

Research article

PERFORMANCE ENHANCEMENT OF CITRIC ACID–CROSSLINKED BAMBOO–STARCH FILMS: EFFECT OF BAMBOO POWDER DOSAGE

Nurul Ain Mohd Amin¹, Siti Fatma Abd Karim^{1,2,✉}, Umami Kalthum Ibrahim^{1,2}, Nurhaslina Che Radzi¹, Noor Harliza Abd Razak¹, Rabiatal Adawiyah Abdol Aziz¹, and Suryani Saallah³

¹Fakulti Kejuruteraan Kimia, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

²Food Process and Engineering Research Group (FOPERG), Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

³Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

✉Corresponding author: Siti Fatma Abd Karim ; E-mail: sitifatma9633@uitm.edu.my ;
Mobile: +60177789215; ORCID : 0000-0002-2894-7859

<https://doi.org/10.34302/crpjfst/OAKP2645>

Article history:

Received:

November 25th, 2025

Accepted:

August 25th, 2026

Published

September 20th, 2026

Keywords:

Bamboo-starch based film;

Food Packaging;

Crosslinker;

Citric acid;

Bamboo dosages.

ABSTRACT

The performance limitations of starch-based biodegradable films remain a major barrier to their application in food packaging, particularly in terms of mechanical strength, moisture sensitivity, and antimicrobial functionality. In this study, citric acid–crosslinked bamboo–starch composite films were developed and systematically evaluated to elucidate the effect of bamboo powder dosage on the film's structure and performance. To the best of our knowledge, this study is the first to systematically evaluate the effect of bamboo powder dosage (3–6 g) in citric acid–crosslinked bamboo–starch films and correlate dosage-dependent changes with mechanical, barrier, morphological, and antibacterial performance. Bamboo powder loadings of 3, 4, 5, and 6 g were incorporated into a gelatinized potato starch matrix using citric acid as a food-grade crosslinker, with a neat starch film serving as the control. The films were characterized for mechanical, chemical, morphological, barrier, and antibacterial properties. Tensile strength increased markedly with the incorporation of bamboo, reaching a maximum of 9.65 MPa at 6 g of bamboo, representing a 170% improvement over the control (3.57 MPa). However, elongation at break decreased due to restricted polymer chain mobility. FTIR analysis confirmed the formation of an ester bond between starch, bamboo cellulose, and citric acid, indicating enhanced crosslinking efficiency with increasing bamboo content. SEM observations revealed optimal filler dispersion at moderate bamboo loading, whereas higher dosages led to fiber agglomeration and structural heterogeneity. Moisture content decreased progressively from 21.57% in the control to 9.96% at 6 g of bamboo, indicating reduced moisture retention as bamboo incorporation increased. Antibacterial evaluation against *Escherichia coli* showed a dose-dependent increase in inhibition activity, with the largest antibacterial ring radius (3 mm) observed for the highest bamboo loading. Overall, citric acid–crosslinked bamboo–starch films exhibit improvements in mechanical strength, moisture-related properties, and antimicrobial activity, highlighting their strong potential as sustainable active food packaging materials, with the bamboo dosage serving as a critical design parameter.

1.Introduction

The environmental burden caused by persistent petroleum-based plastics has created an urgent need for cleaner packaging solutions. Materials such as polyethylene (PE) continue to dominate the industry due to their mechanical reliability, barrier strength, and low cost. However, their non-biodegradable nature has made them a significant contributor to long-term pollution (Khandeparkar et al., 2024). This has spurred global interest in biodegradable packaging films derived from renewable sources. Although biobased polymers offer clear sustainability advantages, many still fall short in performance, particularly in terms of moisture resistance and mechanical robustness, limiting their direct replacement of conventional plastics (Abdul Khalil et al., 2018). This gap has driven the development of composite systems, in which natural fillers reinforce biopolymer matrices to create stronger, more stable, and functionally improved materials suitable for food packaging applications.

Starch has long been recognized as one of the most promising biodegradable film-forming materials due to its abundance, low cost, and ease of processing. However, its high hydrophilicity, susceptibility to deformation in humid conditions, and generally weak mechanical strength restrict its standalone use (Dirpan et al., 2023; Mohammad et al., 2022). To overcome these limitations, reinforcement from natural fibers or particulate fillers is often required. Bamboo offers distinct advantages in this regard. Its composition, which is rich in cellulose, hemicellulose, and lignin, provides a unique balance of rigidity, thermal stability, and biodegradability (Kumar et al., 2022). Bamboo's microfibrillar structure contributes to better hydrophobicity and barrier behavior, while its rapid growth and natural antimicrobial attributes present additional benefits (Abdul Khalil et al., 2018; F. Song et al., 2015). Compared with other agricultural residues such as banana pseudostem, coconut husk fiber, and bagasse, bamboo provides more consistent strength, durability, and water resistance, making it an attractive candidate to improve starch-based films (Nyerere et al., 2024; Sangeetha et al., 2024; Vinay et al., 2024).

Broad research has explored the potential of

bamboo for film and composite fabrication. Previous studies have demonstrated that bamboo veneer films strengthened by hydrogen bonding (X. Liu et al., 2024), active films incorporating bamboo leaf antioxidants with ZnO nanoparticles (J. Liu et al., 2021) and bamboo powder reinforced matrices modified using chitosan, ZnO or TiO₂ (Guan et al., 2019). Bamboo cellulose blended with polyphenols and polyvinyl alcohol (PVA) has also shown potential for extending food shelf life (D. Song et al., 2023), while bamboo chips have been converted into antimicrobial films effective against multiple pathogens (Sobri et al., 2021). Although these findings consistently highlight bamboo's versatility and functional improvements, few studies have specifically examined bamboo–starch composite films, and fewer still have investigated how different bamboo powder dosages, in combination with a food-safe crosslinker, affect film strength, structure, and antimicrobial performance.

Alongside the choice of reinforcement, the crosslinking strategy used during film formation plays a crucial role in determining the stability and behavior of bamboo-based composites. Various crosslinkers, including maleic anhydride (MAH), zinc oxide nanoparticles (ZnO NPs), propanediol diglycidyl ether (PTGE), and deep eutectic solvents (DES) have been applied to improve adhesion, reduce water uptake, and enhance thermal or antibacterial properties (J. Liu et al., 2021; Qian et al., 2015; Su et al., 2023). Among these, citric acid is particularly notable for its food-grade safety, low toxicity, and strong reactivity with hydroxyl-containing polymers, allowing esterification under mild processing conditions (Jin et al., 2017; Widyorini et al., 2017). Citric acid has been shown to strengthen film networks, improve water resistance, and contribute antibacterial effects. While considerable research has explored citric acid-mediated crosslinking in starch-based films, the effect of bamboo powder dosage on the resulting crosslinked network has not been systematically investigated. There is limited understanding of how increasing bamboo content affects crosslinking efficiency, microstructural development, and the mechanical, barrier, and antibacterial properties of bamboo–starch

composite films.

