

Min Wang, Ruocheng Li, Quanliang Wang*, Tao Xing* and Liping Cai

Rapid extraction of lignin from bamboo with high yield by microwave-assisted choline chloride-formic acid method

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Abstract: Efficient extraction of lignin from lignocellulose by low-cost and eco-friendly way has been a key focus in the lignin-first biorefinery strategy. The bamboo lignocellulose consists of three major components, in which the complex crosslinking structure greatly hindering the rapid and large-scale extraction of lignin. To address this issue, an innovative method of microwave-assisted deep eutectic solvent (MADES) was developed to extract lignin from bamboo. The DES emerging as promising alternatives to conventional solvents, offers outstanding selective extraction capabilities. The effect of DES types on the lignin yield was investigated. The DES containing choline chloride-formic acid (1:6 Molar ratio), and the 1:40 solid-liquid ratio of bamboo powder to solution were selected as optimal conditions for the high lignin yield. Under the microwave radiation, the optimum lignin yield (89.5 %) was achieved in 10 min. The bamboo fibers and extracted lignin were analyzed by X-ray photoelectron spectroscopy (XPS), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and thermogravimetric (TG) techniques. The extracted lignin was granular and irregular cluster morphology. The crystallinity index of bamboo fibers increased by 20.4 % after treatment, thus offering a new strategy for optimizing extraction procedures for producing lignin.

Keywords: lignin; bamboo powder; deep eutectic solvent; microwaves; extraction

*Corresponding authors: Quanliang Wang and Tao Xing, College of Mechanical and Electrical Engineering, Northeast Forestry University, Harbin, Heilongjiang, 150040, China, E-mail: wqlunt@126.com (Q. Wang), xingtaohit@126.com (T. Xing)

Min Wang, Material Science and Engineering College, Northeast Forestry University, Harbin, Heilongjiang, 150040, China

Ruocheng Li, College of Mechanical and Electrical Engineering, Northeast Forestry University, Harbin, Heilongjiang, 150040, China

Liping Cai, Jiangsu Co-Innovation Center of Efficient Processing and Utilization of Forest Resources, International Innovation Center for Forest Chemicals and Materials, College of Materials Science and Engineering, Nanjing Forestry University, Nanjing, Jiangsu, 210037, China

1 Introduction

The bamboo is particularly promising because of its abundance in China. It faces a growing supply-demand imbalance due to the banning logging policy. The bamboo has high potential as a biomass feedstock due to its fast growth, high carbon content, and easy cultivation on non-arable land (Emamverdian et al. 2020). Compared to woods, the bamboo is easier to obtain, showing higher sustainable utilization rate. The unique characteristics make bamboo widely applicable in various industries, e.g., building, furniture manufacturing, handicrafts, and papermaking (Fan et al. 2023; Yuan et al. 2022; Zhu et al. 2022). The bamboo has been recognized as a promising lignocellulosic resource in the global bioeconomy. As a biorefinery resource, bamboo contains lignin with proportion of 38–50 % (Zhang et al. 2021). The lignin can be used to provide high-value products as the most abundant renewable aromatic compound in bamboo fibre. Lignin primarily comprises three monolignol units including p-hydroxyphenyl, syringyl, and guaiacyl phenylpropanoid units that are linked by ether and carbon carbon bonds (Liu et al. 2019a,b). The abundant functional groups such as carbonyl, aliphatic, phenolic hydroxyls, and carboxyl groups, endow lignin with high hydrophobicity, UV-shielding properties, antioxidant activity, thermal stability, flame retardancy, and biocompatibility (Figueiredo et al. 2018; Zhang et al. 2021). Therefore, efficient separation of lignin is the key to the utilization of bamboo resources.

