Preparation of a Canola-based Polyethyleneimine-crosslinked Bioadhesive for Particleboards Production

Lawrence Tene Tayo ,* Fabian Dönni, and Markus Euring ,*

Over the past decades, consistent efforts have been dedicated to addressing the challenge of low performance of protein-based wood adhesives. This study explored the potential of polyethyleneimine (PEI) as a crosslinker for improving the bonding strength of canola protein isolate (CPI) and canola meal (CM) bio-adhesive. Simultaneously, the effect of the pH value of the slurry was investigated. Three-layer particleboards were manufactured using the canola-based adhesives and subjected to testing for their internal bonding strength (IB), bending strength (BS), and modulus of elasticity (E-modulus). Results showed that, despite the low PEI ratio utilized, notable enhancements in the mechanical properties of the boards were observed. The IB values increased by 17% and 15% for CPI and CM-based adhesive formulations, respectively; while the BS exhibited rose by 13% and 9%, respectively. It was observed that an increase in pH contributed to enhanced bonding properties of the bio-based adhesive. By enhancing the denaturation of the protein and increasing the free reaction group in the protein chain, this improves the crosslinking mechanism of PEI, leading to improved mechanical properties.

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INTRODUCTION

Conventionally, particleboards are produced using formaldehyde-containing adhesives such as urea, phenol, and melamine (Dorieh *et al*. 2022). While these synthetic binders provide superior efficiency and performance, they are not only associated with environmental and health risks, but are also unsustainable (Li *et al*. 2012; Tene Tayo *et al*. 2022). In recent decades, growing societal awareness and concerns regarding indoor air quality, health, and environmental impact have urged a transition toward the development of more eco-friendly and sustainable alternatives to traditional formaldehyde-based wood adhesives (Cárdenas-Oscanoa *et al*. 2024; Tene Tayo *et al*. 2024, 2025). In order to tackle the issue of indoor pollution and achieve sustainable particleboard production, the development of bio-based adhesives has emerged as a key priority (Hussin *et al*. 2022). While this facilitates the transition towards a more sustainable and resilient bioeconomy (Cárdenas-Oscanoa *et al*. 2024, 2025; Tene Tayo *et al*. 2024, 2025), it contributes to transforming the linear industrial economy into more circular systems that minimize pollution and waste generation. This shift promotes enhanced sustainability and inclusivity, effectively addressing climate change while reducing dependence on fossil-based materials

(Antov *et al*. 2023). Over recent decades, plant proteins have been shown to be a reliable alternative for the development of natural and sustainable adhesive systems for the wood industry, sometimes offering bonding properties comparable to that of synthetic adhesives. Thus far, numerous protein sources have been studied, with soy and wheat being the most extensively studied. Indeed, several soy-based adhesive formulations have been developed, with some reaching the level of commercialization (such as Soyad™ from Solenis). Despite the progress, the fact that soy is also a vital food and feed sources, limits the utilization in the bio adhesive industry. Moreover, the potential variability in agricultural yields due to climate change and global market demands (Chen *et al*. 2023) makes it more difficult to rely on a single protein source. Therefore, there is a growing understanding of the importance of diversifying protein sources (Dunky 2021; Frihart 2023) to ensure the long-term production and supply of protein-based bioadhesives. This diversification effort is essential for enhancing the resilience of the bio adhesive industry (Tene Tayo *et al*. 2024). Unlike soy or wheat, canola meal does not compete with the food supply, making it a more sustainable candidate for adhesive development (Chen *et al*. 2024). This reduces ethical concerns over diverting essential food resources for industrial applications (Huang *et al*. 2023).

The expansion of agricultural activities has significantly increased canola production, making it the second most abundant oilseed after soy (Goyal *et al*. 2021). According to USDA (2024), global rapeseed production reached over 89 million metric tons during the 2023/2024 growing season. Primarily cultivated for its oil, canola processing generates substantial amount of by-products, which are predominantly relegated to low-value applications such as animal feed or fertilizer (Wang *et al*. 2014), due to the presence of glucosinolates, erucic acid, phytates, and phenolics, which render it unsuitable for human consumption (Hale 2013). Therefore, utilizing canola protein for high-value applications, such as bio-adhesive production for wood composites, could significantly enhance the economic sustainability of the canola oil industry (Manamperi *et al*. 2010), while providing a sustainable solution to the wood panel industry.

With a unique protein composition (cruciferin and napin), canola exhibits structural features conducive to adhesion. Cruciferin has a hexameric structure with abundant hydrophilic functional groups that facilitate hydrogen bonding, while napin contributes to cohesive strength due to its smaller size and sulfur-rich disulfide bonds (Bandara *et al*. 2017). These features allow canola proteins to form strong bonds with lignocellulosic materials when properly modified (Aladejana *et al*. 2023). To date, only a limited number of studies have investigated the use of canola by-products in bio-adhesive formulations, utilizing either canola protein isolates (Wang *et al*. 2014; Bandara *et al*. 2017) or canola meal (Yang *et al*. 2011; Ostendorf *et al*. 2021). Among these, adhesive formulations based on protein isolates have demonstrated superior bonding strength compared to those utilizing crude canola meal. However, the use of canola meal could as well offer a more cost-effective and sustainable alternative (Tene Tayo *et al*. 2022), as it eliminates the high costs and low yields associated with protein isolation processes (Elstner and Stein 1982).