Evaluating these interactions requires close attention to performance indicators repeatedly highlighted in earlier research. Past studies show that morphology, mechanical strength, chemical bonding, barrier performance, and antimicrobial activity are the most critical parameters for determining the suitability of bamboo-reinforced films in food packaging (C. Liu et al., 2024; Sobri et al., 2021; K. Song et al., 2017). SEM findings generally reveal that moderate bamboo incorporation produces a denser, more uniform microstructure, whereas excessive filler leads to fiber agglomeration, voids, and structural defects that compromise strength (Guan et al., 2019; Sobri et al., 2021). FTIR analyses often confirm ester formation in citric acid-treated systems through intensified carbonyl and C–O–C peaks, indicating the development of a more tightly crosslinked network (Jin et al., 2017; Widyorini et al., 2017). Mechanically, bamboo has been shown to increase tensile strength and stiffness when well dispersed, though excessive loading tends to restrict polymer mobility and reduce elongation-at-break (Abdul Khalil et al., 2018; Kumar et al., 2022). Barrier studies similarly report reduced moisture uptake in bamboo-reinforced or citric acid-crosslinked films due to the presence of hydrophobic lignocellulosic components and a denser internal matrix (Chen et al., 2019; F. Song et al., 2015). In biological assessments, films containing bamboo extracts, metal oxides or chemically modified fibers often exhibit enhanced antibacterial behavior, particularly against *E. coli* and other foodborne pathogens (J. Liu et al., 2021; Sobri et al., 2021). These consistent patterns highlight the need for a structured investigation into how different bamboo powder dosages affect key film properties in citric acid-crosslinked starch matrices.

Although bamboo-based films have been widely explored for food packaging applications, existing studies have primarily focused on different bamboo forms, crosslinking agents, and film formulations, with limited attention given to the effect of bamboo powder dosage in citric acid-crosslinked systems. Consequently, the influence of bamboo loading on the morphological, chemical, mechanical,

barrier, and antibacterial properties of bamboo–starch composite films remains insufficiently understood. Therefore, this study aimed to investigate the effect of different bamboo powder dosages (3–6 g) on citric acid-crosslinked bamboo–starch films using SEM, FTIR, tensile, moisture content, and antibacterial analyses. The findings provide new insights into dosage-dependent structure–property relationships and lay the groundwork for optimizing bamboo-reinforced starch films as sustainable food packaging materials.

2. Methodology

2.1. Material

The materials used in this research were raw bamboo (Semantan) purchased from Kelantan, sodium chlorite (NaClO_2 , 80%) brand SIGALD from Sigma-Aldrich, glacial acetic acid (CH_3COOH , 99.5%) brand SIGALD from Sigma-Aldrich, sodium hydroxide (NaOH pellets, 95%) from Thermofisher Scientific, citric acid brand Merck from Sigma-Aldrich (MW: 192.12 g/mol), and ethanol from Thermofisher Scientific provided by Universiti Teknologi MARA.

2.2. Preparation of bamboo powder

The raw Semantan bamboo was first cut into smaller sections using a band saw and oven-dried at 70 °C for 24 h to remove free moisture, which is essential for preventing microbial degradation and improving milling efficiency (Su et al., 2023). The dried sections were then processed with a light-duty cutter to obtain coarse sawdust, after which a second drying step at 60 °C for 24 h was carried out to eliminate bound moisture that could otherwise interfere with subsequent chemical treatments and reduce powder stability (Qian et al., 2015). The fully dried material was further ground using a high-speed laboratory blender to produce fine particles, increasing the surface area and enhancing reactivity during pre-treatment and crosslinking. The powder was then passed through a 100-mesh sieve to obtain a uniform particle size distribution, a critical factor for ensuring consistent dispersion within the starch matrix and reproducible film properties (Huang et al., 2024). The sieved bamboo powder was stored in airtight containers at ambient

temperature to prevent moisture uptake and preserve its physicochemical integrity prior to further processing.

2.3.Preparation of delignified and crosslinked bamboo powder

The bamboo powder (100-mesh fraction) was first subjected to an alkaline pre-treatment, following the approach of Huang et al., (2024), to selectively remove non-cellulosic components and increase the accessibility of reactive functional groups. In this step, the powder was dispersed in 250 mL of distilled water containing 2.5 g of NaOH (1% w/v) and heated at 80 °C for 1 h under continuous agitation. The alkaline environment promotes the swelling of lignocellulosic fibres and cleaves ester and ether linkages associated with hemicellulose and residual waxes, thereby improving subsequent chemical reactivity. The suspension was filtered and rinsed repeatedly with hot distilled water until neutral pH was achieved to ensure complete removal of solubilised hemicellulose and extractives, preventing alkaline residues from interfering with later reactions.

A delignification treatment was then performed by immersing the pre-treated powder in 250 mL of distilled water containing 2.5 g of NaClO₂. The pH was adjusted to ~4.6 using glacial acetic acid, and the mixture was heated at 80 °C for 1 h, in accordance with Liu et al., (2024). Under acidic conditions, NaClO₂ acts as a strong oxidising agent, selectively degrading lignin's aromatic structure while leaving cellulose chains intact. The removal of lignin is crucial for increasing fibre–matrix compatibility and exposing additional hydroxyl groups that participate in crosslinking. The characteristic whitening of the material signified the completion of lignin oxidation. The solid fraction was filtered and washed multiple times with hot distilled water and ethanol to eliminate chlorite by-products and residual organic impurities, preventing unwanted side reactions during crosslinking.

For the crosslinking step, the delignified bamboo powder was immersed in a citric-acid-based solution comprising distilled water, ethanol, and NaOH, as adapted from Liu et al., (2024). 50 mL of 50% (w/v) citric acid solution

prepared from anhydrous citric acid was mixed with 80 mL of distilled water and 80 mL of ethanol, then 20 mL of 6.5 M NaOH was added. The delignified bamboo powder was then immersed in the prepared solution. NaOH partially neutralises citric acid, forming citrate salts that enhance solubility and promote ester formation during heating. The reaction mixture containing the bamboo powder was heated to 90 °C and stirred gently for 1 h. At this temperature, citric acid undergoes dehydration to form an anhydride intermediate, which readily reacts with cellulose hydroxyl groups to form covalent ester bonds. This step is essential for improving the dimensional stability, water resistance, and interfacial adhesion of the bamboo filler when incorporated into the starch film. After crosslinking, the powder was washed repeatedly with deionized water at 80 °C until neutral pH was reached to remove unreacted citric acid and sodium citrate, ensuring chemical purity and stable functional performance. The crosslinked material was then dried and kept at ambient temperature before use.