Lignin as the most abundant biopolymer is an abundant renewable resource that can provide a sustainable substitute for energy and chemicals. In recent decades, more emphasis has been given on developing lignin conversion processes for commercialization, such as heating and electricity generation, phenolic modified adhesives (Wang et al. 2023). It presents variable extraction rate, purity, and reaction activity for different extraction conditions, which hinders its high value-added utilization (Hiroshi et al. 2018). The common treatment for extracting lignin include using acids, alkalis, ionic liquids, organic, and deep eutectic solvents now. The acid method can preliminarily separate lignin (Liao et al. 2024). The β -O-4 structure was maintained in the primary lignin. The alkali treatment resulted in lignin with

increased purity and higher molecular weight. Whereas, there will be residual chemical linkages between lignin and cellulose (Wang et al. 2024). The ionic liquid can be reused in separating lignin. However, its efficiency decreased over subsequent cycles (Mohtar et al. 2017). High cost and complex synthesis process of ionic liquid hinder its feasibility in large scale applications. The organosolv method is among the most user-friendly and economically interesting because many of the solvents involved can be easily recycled. The presence acid residues would require additional purification treatments (Florian et al. 2019). The deep eutectic solvent (DES) treatment can selectively dissolve lignin during biomass fractionation for the value-added conversion of the carbohydrates (Feng et al. 2024). The advantages of DES methods over the convention include low toxicity, nonflammability, biocompatibility, and chemical tunability by varying the molar ratio of the parent components (Smith et al. 2014). The DES can break down the β -O-4 and β - β bonds in lignin, which removes fat and methoxy hydroxyl groups from lignin side chains (Uddin et al. 2024). Therefore, the efficient, and green preparation of lignin from lignocellulose is of great significance.

The DES is a system formed by hydrogen bond or self-association interactions between hydrogen bond acceptors and hydrogen bond donors. In recent years, the DES has emerged as promising solvents for lignocellulosic biomass fractionation. DES was recognized on lignin extraction and biomass saccharification enhancement, which attributed to the DES ability to selectively dissolve lignin and hemicellulose (Wang and Lee 2021). Compared to other solvents, preparation of high purity DESs using low cost sources is relatively simple. Most DESs do not deactivate enzymes, making DES advantageous in biomass conversion (Gorke et al. 2010). Several crucial factors influence extraction efficiency and extracts properties for DES-assisted/enhanced methods. These factors include DES types, molar ratio, solid-liquid ratio, extraction temperature, and extraction time (Yahaya et al. 2024). DESs obtained with choline chloride and carboxylic acids are particularly promising for lignin extraction and solubilization. The formic acid (FA) has been reported as a sustainable and effective strategy for deconstruction of bamboo fibers. The simplest organic carboxylic acid and can be derived from biomass (Zhang et al. 2025). Due to the good lignin solvency, the formic acid can make hemicellulose more accessible, thus accelerating the dissolution of lignin out from cellulosic feedstocks (Wang et al. 2019). FA can be efficiently recovered by vacuum distillation due to its lower boiling point (100.8 °C) to guarantee a clean process. Thus, the choline chloride-formic acid system was chosen for lignin extraction.

Despite substantial progress has been made in DES treatment techniques, the majority processes suffer from prolonged durations. It seriously impacts treatment efficiency. It requires a certain reaction temperature to proceed. Heating can reduce reaction time for faster biomass dissolution. The conventional heating methods (e.g., water bath, oil bath, and electric furnace) heat materials from the surface to inside by the radiation, convection, and conduction ways. In comparison, the microwave irradiation is introduced to the improve heating uniformity and reduce the treatment time. Microwaves are longer than other electromagnetic waves used for radiant heating and therefore it has better penetration ability. Many polar molecules vibrate, rub, and collide in high-frequency electromagnetic fields, so that the temperature of the material rises rapidly. The microwave hydrothermal treatment is a more efficient, eco-friendlier method, due to it consumes less energy than traditional heating (del Río et al. 2022).

Herein, this study aims to explore how to efficiently extract lignin from bamboo by MA-DES method. Specifically, it explores the effects of different DES types, microwave time, DES molar ratio, and solid-liquid ratio on the lignin rate under microwave assistance. The extraction optimisation was achieved in a comprehensive and systematic manner by investigating the effect of various extraction conditions. The chemical compositions of bamboo fiber before and after treatment were analyzed. The proportion of each component was calculated to demonstrate the efficient extraction of lignin. The changes in surface compositions, crystallinity, and microstructure of bamboo fibers before and after MA-DES treatment, and the extracted lignin were also investigated by Thermogravimetric (TG), X-ray photoelectron spectroscopy (XPS), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM), respectively. Considering the extracted lignin rate and economy, the optimal solution was obtained.