To overcome the issue of limited performance, especially when it comes to the bonding properties and the water resistance of protein-based bioadhesives, several crosslinking agents, such as urea-formaldehyde (UF), phenol-formaldehyde (PF), and polymeric methylene diisocyanate (pMDI) (Hemmilä *et al*. 2019; Bacigalupe *et al*. 2020; Bekhta *et al*. 2021) have been used. Although these crosslinking agents help improve considerably the performance, as well as the water resistance of the bio-adhesives, they remain problematic, due to their nature and source. Consequently, continued research

efforts are necessary to devise approaches that enhance the adhesive properties (Kristak *et al*. 2023). A challenge lies in attempting to develop viable crosslinkers capable of simultaneously enhancing reactivity, improving mechanical properties, and augmenting moisture resistance (Hemmilä *et al*. 2017). Numerous studies have highlighted the effectiveness of polymers such as polyethyleneimine (PEI) in enhancing the bonding strength of wood adhesives (Li *et al*. 2020; Mousavi *et al*. 2021; Averina *et al*. 2023; Zhang *et al*. 2023). PEI, a highly water-soluble polymer, exhibits exceptional reactivity due to its abundant amino groups, which readily participate in chemical reactions with the functional groups of the protein. Its branched structure further enhances reactivity and crosslinking density, making it a highly efficient crosslinking agent (Yuan *et al*. 2022). PEI also contains both polar (amino) and hydrophobic groups, enabling versatile crosslinking with a wide range of substances (Zeng *et al*. 2023). The amino groups in PEI can form strong hydrogen and ionic bonds by reacting with carboxyl groups, which is particularly advantageous for adhesive formulations (Li *et al*. 2004). These properties have led to its extensive application in adhesives formulation and coatings (Song *et al*. 2023). In wood adhesive production, PEI is frequently used due to its ability to undergo deamination polycondensation, thereby forming polyurea structures when combined with urea (Yang *et al*. 2021). It is also often employed alongside acid anhydrides or glutaraldehyde in various adhesive applications to enhance crosslinking and performance (Xi *et al*. 2021). In this work, PEI was utilized as an additive to strengthen the crosslinking network of in canola protein-based wood adhesive formulations, with the aim of improving the adhesive's bonding properties. The adhesive formulations were designed for the production of P2 grade particleboards (indoor application in dry area).

EXPERIMENTAL

Materials

The canola protein (Puratein® G) isolate (CPI) with a protein concentration of 90% was purchased from Merit functional Foods, Winnipeg, Canada. The canola meal (CM) was offered by Kleeschulte GmbH & Co. KG (Büren, Germany). This by-product of the canola oil manufacturing process arrived in the form of pellets. Sodium dodecyl sulfate (SDS), urea, sodium bisulfate (92%), sodium chloride, and sodium nitrite (99%) were sourced from VWR International in Darmstadt, Hesse, Germany. Sodium bisulfate, an acidic salt produced by partially neutralizing sulfuric acid with sodium hydroxide or sodium chloride, appears as a dry granular substance with hygroscopic properties. In contrast, sodium nitrite, an inorganic compound with the chemical formula $NaNO_2$, presents as a white to slightly yellowish crystalline powder that readily dissolves in water and exhibits hygroscopic characteristics. The Gelatine (180 Bloom) was obtained from Carl Roth GmbH + Co KG in Karlsruhe, Germany. The industrial wood particle material obtained from a residual process was supplied by Pfleiderer in Arnsberg, North Rhine-Westphalia, Germany.

Adhesive Preparation

The preparation of the different adhesive variants followed the procedure described in Tene Tayo *et al*. (2022), with some modifications. The canola meal (CM) was obtained by crushing the canola pellets and sieving to pass through a 400-µm mess. A gel mixture (gelatine-urea-water) was made following these proportion, 35:15:50 respectively. The

mixture was then conditioned in an oven set at 25 ° C for 72 h before being used to prepare the adhesives. A 1% SDS solution was prepared and used as a solvent and denaturant. Next, the necessary amount of NaCl and $NaHSO_4$ was dissolved in the corresponding quantity of SDS solution (see Table 1). Then, the gel mixture was added, followed by the slow supplement of the CPI/CM while stirring with a RW 20 laboratory stirrer from IKA®-Werke GmbH & Co. KG rotating 10000 rpm. To obtain a homogeneous slurry, the mixture was stirred for about 10 min. The glycerine was afterwards added, and the PEI came at the very end. Upon adding the PEI, the mixture was allowed to stir again for about 2 min, after which the pH was adjusted to 8, 9 or 10, using a 10 mol NaOH solution. The different adhesive formulations were immediately used to produce three-layer particleboards.

Table 1. Adhesive Formulation

Components	Proportions (%wt)	
	CPI-based	CM-based
Canola	23.4	16.81
SDS solution	15.63	39.4
Sodium chloride	0.93	0.73
Sodium bisulfate	1.01	0.73
Gel mixture	52	37.36
Glycerine	7.02	5.04
PEI*	0; 0.5; 1	
Total solid content of the adhesive (%)	58	40

* PEI was added as an additive to the slurry

Particleboards Productions at Lab Scale

The three-layer particleboards were produced on a pilot scale in the Biotechnikum laboratory of the University of Goettingen, Germany (see experimental flowchart). Prior to the production, the wood chips were dried to about 2% moisture content of using universal oven from Memmert (model UN45). The necessary amount of wood chips was weighed, as well as the amount of resin needed. A resin load of 12% based on the oven-dried wood material was applied onto the wood particles in a rotative blending drum using the air-pressure atomizer nozzle (Düsen-Schlick GmbH, Coburg, Germany). The boards were preformed using a 0.45 m x 0.6 m mat former and pre-pressed by body weight. The target density of the produced board was 640 kg/m³. The hot-pressing was performed using a semi-automatic laboratory-scale hydraulic single-opening hot-press (Siempelkamp Hydraulic Lab Press A 308/1988). The final thickness was adjusted to 20 mm using stop control bars placed between the pressing plates. The production parameters are shown in Table 2.

Table 2. Particleboard Production Parameters

Board Type	Three-Layer Particleboard
Target density (kg/m³)	640 kg/m³
Board thickness (mm)	20 mm
Binder content (%) *	12 based on the oven-dried wood material
Press temperature (° C)	210
Press time/press factor*	6 and 9 min /18 and 27 $s.mm^{-1}$
Boards per variant	4

* Due to the difference in the mat's moisture, a higher press time factor (27 $s.mm^{-1}$) was used for the canola meal variant, while 18 $s.mm^{-1}$ was used to press the CPI-bonded boards.