2.4.Preparation of Bamboo-starch-based film

For the preparation of the bamboo–starch film-forming matrix, the procedure described by Nadia et al., (2022) was adapted. Initially, 3 g of potato starch was dispersed in 100 mL of distilled water and heated to 70 °C for 30 min under continuous stirring. This temperature range is critical for starch gelatinisation, during which granules swell, lose crystallinity, and release amylose, forming a homogeneous hydrocolloid matrix capable of embedding solid fillers. After gelatinisation, glycerol was incorporated at 25% (w/w) relative to the dry starch content. Glycerol functions as a plasticiser, reducing intermolecular hydrogen bonding within the starch network and thereby improving film flexibility and preventing brittleness during drying. The crosslinked bamboo powder was then introduced into the gelatinised starch matrix. Continuous high-speed stirring for 1 h was employed to ensure uniform dispersion of the filler, a necessary condition for achieving consistent mechanical reinforcement and preventing aggregation, which could otherwise create weak points in the final film. A fixed volume of 25 mL of the

resulting film-forming mixture was cast into each Petri dish (90 mm diameter) and dried at 70 °C for 24 h. This drying protocol promotes gradual solvent evaporation, allowing the polymer network to reorganize without cracking and facilitating strong interfacial adhesion between starch and the citric-acid-modified bamboo particles. The same procedure was performed for all formulations containing 3 g, 4 g, 5 g, and 6 g of bamboo powder to maintain process consistency. A control film was also prepared using only starch and glycerol, without the addition of crosslinked bamboo, to allow direct assessment of the reinforcement and barrier contributions introduced by the modified bamboo filler.

2.5. Bamboo-starch based film characterization

2.5.1. Mechanical Properties

Mechanical performance was evaluated using a universal tensile testing machine, operated at 25 °C and 50% relative humidity to ensure standardised testing conditions. A 5 kN load cell and a strain rate of 10 mm/min were applied. Prior to tensile testing, the thickness of each film specimen was measured and entered the tensile-testing software together with the specimen width. Tensile strength was calculated based on the maximum force divided by the initial cross-sectional area of each specimen. Tensile strength and elongation at break were measured to assess the influence of crosslinked bamboo powder on the films' mechanical performance.

2.5.2. Chemical Properties

Chemical structure and functional group interactions were examined using Fourier Transform Infrared Spectroscopy (FTIR). Small sections of the films were placed in the sample holder, and spectra were recorded across 4000–400 cm⁻¹ to detect characteristic absorbance peaks associated with starch, bamboo cellulose, and citric-acid-induced esterification. Changes in peak position or intensity were used to confirm chemical bonding and structural modifications resulting from crosslinking.

2.5.3. Morphological Properties

Surface morphologies were analysed using SEM operated at 5 kV, allowing high-resolution imaging of fibre distribution, filler–matrix

compatibility, and structural defects. The electron beam scans the film surface and generates detailed microstructural information critical for evaluating dispersion quality, interfacial adhesion, and the impact of increasing bamboo content on film uniformity.

2.5.4. Barrier Properties

Moisture content was determined using an oven-drying method (Murillo & López, 2015), where samples were weighed (W_1), dried at 80 °C for 24 h, cooled, and reweighed (W_2). The moisture content was calculated using Equation (1):

$$\text{Moisture content (\%)} = \frac{(W_1 - W_2)/W_1}{100} \times 100 \quad (1)$$

where W_1 is the initial weight of the film before drying (g), and W_2 is the final weight of the film after drying (g). This test was used to evaluate the moisture-retention characteristics of the films, where a lower moisture content indicates reduced moisture retention associated with crosslinking and the incorporation of bamboo.

2.6. Antimicrobial Properties

The antibacterial activity of the films was assessed using an agar diffusion assay (Guan et al., 2019). First, 5.6 g of agar was dissolved in 200 mL of distilled water for 5–10 minutes to obtain a homogeneous medium. The prepared medium was then sterilized by autoclaving at 121 °C for 15 minutes to eliminate microbial contaminants. The sterilized agar was cooled to 40–50 °C, an essential temperature range that prevents thermal degradation of bacterial cells while maintaining the agar in a pourable state, and 25 mL of the medium was dispensed into sterile petri dishes and allowed to solidify at room temperature. The agar surface was uniformly inoculated with an *Escherichia coli* suspension using a sterile cotton swab to obtain a uniform bacterial lawn. Film samples were cut into 15-mm discs and placed on the inoculated agar. The control starch film without bamboo powder was used as the negative control. No separate positive antimicrobial control was included in the original experimental design. The plates were incubated at 37 °C for 24 h, after

which the inhibition zones were measured, and the antibacterial ring radius was calculated to quantify the films' antibacterial activity. The antibacterial ring radius was calculated using Equation (2):

$$\text{Antibacterial ring radius (mm)} = (d_1 - d_2)/2$$

→ Equation 2

where d_1 is the outer diameter of the inhibition zone (mm), and d_2 is the diameter of the film disc (mm).

2.7. Statistical analysis

Statistical analysis of mechanical performance was performed in Microsoft Excel using one-way analysis of variance (ANOVA).

Post hoc Tukey's tests were performed using IBM® SPSS® Statistics to detect significant differences at $p < 0.05$.

3. Results and discussion

Figure 3.1 shows the visual appearance of bamboo-starch-based films crosslinked with citric acid, Figure 3.1(a) represents the control film containing only potato starch and glycerol, 3.1(b) - (e) show films incorporating increasing amounts of bamboo powder, which are 3g (b), 4g (c), 5g (d), and 6g (e). The films were obtained by peeling them from the petri dish after the drying process was completed during the bamboo-starch-based film formation.

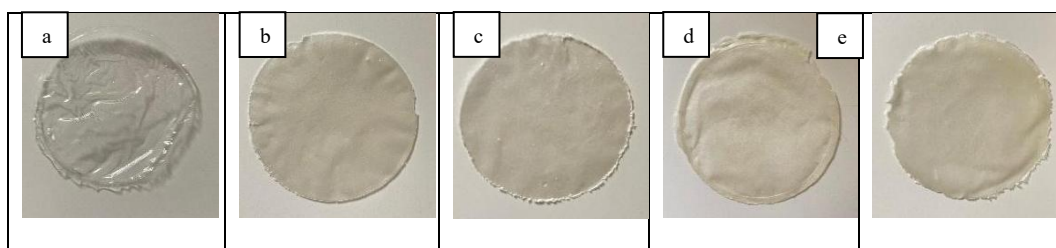


Figure 3.1. Bamboo-starch based film crosslinked with citric acid, a) control sample, b) 3g of bamboo, c) 4g of bamboo, d) 5g of bamboo, e) 6g of bamboo