2 Materials and methods

2.1 Materials

The bamboo of 3–5-year-old Moso bamboo (*Phyllostachys edulis*) were sourced from Zhejiang, China. The bamboo was first cut and then mechanically crushed to form a uniform powder of particles. The powder was dried in an oven at 105 °C to a constant weight. All the chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., Beijing, China.

2.2 Determination chemical compositions of bamboo fiber

To calculate the extraction yield of lignin, the content of each component in bamboo fibers was determined. The moisture content was measured using the MB23 moisture analyzer (Shanghai Ohhaus Instrument Co., Ltd). The organic solvent extract content was determined according to GB/T 2677.6-94. The holocellulose content was determined according to GB/T 2677.10-1995. The lignin content was obtained by calculating the contents of acid insoluble lignin and acid soluble lignin. The content of acid insoluble lignin and acid soluble lignin in bamboo fiber were determined according to the modified method of Pinnarat et al. (Pinnarat et al. 2024). The ash content in the sample was determined according to GB/T 742-2018.

2.3 Preparation of DES

The DES can be prepared by the mixture of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). The DES can rupture cell wall by choline group. Three types of DESs were prepared, where the chloride choline (ChCl) was chosen as HBA, and formic acid (FA), oxalate (OA), and urea (U) were used as the HBD, respectively. All the DESs were produced by mixing HBA and HBD in molar ratios of 1:2. The mixture was heated at 60 °C with continuous stirring for 2 h until a homogeneous colorless liquid obtained. The synthesized DESs were stored in the desiccator prior before use.

2.4 Lignin separation by MA-DES treatment

The bamboo powder and DES were mixed at a ratio of 1:10 (g/g) until obtaining uniform liquid. Using microwave-assisted heating mixed liquid in WD700A microwave reactor (Shunde Galanz Microwave Appliance Co., Ltd) at various times (5 min, 10 min, 15 min, 20 min, and 25 min), molar ratio of choline chloride to formic acid (2:1, 1:1, 1:2, 1:4, and 1:6) and DESs and bamboo powder solid-liquid ratio (1:10, 1:20, 1:30, 1:40) (g/g).

The extraction yield X (%) of lignin from bamboo fiber were calculated as following equations:

$$X = \frac{m - m_0}{m} \times 100 \% \quad (1)$$

where m is weight of lignin in untreated bamboo fiber (g), and m_0 is the weight of Lignin in bamboo fibers treated with deep eutectic solvents (g).

2.5 Analytical methods

Thermal stabilities of bamboo fibers and extracted lignin were characterized using thermogravimetric analysis SDT Q600 (Waters Technology Co., Ltd., USA) with a temperature range from 30 to 600 °C in nitrogen atmosphere with a heating rate of 10 °C/min. The surface morphology was observed by a Hitachi SU8010 scanning electron microscope (Shanghai Jiexing Biotechnology Co., Ltd) with an accelerating voltage of 5 kV before and after treatment. The sample was performed golden spraying treatment under vacuum conditions before X-ray analysis. The bamboo fibers were investigated using an X-ray diffractometer (Bruker GmbH, Germany) ranging from 5 to 40° at 40 kV and 30 mA. The crystallinity index (C_rI) was calculated according to (Segal et al. 1959) in Eq. (2):

$$C_rI = \frac{I_{002} - I_{amorph}}{I_{002}} \times 100 \% \quad (2)$$

where I_{002} is the crystalline peak at $2\theta = 22.0^\circ$. and I_{amorph} is the amorphous peak at $2\theta = 18.0^\circ$.

The bamboo fibers were determined using an AXIS SUPRA X-ray photoelectron spectrometer (SHIMADZU Corporation, Japan). The qualitative analysis of lignin was performed using a Nicolet iN10 Fourier transform infrared spectrometer (Thermo Fisher Scientific, Inc), with a wavelength scanning range of 4,000–400 cm^{-1} , a resolution of 4 cm^{-1} , and a frequency of 32 times. The OMNIC software was used for processing and analysis.