For each treatment, four boards were produced. After production, the boards were conditioning at room temperature for 24 h. They were next trimmed to avoid edge effects and sanded on both sides by using a wide- belt sanding machine (Felder type FW 950 C from Felder Group, Hall In Tirol, Austria) before being tested. The thickness of the boards after sanding was about 18.4 mm.

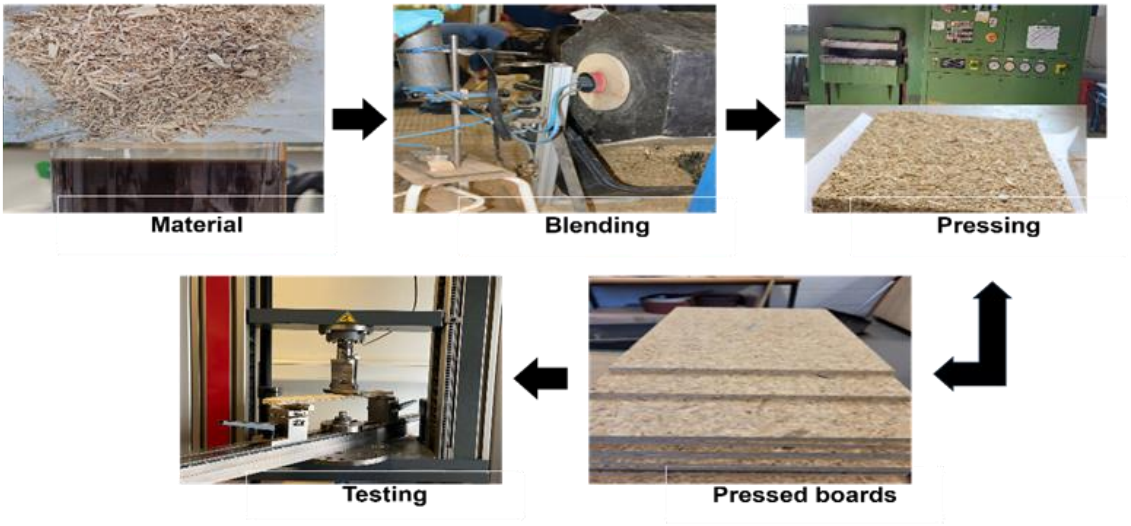

Fig. 1. Flowchart of the experimental setup

Testing the Mechanical Properties of the Produced Particleboards

The mechanical performance of the manufactured particleboards was evaluated using standardized testing methods. The internal bond strength (IB) was measured following EN 319 (1993), while the modulus of rupture (MOR) and the modulus of elasticity (MOE) were assessed in accordance with EN 310 (1993). The universal testing machine (ZWICK/ROELL type 10) from MFC Sensortechnik GmbH, Wuppertal, Germany, was employed for these evaluations. Five specimens, each measuring 50 × 500 mm, from each board, were used to assess the MOR and MOE. Similarly, IB testing involved preparing five samples per board. The selection of specimens was density-based. After testing the MOR, the samples were sectioned into 50 × 50 mm pieces, and their weight and dimensions were recorded to calculate density. Test samples with density values closest to the target density were chosen for IB analysis.

Data Analysis

The data analysis was performed for the mechanical properties of particleboards. An analysis of variance (ANOVA) ($p < 0.05$) was conducted to test the significance of the influence of the factors on the mechanical properties of the particleboards followed by a pair wise mean comparison when necessary.

RESULTS AND DISCUSSION

Figure 2 presents the density profile of the produced particleboard with varying PEI (polyethyleneimine) contents: 0%, 0.5%, and 1%. As thickness increased, density generally decreased, but higher PEI content (1%) mitigated this decline, maintaining higher density due to improved binding. At 0% PEI, density dropped significantly with greater

thickness, while 0.5% and 1% PEI showed more stable densities, especially beyond 15 mm. This indicates that PEI enhanced structural integrity, particularly for thicker boards, making it crucial for optimizing particleboard performance in applications requiring strength and durability.