3.1. Mechanical performance

Figure 3.2 presents the influence of bamboo powder loading on the tensile strength of citric acid-crosslinked bamboo-starch films. A pronounced increase in tensile strength was observed with increasing bamboo content, reaching a maximum of 9.65 ± 1.18 MPa at 6 g bamboo, corresponding to approximately a 170% increase relative to the control film (3.57 ± 0.45 MPa). According to Tukey's HSD test ($p < 0.05$), the control and 4 g films belonged to the same statistical group (a), while the 3 g and 6 g films formed a higher tensile strength group (b). The 5 g film exhibited intermediate behavior (ab) and was not significantly different from either group. This improvement is attributed to the reinforcing effect of bamboo-derived cellulose, which increases load-bearing capacity by enabling efficient stress transfer and enhancing intermolecular interactions within the polysaccharide network. At lower filler loading, the incorporation of 3 g bamboo significantly increased tensile strength (7.68 ± 0.52 MPa), indicating favorable dispersion and effective filler-matrix adhesion. In contrast, a marked

reduction in tensile strength at 4 g of bamboo (3.66 ± 1.65 MPa) suggests disruption of network continuity, which is likely caused by the local aggregation of bamboo particles acting as stress concentrators. Such non-monotonic behavior is characteristic of polysaccharide-based composites in which mechanical performance is governed by a balance between filler content and dispersion state (C. Liu et al., 2024). The subsequent recovery in tensile strength at higher bamboo loadings (5–6 g) implies that increased availability of hydroxyl functionalities from bamboo cellulose promotes more extensive citric acid-mediated ester crosslinking and hydrogen bonding. This results in a denser and more interconnected polymer network, thereby compensating for dispersion-related defects and improving mechanical integrity (Abdul Khalil et al., 2018). Overall, these results underscore that the tensile performance of citric acid-crosslinked starch composites is controlled by the interplay between filler-induced reinforcement and network homogeneity, rather than filler content alone.

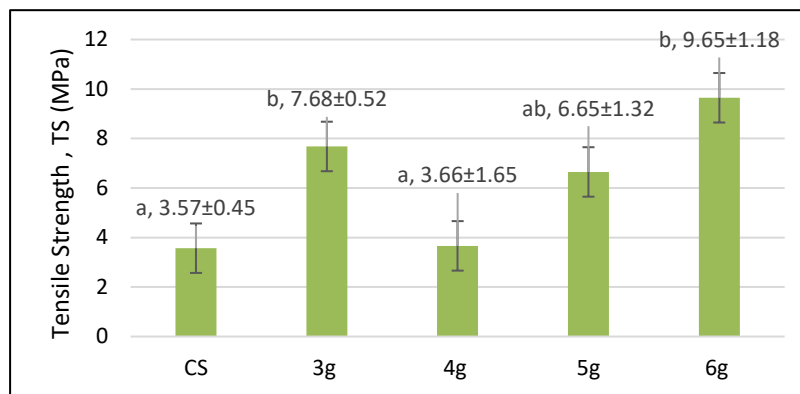


Figure 3.2. Effect of bamboo powder dosage on the tensile strength of citric acid-crosslinked starch film. Data presented as mean $\pm$ S.D. ($n = 3$). Different alphabets between columns represent significance at $p < 0.05$ after applying post hoc Tukey's test.

Figure 3.3 illustrates the effect of bamboo powder dosage on the elongation at break of citric acid-crosslinked bamboo-starch films. The elongation at break of all bamboo-containing films was significantly lower than that of the control film ($p < 0.05$), whereas no significant differences were observed among the bamboo-containing formulations. The control film exhibited the highest elongation at break ($31.40 \pm 5.52\%$), reflecting the high flexibility and unrestricted chain mobility of the starch matrix in the absence of rigid lignocellulosic fillers. Upon incorporation of bamboo powder, a pronounced reduction in elongation was observed, indicating a progressive loss of film ductility. At 3 g bamboo, the elongation at break decreased sharply to $6.70 \pm 0.18\%$, corresponding to a reduction of approximately 78.7% relative to the control. Further increases in bamboo loading resulted in continued decreases in elongation, with the lowest value

recorded at 6 g bamboo ($4.44 \pm 2.15\%$, ~85.9% reduction). This monotonic decline suggests that bamboo incorporation significantly restricts polymer chain mobility, primarily due to the rigid and fibrous nature of bamboo cellulose, which limits segmental motion within the starch matrix. In addition, the formation of citric acid-mediated ester crosslinks and hydrogen bonding further increases network density, thereby enhancing stiffness at the expense of extensibility. Similar reductions in elongation at break have been reported for starch- and cellulose-reinforced polysaccharide composites, where increased filler content and crosslink density promote brittleness and suppress ductile deformation (Abdul Khalil et al., 2018; Rashid et al., 2023). Overall, the results highlight a clear strength-ductility trade-off in citric acid-crosslinked starch films, in which improved mechanical reinforcement is accompanied by reduced flexibility as bamboo dosage increases.

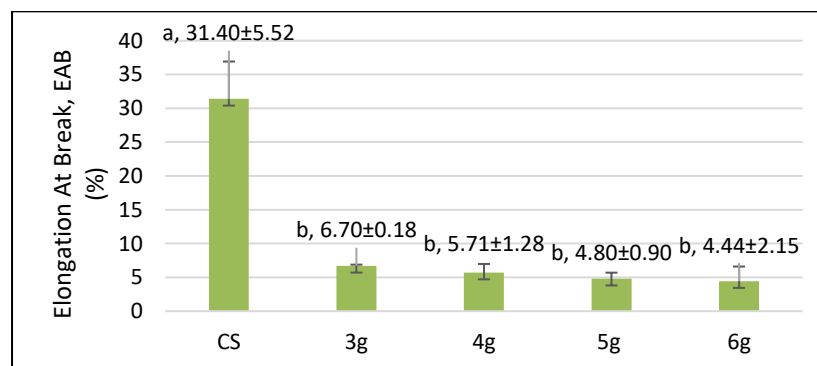


Figure 3.3. Effect of bamboo powder dosage on the elongation at break of citric acid-crosslinked starch films. Data presented as mean $\pm$ S.D. ($n = 3$). Different alphabets between columns represent significance at $p < 0.05$ after applying post hoc Tukey's test.

3.2. Chemical Properties

Figure 3.4 presents the FTIR spectra of bamboo–starch–based films with varying bamboo loadings (3–6 g) alongside the control sample. Clear compositional and structural modifications were observed as a function of bamboo content in films crosslinked with citric acid. In Region I ($3700\text{--}3000\text{ cm}^{-1}$), all samples exhibited a broad O–H stretching band. The control film exhibited an intense and broad peak centered at approximately 3300 cm^{-1} , reflecting the high density of hydroxyl groups inherent to potato starch. With increasing bamboo incorporation (3–6 g), this band became broader and exhibited slight peak shifts, indicating enhanced hydrogen bonding and partial esterification between hydroxyl groups of starch and bamboo cellulose with the carboxyl groups of citric acid (Omar et al., 2022). As the citric acid concentration was maintained constant, these spectral changes are attributed to the increased availability of hydroxyl groups introduced by bamboo, which promoted more effective crosslinking within the polymer matrix.