3 Results and discussion

3.1 Lignin extraction process and structure changes of bamboo fibers in MA-DES method

The deep eutectic solvent (DES) method is green and recyclable in extracting lignin, which has received widespread attention from scholars. This work proposes a microwave-assisted (MA) DES method for lignin separation from bamboo fibers. The lignin extraction process is illustrated in Figure 1a. The bamboo was mechanically crushed into powder, and then mixed with DES under MA heating. After MA treatment, the solid-liquid mixture exhibited dark brown appearance. The solids rich in cellulose was obtained by vacuum filtration. The regenerated lignin was then precipitated from the remaining mixture.

In addition to 6.2 % of benzyl alcohol extract, the bamboo fiber contained 61.3 % of holocellulose and 30.7 % of lignin

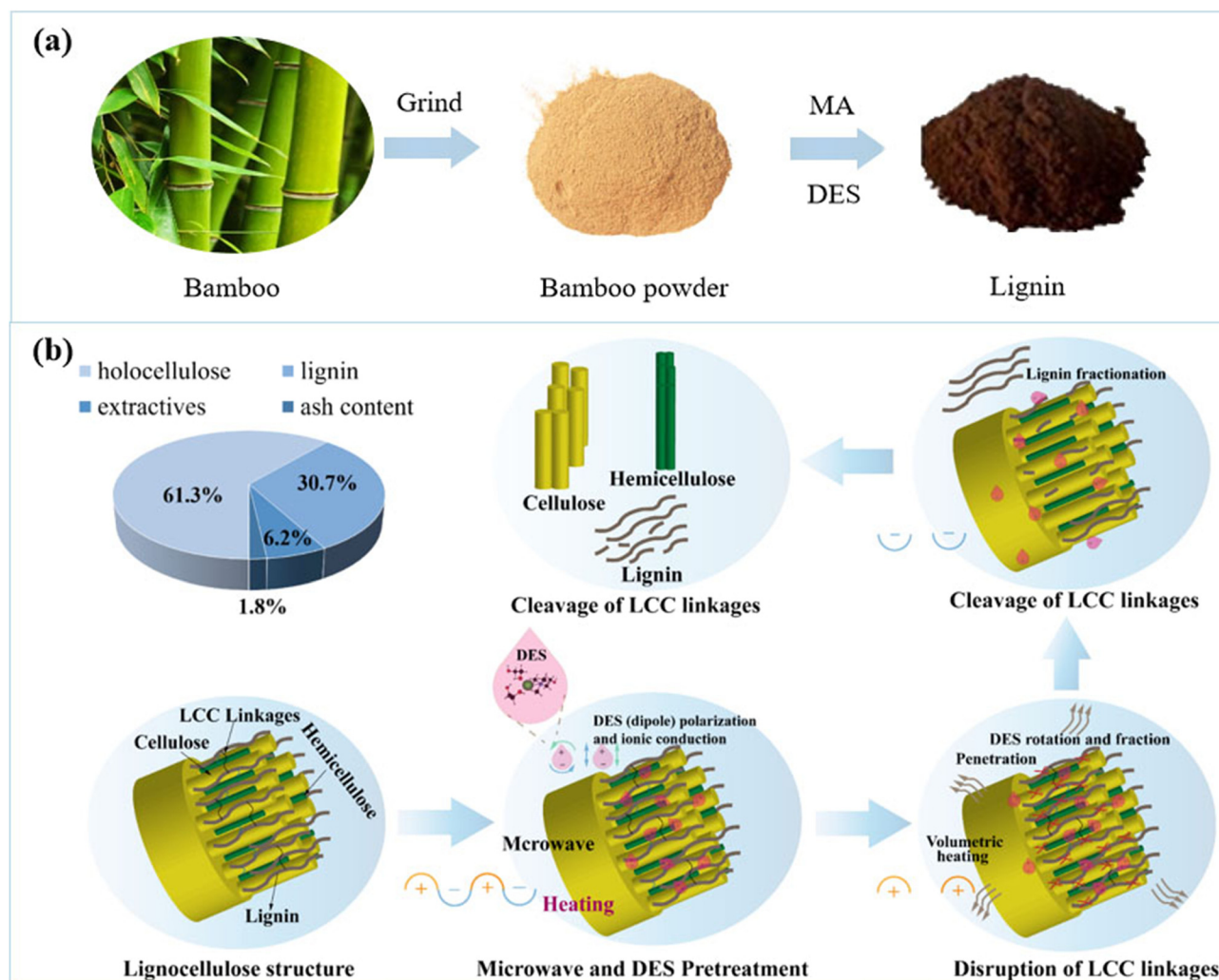


Figure 1: Schematic of (a) lignin extraction process and (b) structure and compositions changes of bamboo fiber under MA-DES.