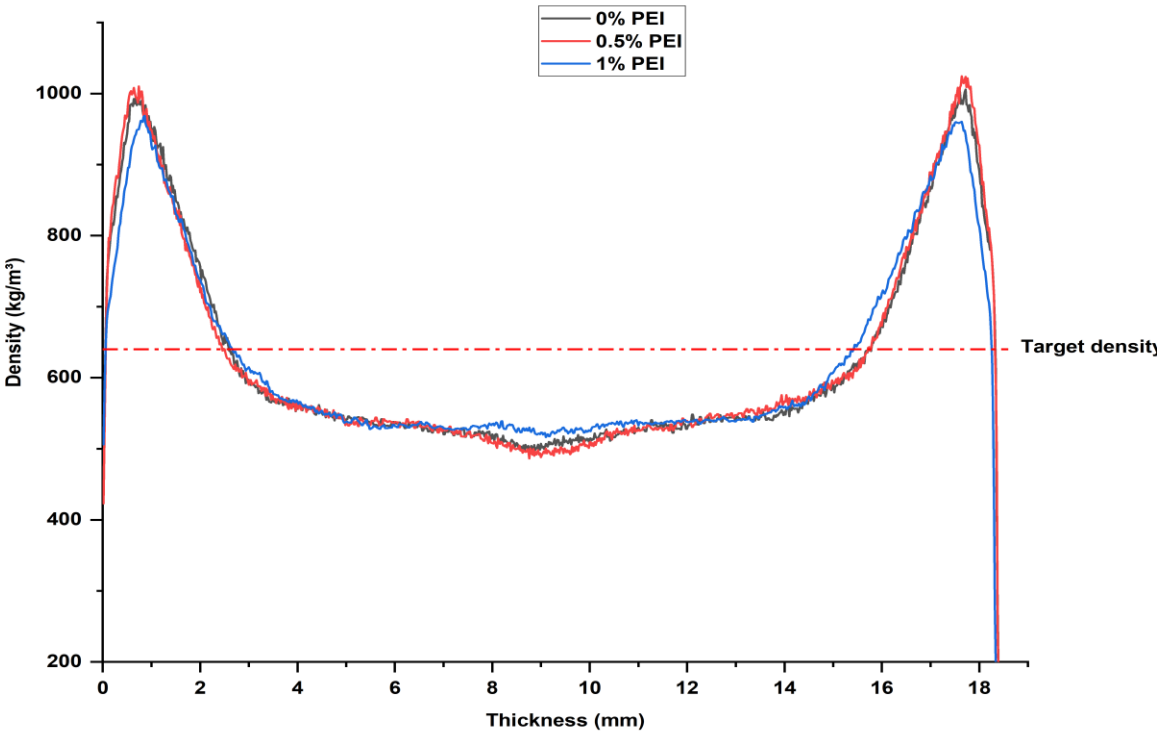

Fig. 2. Density profile of the three-layer particleboards

The internal bonding strength (IB), static bending strength (BS), and modulus of elasticity (E-modulus) of the produced three-layer particleboards bonded with canola protein- and canola meal-based adhesives are presented in Fig. 3. The results demonstrate that increasing the pH significantly impacted the internal bonding strength of both adhesive systems. Proteins are known to undergo denaturation under alkaline conditions (Zhao *et al*. 2014). This process disrupts the native structure of the protein, exposing functional groups such as carboxylic and amine groups, which can then participate in crosslinking reactions. This structural modification enhances the bonding strength of the adhesive. For the canola meal-based adhesive, the IB increased by 25% as the pH rose from 8 to 9, but no further improvement was observed at pH 10. In contrast, the canola protein isolate adhesive exhibited a less pronounced but still notable IB improvement of 3.3% and 13.3% as the pH increased from 8 to 9 and 10, respectively.

Similarly, the bending strength (BS) of the canola protein-bonded boards improved with higher pH values, increasing from 9.65 N/mm² at pH 8 to 10.14 N/mm² and 10.53 N/mm² at pH 9 and 10, respectively. Conversely, the BS of the canola meal-bonded boards showed no significant change across the same pH range. For both adhesives, the E-modulus remained unaffected by changes in pH. This indicates that while alkaline conditions may influence adhesive penetration and bond strength, they may not significantly alter the stiffness of the resulting boards.

The addition of 0.5% polyethyleneimine (PEI) to the adhesive formulations shortly before application yielded negligible improvements in the mechanical properties of the produced particleboards, except for a modest increase in the IB of the protein isolate-based adhesive. This suggests that the PEI concentration was insufficient to induce substantial crosslinking effects. However, when the PEI content was increased to 1%, significant improvements were observed in the IB, BS, and E-modulus of the boards. For the canola protein isolate-based adhesive, the IB increased by 17.24%, the BS by 12.9%, and the E-modulus by 10.7%. Similarly, the canola meal-based adhesive saw enhancements of 15.4% in IB, 8.6% in BS, and 7.9% in the E-modulus.

Despite these improvements, the particleboards bonded with both adhesive formulations failed to meet the European standards for P2 particleboard type concerning IB and BS. This outcome suggests that the PEI content was still insufficient to achieve the desired crosslinking effect for industrial applications. Previous studies support the potential of PEI as an effective crosslinker. For instance, Wang *et al*. (2021) reported significant enhancement of a urea-glyoxal adhesive crosslinked with PEI, and Zeng *et al*. (2023) observed a similar improvement in glucose-based wood adhesives. In Zeng *et al*.'s study, the inclusion of PEI improved water resistance due to the formation of a stable, high crosslinking density network within the adhesive. The introduction of N-H, C=N, and C=O functional groups in the crosslinked structure, along with hydrogen bonds formed at the intramolecular, intermolecular, and wood-adhesive interface, increased both adhesive cohesion and wood surface adhesion.

These findings indicate that optimizing the PEI content could yield superior results, enhancing the mechanical properties and water resistance of canola-based adhesives to meet industrial standards. A higher PEI concentration could potentially improve crosslinking density, resulting in better bonding strength and durability while addressing the current limitations of these adhesive formulations.

Similar studies on PEI-crosslinked bio-adhesives have demonstrated a more pronounced effect when higher PEI content is used. For instance, Liu and Li (2007) reported a significant increase in the dry shear strength of plywood when the PEI content was raised. The dry shear strength improved from 2.3 MPa at 5 wt.% PEI to 4.5 MPa at 10 wt.% and 6.8 MPa at 20 wt.%. However, the shear strength dropped slightly to 6.2 MPa when the PEI content reached 30 wt.%, indicating a threshold beyond which the additional PEI content negatively impacts bonding properties. A similar trend was observed in a tannin-based adhesive system crosslinked with PEI. In that study, as the tannin/PEI ratio increased from 4:2 to 2:1, the properties of plywood improved significantly, but they began to decline at a 1:1 ratio or higher Li *et al*. (2004). These findings suggest that while increasing PEI content enhances mechanical properties up to a certain point, excessive amounts of PEI (*e.g.*, 30 to 40 wt.%) can lead to diminishing returns or even a decline in adhesive performance.

The decrease in mechanical properties observed at high PEI concentrations is likely due to the saturation of functional groups available for bonding, which limits the crosslinking mechanism with the wood substrate. When the adhesive matrix becomes oversaturated with PEI, the excess molecules may no longer effectively participate in bonding, potentially creating a weaker or less cohesive adhesive network. This is consistent with the observation that PEI's high reactivity relies on its ability to interact with functional groups in both the protein-based adhesive and the wood material.