In Region II ($3000\text{--}2800\text{ cm}^{-1}$), C–H stretching vibrations displayed minor peak sharpening and shifts with increasing bamboo content, suggesting interactions between the aliphatic components of bamboo and the starch matrix, consistent with previous reports on bamboo-reinforced biopolymer films (Abdul Khalil et al., 2018). Region III ($1750\text{--}1600\text{ cm}^{-1}$) exhibited a pronounced intensification of carbonyl (C=O) stretching bands in the range of $1720\text{--}1745\text{ cm}^{-1}$, characteristic of ester linkages formed between the carboxyl groups of citric acid and the hydroxyl groups of lignocellulosic constituents. It is suggested that the addition of bamboo enhanced the esterification degree, as the greater availability of hydroxyl groups in bamboo cellulose provides more active sites for reaction with citric acid, promotes the formation of ester bonds, and thereby increases the crosslinking density. The observed peak shift from approximately 1640 to 1646 cm^{-1} further confirms the successful formation of the ester bond, with bamboo cellulose contributing additional reactive sites for crosslinking

(Widyorini et al., 2017).

In Region IV ($1200\text{--}900\text{ cm}^{-1}$), a progressive increase in the intensity of C–O–C stretching vibrations was detected with higher bamboo loading, indicating enhanced polysaccharide interactions and the formation of a more organized and interconnected polymer network (Huang et al., 2024). This structural densification was further supported by a consistent decrease in transmittance intensity with increasing bamboo content. Specifically, transmittance at 3315 cm^{-1} decreased from 74.02% in the control film to 53.08% in the 6 g bamboo film, while in Region IV ($\approx 1000\text{ cm}^{-1}$) it declined from 51.45% to 13.08%. These reductions in light transmission reflect increased molecular packing density and reduced free volume arising from bamboo-enhanced crosslinking.

Overall, the FTIR results confirm that, despite a constant citric acid concentration, increasing bamboo content significantly enhances crosslinking efficiency, leading to pronounced modifications in chemical bonding, matrix organization, and optical behavior of the bamboo–starch–based films.

3.3. Morphological structure

Figure 3.5 presents the surface morphology of bamboo–starch–based films containing different bamboo powder loadings and crosslinked with citric acid, as observed by SEM at $100\times$ magnification. The control film, composed solely of gelatinized potato starch, exhibited a relatively smooth and continuous surface with limited surface defects. Minor microcracks and faint outlines of starch granules were observed, which are commonly associated with the brittle nature of uncrosslinked starch films due to weak intermolecular cohesion, as previously reported by Hirpara et al., (2021). The control film, composed of gelatinized potato starch and glycerol without crosslinked bamboo powder, exhibited a relatively smooth, continuous surface with few surface defects. Minor microcracks and faint outlines of starch granules were observed, which may be associated with the inherent structural characteristics of starch-based films.

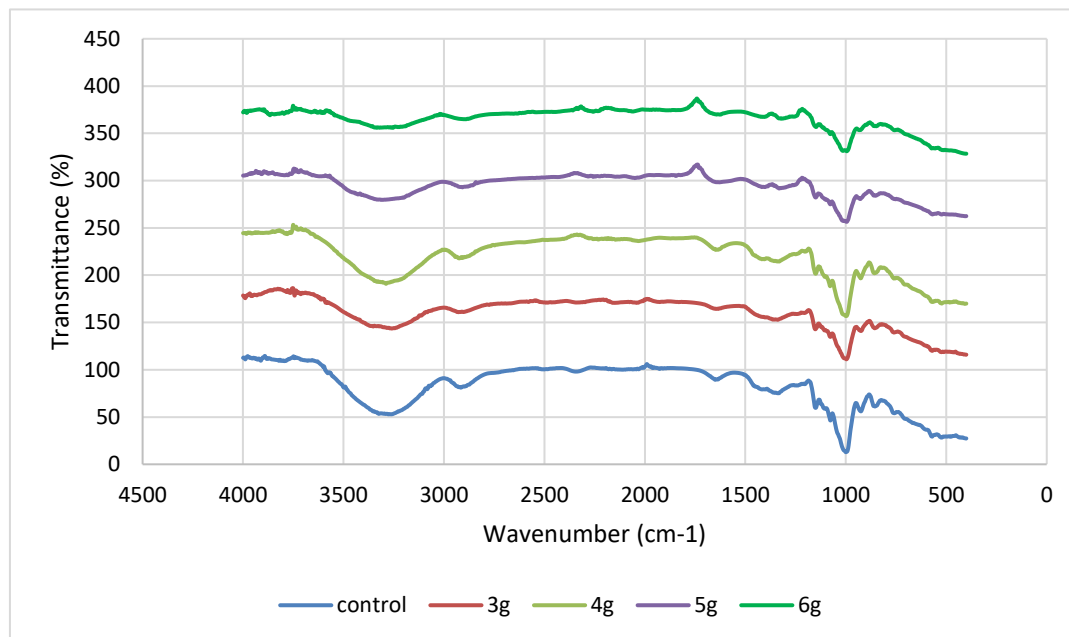


Figure 3.4. FTIR spectra of different bamboo powder dosages on citric acid-crosslinked starch films

Upon incorporation of 3 g of bamboo powder, the film surface became noticeably rougher, with dispersed fibrous features embedded within the starch matrix. This morphology suggests an effective interfacial interaction between bamboo cellulose and starch, facilitated by citric acid acting as a compatibilizing cross-linker between the hydroxyl groups of both components. Increasing the bamboo content to 4 g further intensified surface roughness; however, the bamboo fibers remained uniformly distributed without visible agglomeration. This sample exhibited the most homogeneous and compact morphology, indicating optimal filler dispersion and effective stress transfer within the polymer network, consistent with observations reported by Abdul Khalil et al., (2018).

In contrast, films containing 5 g of bamboo powder exhibited pronounced surface irregularities, characterized by fiber clustering and voids within the matrix. These features indicate the onset of filler agglomeration and reduced matrix continuity, suggesting that the bamboo content exceeded the dispersion capacity of the starch-citric acid system. Such agglomerated regions can act as stress

concentration points during tensile loading, promoting localized crack initiation and reducing the efficiency of stress transfer between the starch matrix and bamboo filler. This effect became more severe at 6 g bamboo loading, where extensive fiber agglomeration, surface fractures, and poorly bonded regions were evident. The formation of large fiber bundles and voids reflects inadequate interfacial adhesion and disrupted polymer continuity, which may hinder uniform stress distribution throughout the composite and ultimately compromise film structural integrity (Omar et al., 2022).

Overall, the SEM analysis confirms that bamboo loading has a strong influence on the morphological characteristics of citric acid-crosslinked starch films. Moderate bamboo incorporation, particularly at 4 g, promotes uniform dispersion and strong interfacial bonding, resulting in a well-structured composite. Conversely, excessive bamboo addition leads to filler agglomeration and matrix disruption, indicating that the effectiveness of citric acid as a crosslinker diminishes when filler content surpasses the matrix's dispersion threshold.