(Figure 1b). The rapid and uniform heating with low energy loss was achieved through the interaction between microwaves and polar molecules. The electric dipoles in DES maximized ionic properties and increased the molecular polarity (Zhou et al. 2022). The movement of polar molecules could enhance the penetration of DES into bamboo fibers, providing energy for lignin solvation. The microwave heating also promoted the selective bond cleavage during lignin depolymerization, such as β -O-4 bond and $C\beta$ -O bond (Muley et al. 2019).

3.2 Microstructure and compositions of bamboo fibers

The morphological changes of bamboo fibers were observed in Figure 2a–c. The original bamboo fibers exhibited a dense and smooth surface morphology. In comparison, the treated fibers underwent tremendous changes with layered protrusions

and pores, resulting in visible cracks and rough surfaces (Figure 2d–f). This phenomenon could be attributed to the partial destruction of fibers. The partial degradation of hemicellulose and lignin occurred during MA-DES treatment. The penetration and delivery of DES into fiber cells enhanced by the microwave irradiation (Yan et al. 2009). The polar molecules under the microwave energy facilitated the fragmentation of weak bonds within bamboo fibers, including ether connections connecting lignin molecules, linkage bonds between lignin and polysaccharides (Chen et al. 2020). The surface compositions were mainly composed of cellulose, which were covered by the amorphous lignin. It confirmed that the MA-DES method were an effective approach to etch the surface amorphous layer, eventually removing the layer. It destroyed the compact fiber structure, retaining plentiful cellulose with crystalline regions. It facilitated the accessibility of DES into bamboo fibers, advantaging for enhancing lignin extraction.

The XPS spectra of bamboo fibers before and after treatment with MA-DES are shown in Figure 2g and h. The spectra of all samples showed two major peaks of C1s (binding energy (BE): 285 eV) and O1s (BE: 532 eV). The theoretical O/C values of carbohydrates are higher than that of lignin, with values of 0.83 and 0.33, respectively (Ju et al. 2013). The O/C ratio increased by 8.3 % after treatment,

reflecting the decrease in lignin concentration (Johansson et al. 1999), confirming the MA-DES treatment significantly dissolved lignin.

The high-resolution XPS spectra of C1s of bamboo fibers are presented in Figure 2i and j. The spectra were deconvoluted into four Gaussian peaks. The quantification results of C1s and O1s are shown in Table 1. For lignin, the C1s