Additionally, the molecular weight of PEI significantly influences its crosslinking efficacy and bonding performance. Liu and Li (2007) reported substantial improvements

in the dry shear strength of plywood when using PEI with varying molecular weights (M_w). Specifically, they tested PEI with molecular weights of 10,000, 70,000, and 75,000. The results showed an increase in shear strength from 3.2 MPa for 10,000 M_w PEI to 6 MPa for 70,000 M_w PEI and 6.8 MPa for 75,000 M_w PEI. These findings highlight that higher molecular weight PEI, which is more branched, is more effective at forming a robust crosslinked network. The branching structure of high-Mw PEI likely increases the density of crosslinking, leading to stronger adhesive performance by providing more active sites for bonding.

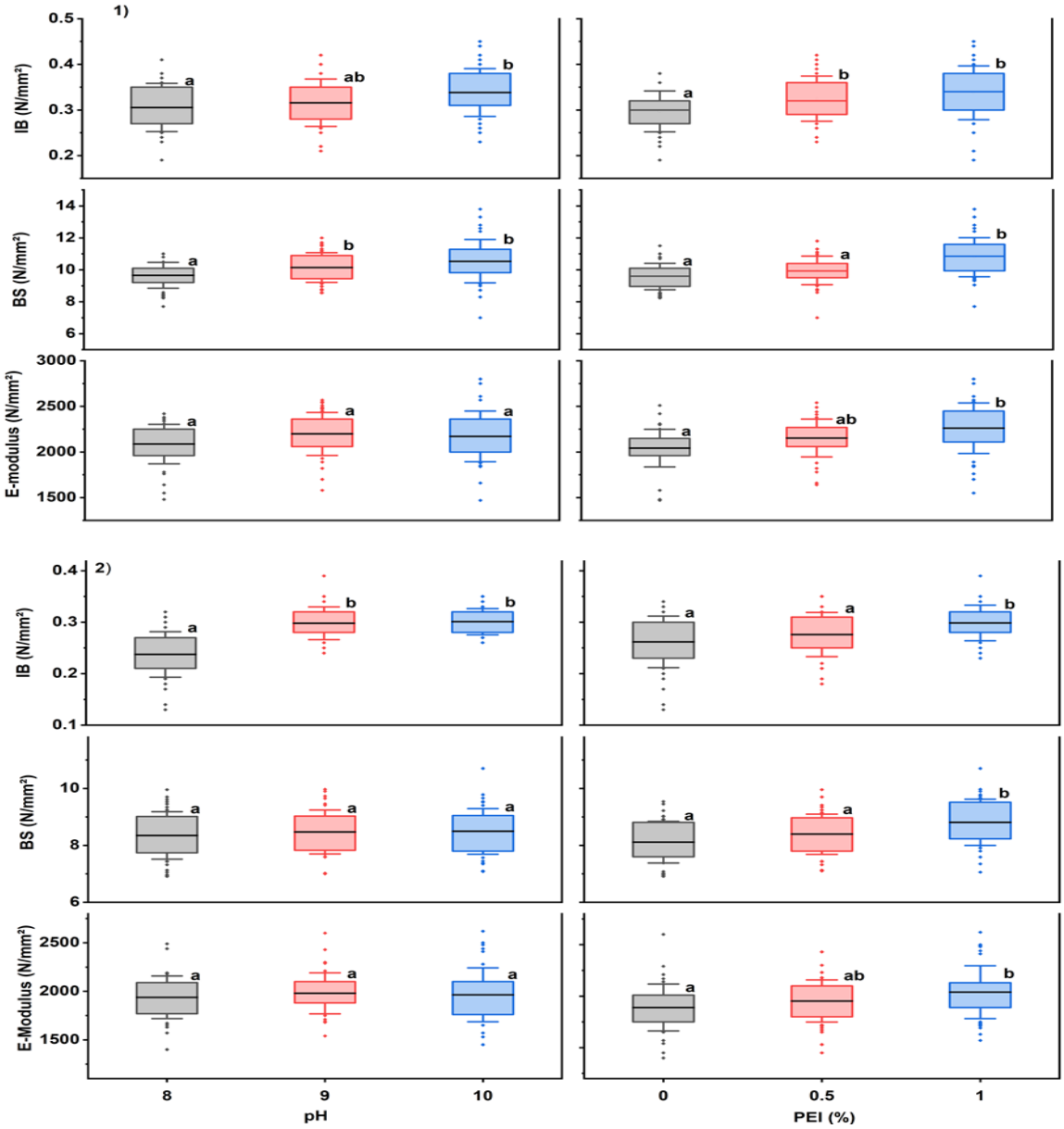

Fig. 3. Effect of pH and polyethyleneimine content on the internal bonding, the bending strength and the E-modulus of the produced three-layer particleboards bonded with 1) CPI and 2) CM adhesives. Different letters indicate a significant difference between values ($p < 0.001$)

Furthermore, PEI's highly branched structure facilitates interactions with various functional groups such as carboxyl, hydroxyl, and amine groups, forming strong covalent

and hydrogen bonds within the adhesive matrix and with the wood surface. This versatility explains its effectiveness in crosslinking diverse bio-based adhesives, including tannin, soy protein, and canola protein systems. However, the balance between PEI concentration and molecular weight is crucial to achieving optimal bonding performance without oversaturating the adhesive matrix or compromising the cohesion of the final product.