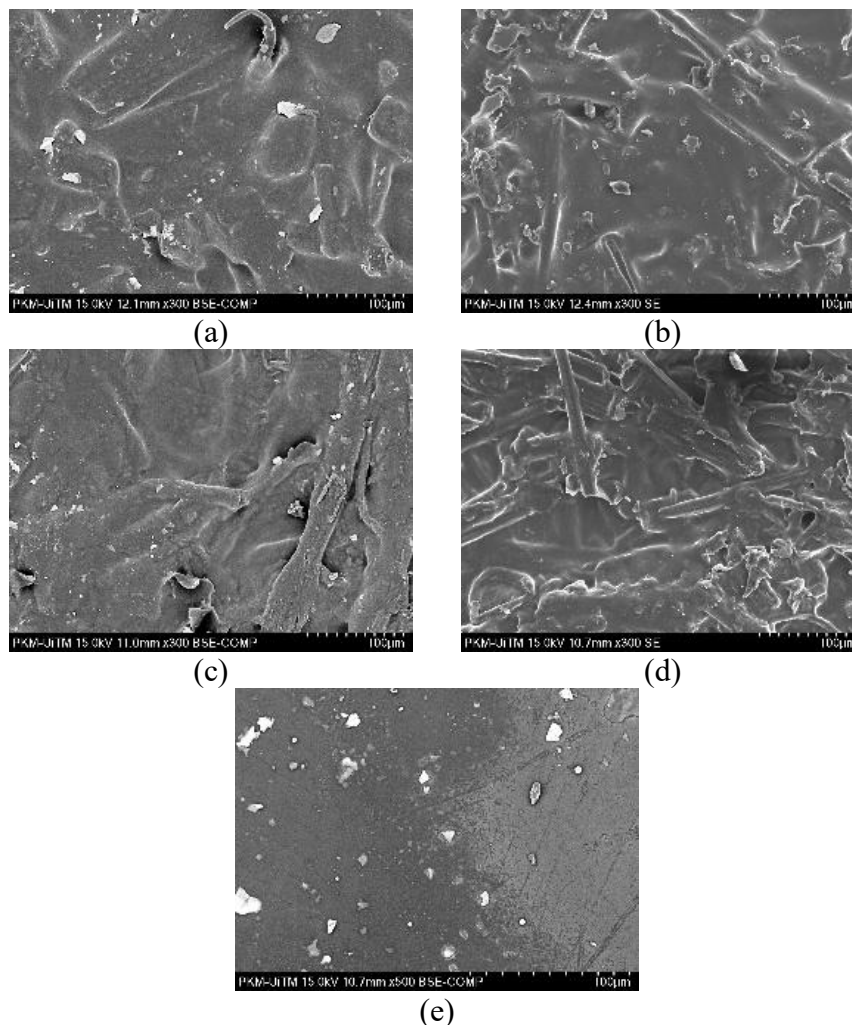


Figure 3.5. Surface morphology of different bamboo powder dosages on citric acid-crosslinked starch films, a) 3g of bamboo dosages, b) 4g of bamboo dosages, c) 5g of bamboo dosages, d) 6g of bamboo dosages, e) control sample

3.4. Moisture Content

Figure 3.6 presents the moisture content of bamboo-starch-based films as an indicator of their moisture-retention behaviour. A clear and monotonic decrease in moisture content was observed with increasing bamboo loading, indicating a progressive reduction in moisture retention. The control starch film exhibited the highest moisture content (21.57%), consistent with the inherently hydrophilic character of starch arising from its high density of hydroxyl (–OH) groups that readily interact with water molecules (Wu et al., 2019). Upon citric-acid crosslinking, partial esterification of these hydroxyl groups reduced the availability of hydrophilic sites and promoted the formation of a more compact polymer network, thereby enhancing water insolubility. The incorporation

of bamboo further reduced moisture retention, with moisture content decreasing from 20.06% at 3 g bamboo to 18.65% at 4 g, 14.62% at 5 g, and reaching a minimum of 9.96% at 6 g bamboo. This substantial reduction reflects increased matrix densification and stronger filler-matrix interactions, which limit free volume and restrict water diffusion pathways within the film. The nearly 54% reduction in moisture content at 6 g of bamboo relative to the control demonstrates a substantial reduction in moisture retention with increasing bamboo incorporation. However, moisture content alone does not directly represent water-vapor barrier performance; therefore, further evaluation using water-vapor permeability or water-absorption measurements is required to establish the barrier properties of these films.

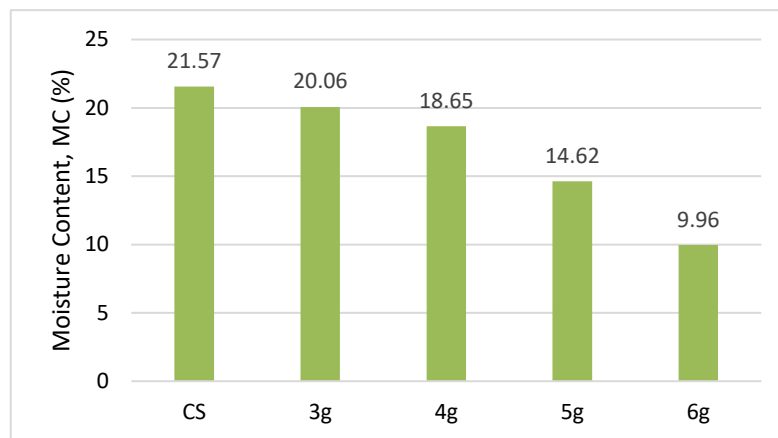


Figure 3.6. Effect of bamboo powder dosage on the moisture content of citric acid-crosslinked starch films.

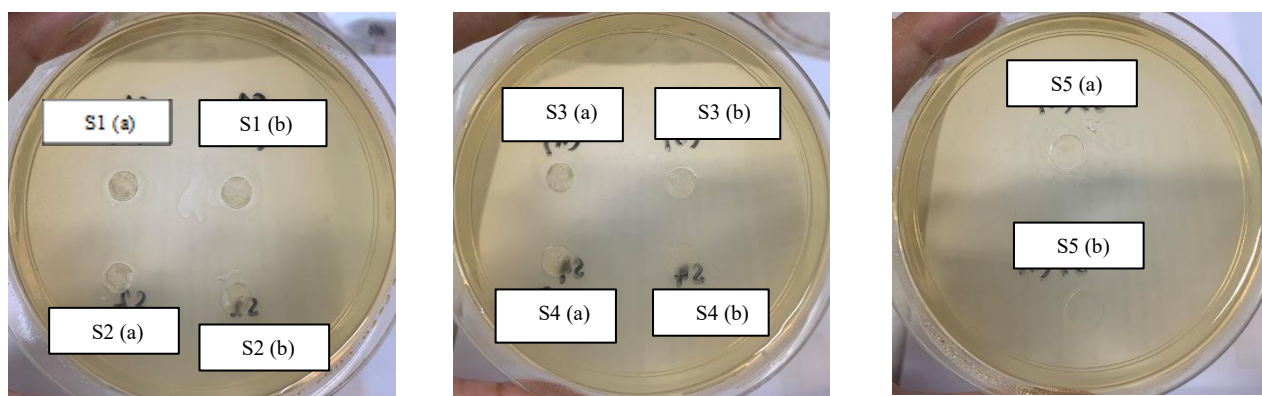


Figure 3.7. Effect of bamboo powder dosage on the antibacterial performance (antibacterial ring radius) of citric acid-crosslinked starch films, S1) 3g of bamboo dosages, S2) 4g of bamboo dosages, S3) 5g of bamboo dosages, S4) 6g of bamboo dosages, S5) control sample.

