellency utilizing wheat gluten protein," *Int. J. Biological Macromol.* 273, article 133081. https://doi.org/10.1016/j.ijbiomac.2024.133081

Frihart, C., Pizzi, A., Xi, X., and Lorenz, L. (2019). "Reactions of soy flour and soy protein by non-volatile aldehydes generation by specific oxidation," *Polymers* 11(9), 1478. https://doi.org/10.3390/polym11091478

Gao, Q., Shi, S.Q., Zhang, S., Li, J., Wang, N., and Meng, X. (2012). "Soybean meal-based adhesive enhanced by MUF resin," *J. Appl. Polym. Sci.* 125(5), 3676-3681. https://doi.org/10.1002/app.36700

GB/T 17657 (2022). "Test methods of evaluating the properties of wood-based panels and surface decorated wood-based panels," Standardization Administration of China, Beijing.

Ghahri, S., and Park, B. D. (2025). "Bio-crosslinking of oxidized hardwood kraft lignin as fully bio-based adhesives for wood bonding," *Int. J. Biol. Macromol.* 309, article 142907. https://doi.org/10.1016/j.ijbiomac.2025.142907

Hao, Z., Xi, X., Hou, D., Lei, H., Li, C., Xu, G., and Du, G. (2023). "A fully bio-based soy protein wood adhesive modified by citric acid with high water tolerance," *Int. J. Biol. Macromol.* 253, article 127135. https://doi.org/10.1016/j.ijbiomac.2023.127135

Hou, M., Zhang, Q., Lei, H., Zhou, X., Du, G., Pizzi, A., Essawy, H., and Xi, X. (2025). "Mildew resistant modified starch adhesive by soybean meal flour crosslinking with excellent bonding properties," *Carbohyd. Polym.* 354, article 123247. https://doi.org/10.1016/j.carbpol.2025.123247

Hu, Y., Bao, Z., Li, Z., Wei, R., Yang, G., Qing, Y., Li, X., and Wu, Y. (2024). "Develop a novel and multifunctional soy protein adhesive constructed by rosin acid emulsion and TiO_2 organic-inorganic hybrid structure," *Int. J. Biol. Macromol.* 277, article 134177. https://doi.org/10.1016/j.ijbiomac.2024.134177

Jiang, K., Dong, X., Chen, Y., Fan, D., and Chu, F. (2024). "A room-temperature curing plant protein-based adhesive with high strength and flame retardancy for heat-free adhesion," *Adv. Funct. Mater.* 34, article 2403490. https://doi.org/10.1002/adfm.202403490

Kan, Y., Chang, J., Wei, S., Li, J., Gui, C., Han, S., and Gao, Z. (2024). "A synergistic strategy for formulating a facile and cost-effective soybean protein-based adhesive via co-crosslinking and inorganic hybridization," *Int. J. Biol. Macromol.* 283, article 137569. https://doi.org/10.1016/j.ijbiomac.2024.137569

Li, C., Hou, D., Lei, H., Xi, X., Du, G., Zhang, H., Cao, M., and Tondi, G. (2023). "Effective and eco-friendly safe self-antimildew strategy to simultaneously improve the water resistance and bonding strength of starch-based adhesive," *Int. J. Biol. Macromol.* 248, article 125889. https://doi.org/10.1016/j.ijbiomac.2023.125889

Li, C., Hou, D., Xi, X., Lei, H., Tondi, G., Shi, J., and Du, G. (2024a). "Eco-friendly biobased adhesives prepared from different polyester-type glucose with high cross-linked structure," *ACS Sustainable Chem. Eng.* 12, 7831-7843. https://doi.org/10.1021/acssuschemeng.4c00669

Li, J., Zhang, M., and Sun, R. (2024b). "High-performance lignin-based phenolic resin adhesives for plywood applications," *Ind. Crops Prod.* 216, article 118915. https://doi.org/10.1016/j.indcrop.2024.118915

Li, X., Shao, J., Bai, M., Chen, S., Song, P., Shi, S., Chu, F., Jiang, S., Cao, J., and Li, J. (2025). "Biomimetic nanozyme chemistry transforms soy meal into high strength and multifunctional adhesive," *ACS Sustain. Chem. Eng.* 13(5), 2011-2022. https://doi.org/10.1021/acssuschemeng.4c07771

Liu, X., Bian, Y., Zhang, X., Liu, Z., Weng, T., Wang, G., Li, J., Chen, H., and Gao, Q. (2023). "Preparation of strong and mildew-resistant soybean meal adhesives with self-assembled core-shell structured nanospheres," *Ind. Crops Prod.* 205, article 117446. https://doi.org/10.1016/j.indcrop.2023.117446

Liu, X., Li, J., Liu, S., Zhang, Z., Qing, Y., Li, X., Liu, M., and Wu, Y. (2025). "Mussel and plant cell wall-inspired soy protein adhesives: High strength, flame retardancy, and mildew resistance," *Int. J. Biol. Macromol.* 320, article 145875. https://doi.org/10.1016/j.ijbiomac.2025.145875