The interaction effect between pH and PEI content provides deeper insights into the behaviour of the adhesive formulations, as illustrated in Figs. 4, 5, and 6. The results indicate that the impact of PEI content was most pronounced at pH 10. For the protein isolate-based variant (Fig. 4a), internal bonding (IB) strength improved by 10% and 26.6% when 0.5% and 1% PEI were applied, respectively, at pH 10. At pH 8 and 9, however, the IB values remained relatively unchanged as the PEI content was increased from 0.5% to 1%. Across the different pH levels, an incremental improvement in IB was observed. At 1% PEI content, the IB values increased by 3.2% and 22.6%, rising from 0.31 N/mm² (pH 8) to 0.32 N/mm² (pH 9) and 0.38 N/mm² (pH 10). Notably, the IB value of the protein isolate variant with 1% PEI at pH 10 exceeded the EN norm for P2 particleboard type, demonstrating the effectiveness of the optimized adhesive formulation. A similar trend was observed for the canola meal-based variant (Fig. 4b), although the improvement was less pronounced. These results suggest that proper denaturation of the protein at higher pH enhances the crosslinking effect of PEI, thereby improving the bonding properties of the bio-adhesive.

The improved performance at pH 10 can be attributed to the well-documented effect of alkaline conditions on protein degradation (Tene Tayo *et al*. 2022, 2024; Xi *et al*. 2021). Under alkaline conditions, the canola protein undergoes better unfolding, exposing functional groups such as carboxyl and amine groups along the protein chain. These reactive groups become more accessible for crosslinking reactions with PEI, enabling stronger chemical bonding and enhancing the adhesive's overall bonding mechanism. This mechanism explains the significantly higher increment in the mechanical properties of particleboards, such as IB, bending strength (BS), and modulus of elasticity (MOE), observed at pH 10 compared to pH 8 and 9.

Similar effects have been observed in other natural adhesive systems. Tannin-based wood adhesives, for instance, demonstrate enhanced adhesive properties under alkaline conditions. Faris *et al*. (2016) reported that increasing pH significantly improved the bonding performance of tannin-based adhesives. This enhancement is primarily attributed to the higher reactivity of tannins in alkaline environments, which facilitates better crosslinking and polymerization, resulting in stronger and more durable adhesive bonds. These findings underscore the critical role of pH optimization in the formulation and application of tannin-based systems.

Soy- and wheat-based adhesives also exhibit improved performance in alkaline environments, supporting the observations made for canola protein-based adhesives. Research shows that under alkaline conditions, these protein adhesives undergo structural denaturation, which unfolds the protein molecules and exposes reactive functional groups such as amine, hydroxyl, and carboxyl groups (Kalapathy *et al*. 1996; Khosravi *et al*. 2014). These groups enhance the adhesive's crosslinking and bonding ability, leading to significant improvements in mechanical properties such as IB and BS. For instance, Kalapathy *et al*. (1996) demonstrated that soy protein adhesives exhibit better water resistance and adhesion strength under alkaline pH due to enhanced crosslinking facilitated by exposed reactive sites.

The enhanced crosslinking effect at pH 10 aligns with findings from studies on glucose-PEI adhesives (Zeng *et al*. 2023). In these systems, the stable and dense crosslinking network formed by PEI increased mechanical properties and water resistance. Additionally, the introduction of functional groups such as N-H, C=N, and C=O, as well as the formation of hydrogen bonds at intra- and intermolecular levels, further enhanced cohesion within the adhesive and adhesion to the wood surface. These interactions likely contributed to the improved performance of the canola protein-based adhesive at higher pH levels.

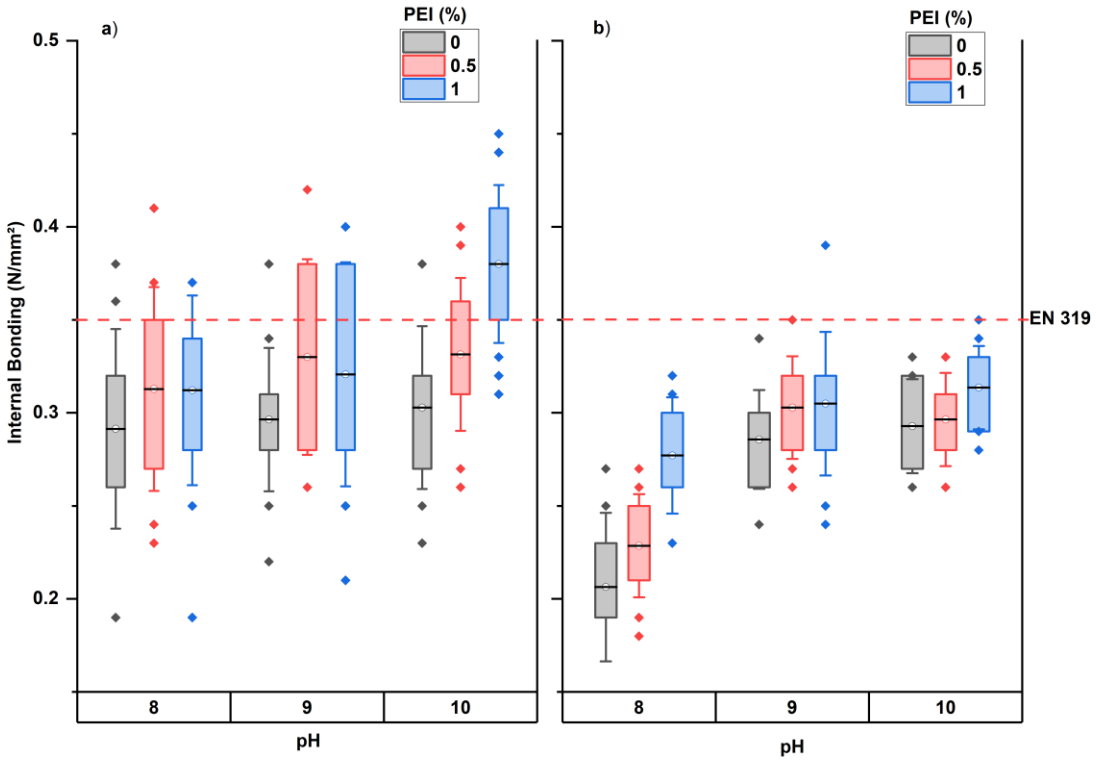

Fig. 4. Effect of the pH and PEI content on the internal bonding strength of a) CPI and b) MC-based adhesive formulations. Box plots (25th quartile, mean and 75th quartile) and whiskers (1 × standard difference) of internal bonding. EN 319 is the European standard for IB (0.35 N/mm²).