3.5. Antibacterial Performance

The antibacterial performance of the films was evaluated against *E. coli* using the agar diffusion method, with activity quantified as the antibacterial ring radius. The control film, consisting solely of potato starch, exhibited no detectable inhibition zone (0 mm), confirming the absence of intrinsic antimicrobial activity in native starch matrices (Dudeja et al., 2023). In contrast, all bamboo-reinforced films demonstrated measurable antibacterial activity, with the antibacterial ring radius increasing with bamboo loading. The films containing 3, 4, and 5 g of bamboo exhibited antibacterial ring radii of 1.0, 1.5, and 2.0 mm, respectively. The 6 g bamboo film exhibited the highest antibacterial activity, with an antibacterial ring radius of 3.0 mm. This dose-dependent enhancement is attributed primarily to the presence of bioactive constituents in bamboo, including phenolic

compounds, lignin, and flavonoids. Phenolic compounds can interact with bacterial cell membranes, increasing membrane permeability and causing leakage of intracellular components, while lignin-derived phenolic structures may inhibit microbial enzyme activity and induce oxidative stress within bacterial cells. As bamboo loading increased, a greater concentration of these bioactive compounds became available within the film matrix, thereby enhancing antibacterial efficacy. Additionally, citric acid, used as a crosslinking agent in all bamboo-containing films, may exert a synergistic antimicrobial effect through residual acidic groups within the film network, creating a localized acidic microenvironment that suppresses bacterial growth. This acidic environment can disrupt membrane transport processes, reduce intracellular pH, and further compromise bacterial membrane integrity,

thereby enhancing growth inhibition against *E. coli* (Zhu et al., 2023). From an antimicrobial performance benchmarking perspective, the threefold increase in antibacterial ring radius observed at the highest bamboo loading compared to the lowest reinforced sample highlights the effectiveness of incorporating bamboo in imparting functional antimicrobial activity. These findings are consistent with

4. Conclusion

This study demonstrated that the incorporation of citric acid–crosslinked bamboo powder significantly affects the structural and functional properties of bamboo–starch-based films. Increasing bamboo dosage improved tensile strength, reduced moisture content, and enhanced antibacterial activity, indicating enhanced intermolecular interactions and effective crosslinking within the polymer matrix. The film containing 6 g of bamboo exhibited the highest tensile strength (9.65 MPa), the lowest moisture content (9.96%), and the greatest antibacterial activity against *E. coli*, confirming the role of bamboo as an effective reinforcing and bioactive component. However, higher bamboo loadings resulted in reduced elongation at break and increased surface irregularities, as observed in SEM analysis, suggesting limitations associated with filler agglomeration and restricted polymer chain mobility. These results indicate a trade-off between mechanical strength and flexibility that must be considered when designing starch-based composite films for food packaging applications. Overall, citric acid proved to be an effective food-grade green crosslinker, promoting ester bond formation and improved interfacial adhesion between starch and bamboo components. The findings highlight the potential of citric acid–crosslinked bamboo–starch films as environmentally friendly, biodegradable materials for food packaging. The improved mechanical strength, reduced moisture content, and antibacterial performance demonstrate the potential of these films for further development as sustainable food-packaging materials. The reduced moisture content may help minimize moisture transfer during storage, while the observed antibacterial activity against *E. coli* warrants further evaluation of the films in actual

previous reports demonstrating improved *E. coli* inhibition in starch-based films containing plant-derived bioactive fillers (Dudeja et al., 2023). Overall, the bamboo-reinforced, citric-acid-crosslinked films developed in this study exhibit promising antimicrobial functionality, supporting their potential application in sustainable active food packaging systems.

food systems. In addition, the biodegradable nature of the bamboo–starch matrix offers a sustainable alternative to conventional petroleum-based packaging materials, supporting current efforts toward environmentally responsible food packaging solutions. Further studies focusing on dispersion optimization and application-specific performance requirements are recommended to enhance film flexibility while maintaining functional properties.

5. References

- Abdul Khalil, H. P. S., Che Mohamad Hazwan, C. I., Khairunnisa, A. R., Owolabi, F. A. T., Asniza, M., Rizal, S., Nurul Fazita, M. R., & Paridah, M. T. (2018). Development and characterization of bamboo fiber reinforced biopolymer films. *Materials Research Express*, 5(8), 085309. <https://doi.org/10.1088/2053-1591/aad2a0>
- Chen, J., Lin, H., Zheng, Q., Zhang, X., Yang, W., & Li, R. (2019). Fabrication of hydrophobic ZnO / PMHS coatings on bamboo surfaces : The synergistic effect of ZnO and PMHS on anti-mildew properties. *Coatings*, 9(15), 1–10. <https://doi.org/10.3390/coatings9010015>
- Dirpan, A., Ainani, A. F., & Djalal, M. (2023). A review on biopolymer-based biodegradable film for food packaging : Trends over the last decade and future research. *Polymers*, 15(2781), 1–33. <https://doi.org/10.3390/polym15132781>
- Dudeja, I., Kaur, R., Singh, A., & Kaur, J. (2023). Citric acid : An ecofriendly cross - linker for the production of functional biopolymeric materials. *Sustainable Chemistry and Pharmacy*, 36, 101307. <https://doi.org/10.1016/j.scp.2023.101307>
- Guan, Y., Gao, H., Li, W., Rao, J., & Zhang, Y.