Müller, B., Schmidt, A., and Li, C. (2024). "Glyoxal in pharmaceutical synthesis: High-efficiency intermediates for cephalosporin antibiotics and vitamin A derivatives," *Org. Process Res. Dev.* 28(3), 1125-1138. https://doi.org/10.1021/acs.oprd.3c00456

Park, J., Stevenson, D., and Martinez, F. (2023). "Molecular structure-reactivity relationship of short-chain dialdehydes: Why glyoxal outperforms glutaraldehyde in functional group density," *Green Chem. Lett. Rev.* 16(2), 189-201. https://doi.org/10.1080/17518253.2023.2189012

Singh, S. K., Ostendorf, K., Euring, M., and Zhang, K. (2022). "Environmentally sustainable, high-performance lignin-derived universal adhesive," *Green Chem.* 24, 2624-2635. https://doi.org/10.1039/d2gc00014h

Watcharakitti, J., Win, E. E., Nimnuan, J., and Smith, S. M. (2022). "Modified starch-based adhesives: A review," *Polymers* 14, article 2023. https://doi.org/10.3390/polym14102023

Wu, Z., Xi, X., Lei, H., and Du, G. (2017). "Soy-based adhesive cross-linked by phenol-formaldehyde-glutaraldehyde," *Polymers* 9(5), article 169. https://doi.org/10.3390/polym9050169

Wu, Z., Xi, X., Lei, H., Liang, J., Liao, J., and Du, G. (2019). "Study on soy-based adhesives enhanced by phenol formaldehyde cross-linker," *Polymers* 11(2), article 365. https://doi.org/10.3390/polym11020365

Wu, Z., Xi, X., Pizzi, A., Zheng, X., and Xiao, Z. (2020). "Effects of *Broussonetia papyrifera* leaf cutting modes on bonding performance of its protein-based adhesives," *Eur. J. Wood Prod.* 78(3), 461-470. https://doi.org/10.1007/s00107-020-01533-w

Xu, D., Li, C., Pizzi, A., Xi, X., Wang, Z., Du, G., Chen, Z., and Lei, H. (2024). "Self-neutralizing citric acid–corn starch wood adhesives," *ACS Sustainable Chem. Eng.* 12, 13382-13391. https://doi.org/10.1021/acssuschemeng.4c05590

Xu, G., Zhang, Q., Xi, X., Lei, H., Cao, M., Du, G., and Wu, Z. (2023). "Tannin-based wood adhesive with good water resistance crosslinked by hexanediamine," *Int. J. Biol. Macromol.* 234, article 123644. https://doi.org/10.1016/j.ijbiomac.2023.123644

Xu, Y., Han, Y., Shi, S.Q., Gao, Q., and Li J. (2020). "Preparation of a moderate viscosity, high performance and adequately-stabilized soy protein-based adhesive via recombination of protein molecules," *J. Clean. Product.* 255, article 120303. https://doi.org/10.1016/j.jclepro.2020.120303

Yang, G., Gong, Z., Luo, X., Chen, L., and Shuai, L. (2023). "Bonding wood with uncondensed lignins as adhesives," *Nature* 621, 511-515. https://doi.org/10.1038/s41586-023-06507-5

Yang, Z., Yan, S., Zhang, B., Huang, H., Gong, T., He, L., Essawy, H., Charrier, B., Pizzi, A., Huang, H., Zhou, X., Du, G., and Chen, X. (2025). "High-performance bio-based wood adhesive derived from *Camellia oleifera* meal with long shelf life and excellent mold resistance," *Ind. Crop. Prod.* 235, article 121712. https://doi.org/10.1016/j.indcrop.2025.121712

Zhang, M., Zhang, Y., Chen, M., and Zhang, S. (2018). "A high-performance and low-cost soy flour adhesive with a hydroxymethyl melamine prepolymer," *Polymers* 10(8), 909. https://doi.org/10.3390/polym10080909

Zheng, L., Tang, Z., Liang, Y., Yang, H., Wei, N., Li, H., Lei, H., and Tian, H. (2024). "Investigation of novel thermosetting amino resins based monomethylolurea, glyoxal and tannin," *Ind. Crops Prod.* 210, article 118092. https://doi.org/10.1016/j.indcrop.2024.118092

Zheng, X., Li, X., Li, P., Mou, Q., Wu, Y., Yu, Z., Li, T., and Li, X. (2025). "High-strength and sustainable bamboo scrimber manufactured with lignin-based biomass adhesive for indoor application," *Ind. Crops Prod.* 233, article 121450. https://doi.org/10.1016/j.indcrop.2025.121450

Zhou, Y., Li, C., Xu, D., Wang, Z., Chen, Z., Lei, H., and Du, G. (2024). "A tannin–oxidized glucose wood adhesive with high performance," *Ind. Crops Prod.* 222, article 119603. https://doi.org/10.1016/j.indcrop.2024.119603

Article submitted: November 19, 2025; Peer review completed: December 8, 2025; Revised version received and accepted: December 16, 2025; Published: December 19, 2025.
DOI: 10.15376/biores.21.1.1207-1223