The reduced effectiveness of PEI crosslinking in canola meal-based variants can likely be attributed to the lower protein content of the meal, which is approximately 36%, as reported by Ostendorf *et al*. (2021b). In addition to its limited protein content, canola meal contains a substantial proportion of carbohydrates, fatty compounds, and residual oils, which may interfere with the crosslinking reactions and reduce the adhesive's overall effectiveness. This limitation particularly affected the bending strength (BS) of the canola meal-based adhesives, as illustrated in Fig. 5b. While the protein isolate-based variants showed a significant increase in BS at every pH level tested, the effect of PEI crosslinking remained negligible for the canola meal formulations.

As a result, none of the canola meal-based variants achieved a BS exceeding 9 N/mm². In contrast, the protein isolate adhesive variant with 1% PEI at pH 10 met the EN requirements for P2 particleboards, underscoring the importance of protein purity in achieving desirable mechanical performance. Furthermore, no statistically significant improvement in BS was observed within or across the pH levels for the canola meal

adhesives, suggesting that the presence of non-protein compounds in the meal matrix may have hindered the formation of a robust crosslinked network.

Regarding the modulus of elasticity (E-modulus), shown in Fig. 6, all adhesive formulations exhibited excellent stiffness, with values exceeding the EN requirement of 1600 N/mm². This suggests that the adhesive systems, even in the canola meal-based variants, provided sufficient stiffness for structural integrity. However, the absence of significant variation in E-modulus across pH levels or PEI concentrations suggests that the parameter is less impactful on the adhesive's crosslinking efficacy compared to BS and internal bonding (IB).

Another crucial factor influencing the effectiveness of PEI crosslinking is the reaction time after the PEI is introduced into the adhesive formulation. Studies, such as those by Zeng *et al*. (2023), have shown that allowing sufficient reaction time for PEI to interact with protein molecules is critical for optimal crosslinking. In this study, the adhesives were used immediately after adding PEI, which likely limited the development of a fully crosslinked network. Allowing the adhesive to react for at least one hour before application would likely have enhanced the crosslinking mechanism, improving the bonding strength and the mechanical interaction with the wood surface.

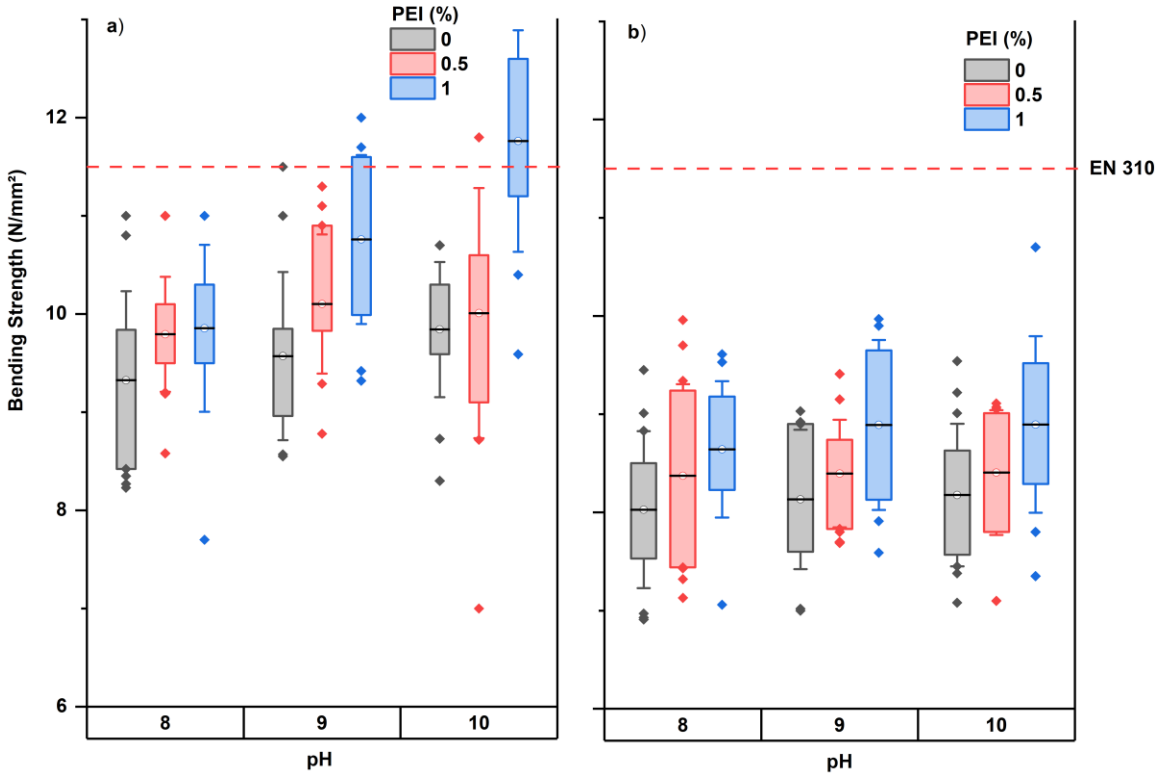

Fig. 5. Effect of the pH and PEI content on the bending strength of a) CPI and b) MC-based adhesive formulations. Box plots (25th quartile, mean and 75th quartile) and whiskers (1 × standard difference) of MOR. EN 319 is the European standard for MOR (11.5 N/mm²).

The delayed reaction time is especially important given PEI's high reactivity due to its numerous amine groups, which require time to form covalent and hydrogen bonds with the protein's functional groups. Without adequate reaction time, the crosslinking network may remain underdeveloped, reducing its mechanical contributions to the

adhesive's bonding properties. The need for sufficient reaction time aligns with findings from Zeng *et al*. (2023), where the PEI-crosslinked glucose-based wood adhesives exhibited significantly improved properties after an extended mixing period, including better water resistance and cohesive bonding due to a stable and high-density crosslinked network.