- (2019). Preparation and characterization of antibacterial films from bamboo. *Wood Research*, 64(5), 821–832.
- Hirpara, N. J., N., D. M., & J., R. P. (2021). Development of potato starch based biodegradable packaging film. *Biological Forum - An International Journal*, 13(1), 529–541.
- Huang, Z., Lou, W., Zhong, T., Zhang, J., Wang, J., Yang, H., Shao, Q., & Cai, M. (2024). Fabrication of bamboo nanocellulose fibril-based food packaging with dual-antimicrobial property. *International Journal of Biological Macromolecules*, 281, 136249. <https://doi.org/10.1016/j.ijbiomac.2024.136249>
- Jin, Q., Zhang, W., Yao, W., Qiu, C., Li, F., Kaima, J., & Jongchansitto, P. (2017). Experimental investigation and analysis of mercerized and citric acid surface treated bamboo fiber reinforced composite. *IOP Conference Series: Materials Science and Engineering*, 225, 012154. <https://doi.org/10.1088/1757-899X/225/1/012154>
- Khandeparkar, A. S., Paul, R., Sridhar, A., Lakshmaiah, V. V., & Nagella, P. (2024). Eco-friendly innovations in food packaging: A sustainable revolution. *Sustainable Chemistry and Pharmacy*, 39, 101579. <https://doi.org/10.1016/j.scp.2024.101579>
- Kumar, K. N., Babu, P. D., Surakasi, R., Kumar, P. M., Ashokkumar, P., Khan, R., Alfozan, A., & Gebreyohannes, D. T. (2022). Review article mechanical and thermal properties of bamboo fiber – reinforced PLA polymer composites: A critical study. *Hindawi*, 2022, 1332157. <https://doi.org/10.1155/2022/1332157>
- Liu, C., Zhang, S., Yan, S., & Pan, M. (2024). Mechanical and antibacterial properties of bamboo charcoal / ZnO-Modified bamboo fiber / polylactic acid composites. *Forests*, 15(371), 1–14. <https://doi.org/10.3390/f15020371>
- Liu, J., Huang, J., Hu, Z., Li, G., Hu, L., & Chen, X. (2021). Chitosan-based films with antioxidant of bamboo leaves and ZnO nanoparticles for application in active food packaging. *International Journal of Biological Macromolecules*, 189, 363–369. <https://doi.org/10.1016/j.ijbiomac.2021.08.136>
- Liu, X., Li, H., Zhang, T., Peng, H., Wang, K. K., Dong, Y., Lu, Y., Wang, K. K., Zhan, X., Liu, Y., Li, Y., & Li, J. (2024). Strong, tough, anisotropic, flexible, and transparent bamboo films. *Industrial Crops & Products*, 207, 117757. <https://doi.org/10.1016/j.indcrop.2023.117757>
- Mohammad, O., Manan, S., Shahzad, A., Ullah, M., Wajid, M., & Yang, G. (2022). Biobased materials for active food packaging: A review. *Food Hydrocolloids*, 125, 107419. <https://doi.org/10.1016/j.foodhyd.2021.107419>
- Murillo, E. A., & López, B. L. (2015). Effect of the maleic anhydride content on the structural, thermal, rheological and film properties of the n-butyl methacrylate – maleic anhydride copolymers. *Progress in Organic Coatings*, 78, 96–102. <https://doi.org/10.1016/j.porgcoat.2014.10.003>
- Nyerere, G., Kyokusiima, S., Nabaterega, R., & Tumusiime, G. (2024). The synergy of maize straw cellulose and sugarcane bagasse fibre on the characteristics of bioplastic packaging film. *Bioresource Technology Reports*, 28, 102007. <https://doi.org/10.1016/j.biteb.2024.102007>
- Omar, N. F., Hafid, H. S., Zhu, J., Kamal Bahrin, E., Mohd Nadzri, F. Z., & Wakisaka, M. (2022). Starch-based composite film reinforcement with modified cellulose from bamboo for sustainable packaging application. *Materials Today Communications*, 33, 104392. <https://doi.org/10.1016/j.mtcomm.2022.104392>
- Qian, S., Mao, H., & Zarei, E. (2015). Preparation and characterization of maleic anhydride compatibilized poly (lactic acid)/ bamboo particles biocomposites. *Journal of Polymers and the Environment*, 3, 341–347. <https://doi.org/10.1007/s10924-015-0715-x>
- Rashid, M. M., Abir, N., & Iqbal, M. I. (2023). Bamboo cellulose: Structure, properties, and

- applications. In *Cellulose Fibre Reinforced Composites* (pp. 23–48). Elsevier Ltd. <https://doi.org/10.1016/B978-0-323-90125-3.00007-0>
- Sangeetha, U. K., Sudhakaran, N., Parvathy, P. A., Abraham, M., Das, S., De, S., & Sahoo, S. K. (2024). Coconut husk-lignin derived carbon dots incorporated carrageenan based functional film for intelligent food packaging. *International Journal of Biological Macromolecules*, 266, 131005. <https://doi.org/10.1016/j.ijbiomac.2024.131005>
- Sobri, Z., Asa'ari, A. Z. M., Yacob, N., H'ng, P. S., Abdullah, L. C., & Zainudin, E. S. (2021). In situ formation of zinc oxide on bamboo bleached pulp in preparation of antibacterial paper: Effect of precursors addition. *BioResources*, 16(3), 6121–6134.
- Song, D., Ma, L., Pang, B., An, R., Nie, J., Guo, Y., & Li, S. (2023). An active bio-based food packaging material of ZnO @ plant polyphenols/cellulose / polyvinyl alcohol: Design , characterization and application. *International Journal of Molecular Sciences*, 24(1577), 1–13. <https://doi.org/10.3390/ijms24021577>
- Song, F., Xu, C., Bao, W., Wang, X., & Wang, Y. (2015). Bamboo (*Neosinocalamus affinis*)-based thin film, a novel biomass material with high performances. *Carbohydrate Polymers*, 119, 167–172. <https://doi.org/10.1016/j.carbpol.2014.11.055>
- Song, K., Ren, X., & Zhang, L. (2017). Bamboo fiber-polymer composites: Overview of fabrications, mechanical characterizations and applications. In *Green Energy and Technology*. Springer International Publishing AG 2017. <https://doi.org/10.1007/978-3-319-49382-4>
- Su, Y., Tang, J., Chen, Y., Ali, A., Toufouki, S., Wang, X., & Zhang, J. (2023). Development and characterization of a bamboo cellulose-based multifunctional composite film by deep eutectic solvent and gelatin. *Industrial Crops & Products*, 204, 117275. <https://doi.org/10.1016/j.indcrop.2023.117275>
- Vinay, G. M., Modi, R. B., & Prakasha, R. (2024). Banana pseudostem : An innovative and sustainable packaging material: A review. *Journal of Packaging Technology and Research*, 8, 95–107. <https://doi.org/10.1007/s41783-024-00167-0>
- Widyorini, R., Umemura, K., Kusumaningtyas, A. R., & Prayitno, T. A. (2017). Effect of starch addition on properties of citric acid-bonded particleboard made from bamboo. *BioResources.Com*, 12(4), 8068–8077.
- Wu, H., Lei, Y., Lu, J., Zhu, R., Xiao, D., Jiao, C., Xia, R., Zhang, Z., Shen, G., Liu, Y., Li, S., & Li, M. (2019). Effect of citric acid induced crosslinking on the structure and properties of potato starch/chitosan composite films. *Food Hydrocolloids*, 97, 105208. <https://doi.org/10.1016/j.foodhyd.2019.105208>
- Zhu, Y., Zhang, J., Wang, D., Shi, Z. Z. Z., Yang, J., & Yang, H. (2023). Preparation of anti-bacterial cellulose nanofibrils (CNFs) from bamboo pulp in a reactable citric acid–choline chloride deep eutectic solvent. *Polymers*, 15(148), 1–11. <https://doi.org/10.3390/polym15010148>

Acknowledgement

The authors gratefully acknowledge the financial support provided by the Conference Support Initiative (CSI), which contributed to the successful dissemination of this research. The authors also sincerely thank the Faculty of Chemical Engineering, Universiti Teknologi MARA, for providing laboratory facilities, technical support, and resources essential for conducting the experimental work in this study.