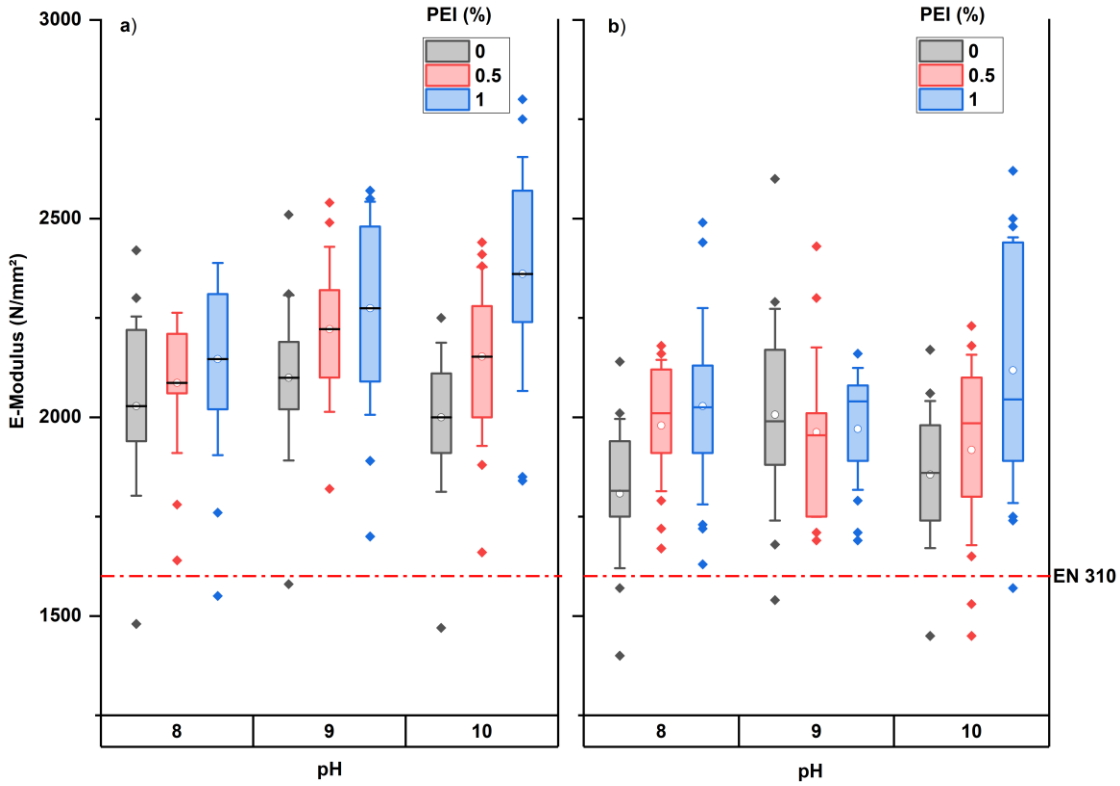

Fig. 6. Effect of the pH and PEI content on the bending strength of a) CPI and b) MC-based adhesive formulation**s**. Box plots (25th quartile, mean and 75th quartile) and whiskers (1 × standard difference) of MOE. EN 319 is the European standard for MOE (1600 N/mm^2)

Moreover, the presence of competing components, such as carbohydrates and lipids in the canola meal, likely diluted the availability of reactive sites in the protein, further limiting the crosslinking efficiency of PEI. These competing molecules might block access to the protein's functional groups or disrupt the formation of a continuous polymeric network, resulting in weaker adhesive properties.

CONCLUSIONS

This study explored the effects of varying polyethyleneimine (PEI) content and pH on the bonding properties of canola-based binder formulations. Three-layer particleboards were produced using the developed adhesives and tested for internal bond strength (IB), modulus of rupture (MOR), and modulus of elasticity (MOE). The findings provide valuable insights into optimizing canola protein-based adhesives for industrial applications. These results underscore the potential of PEI-crosslinked canola protein adhesives, particularly when production parameters such as pH and crosslinker content are

carefully optimized. This study provides a foundation for further innovation in bio-based adhesive technologies.

1. *Effect of poly(ethyleneimine) (PEI) content:* Incorporating PEI into the canola-based adhesive formulation led to notable improvements in bonding properties, particularly at higher concentrations. While 0.5 wt.% PEI showed no significant improvement compared to the reference (0% PEI), increasing the PEI content to 1% significantly enhanced the adhesive's performance. However, the limited reaction time and low protein purity of the canola meal, coupled with non-protein constituents, constrained the mechanical performance of the meal-based adhesives.
2. *Impact of pH on binder performance:* Increasing the pH of the adhesive positively influenced the binder's properties, particularly internal bond (IB) and bending strength (BS). At pH 10, more functional groups of the protein became available for crosslinking with PEI, leading to the best mechanical performance when paired with 1% PEI content. These results confirm that alkaline conditions are critical for optimizing PEI-crosslinked bio-adhesives, as they promote protein unfolding, enhance crosslinking efficiency, and improve the mechanical properties of the adhesive.
3. *Crosslinking mechanism of PEI:* The observed improvements in bonding properties can be attributed to the interactions between the amine groups of PEI and the amine and carboxylic groups of the canola protein. This highlights PEI's potential as a promising crosslinking agent for bio-based wood adhesives. However, the study suggests that the effectiveness of PEI depends on its concentration, molecular weight, and branching structure.

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Author Contributions

Conceptualization, L.T.T. and M.E.; Experiments, L.T.T. and F.D.; Formal data analysis and visualization, L.T.T.; Writing - original draft preparation, L.T.T.; Writing—review and editing, L.T.T. and M.E.; Funding acquisition, M.E. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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