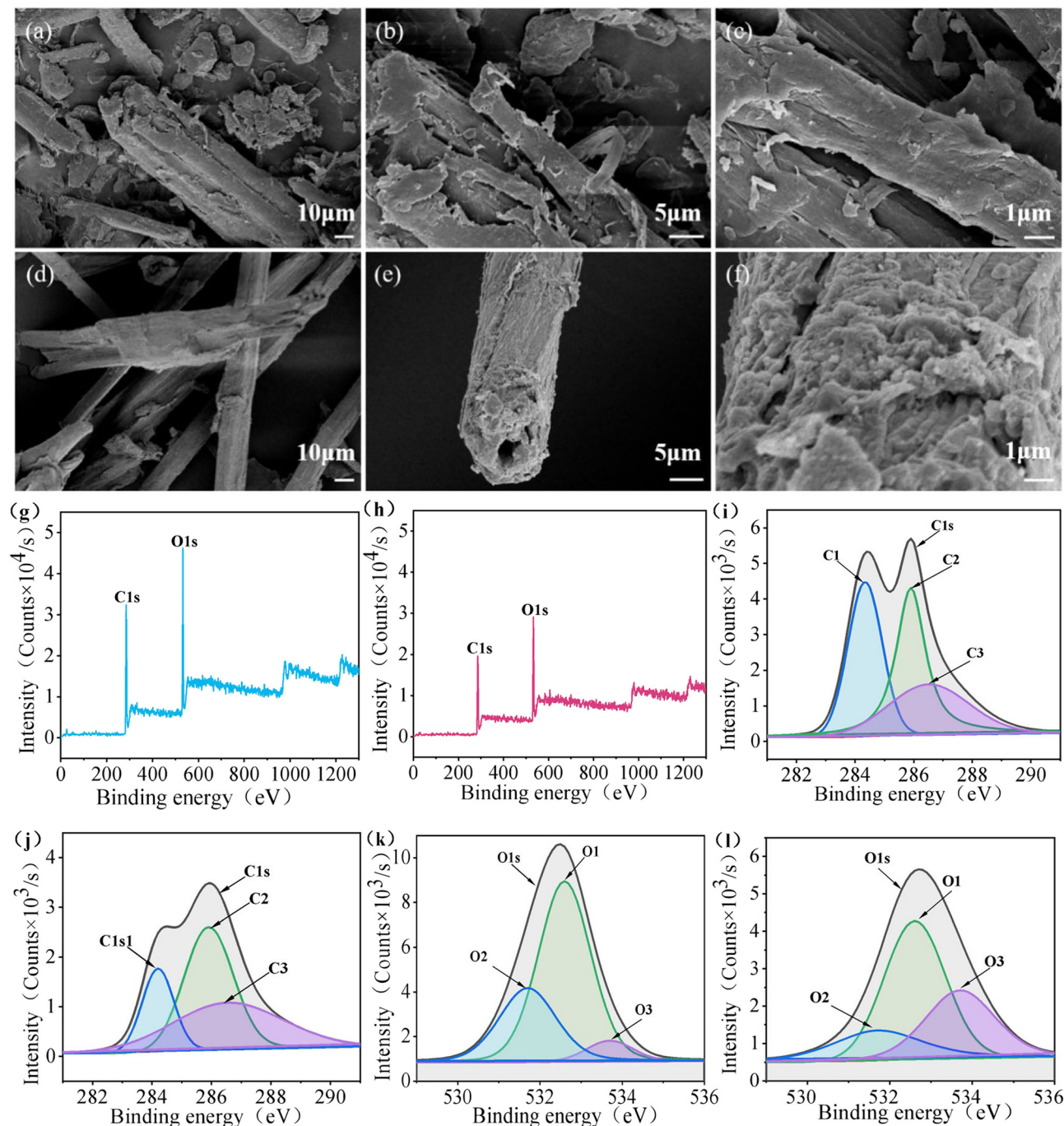


Figure 2: SEM images of (a–c) untreated bamboo fibers and (d–f) DES treated fibers; XPS spectra of (g) untreated and (h) DES treated fibers; XPS spectra of C1s for (i) untreated bamboo fiber and (j) treated fibers; XPS spectra of O1s for (k) untreated and (l) DES treated fibers.

Table 1: Surface atomic concentration of bamboo fibers treated with MA-DES method.

Samples	C1	C2	C3	O1	O2	O3
Untreated fiber (%)	36.9	35.7	27.4	65.0	28.6	6.4
DES treated fibers (%)	20.2	45.0	34.8	55.2	15.6	29.2

spectrum of the sample deconvoluted into three peaks corresponding to C1: C–C at 284.5 eV, C2: C–O/C–OC at 286.2 eV, C3: O–C–O/C=O at 287.7 eV, respectively (Arun et al. 2020; Chirila et al. 2013; Sreedhar et al. 2006). The C1 is connected to groups of hydrogen (C–H) or carbon (C–C), that are primarily aliphatic and aromatic (Wei et al. 2018a,b). The relative content of C1 decreased from 36.9 % to 20.2 %, with that of C2 increased from 35.7 to 45.0 % after DES treatment, indicating that carbon chains on fiber surface became less exposed, while hydroxyl groups become more exposed after the treatment.

The high-resolution O1s spectra of bamboo fibers are presented in Figure 2k and l. The O1 spectrum was composed of three highly intense peaks that corresponding to three oxygen chemical functional groups: O1 peak represents C=O at 531.7 eV, the O2 peak is –OH at 532.9 eV, and O3 peak is C–O at 533.7 eV (Peng et al. 2017; Wei et al. 2018a,b). The O1 peak (C=O) corresponds to lignin structure of bamboo fibers. The O3 peak (C–O) is closely related to hemicellulose and cellulose (De et al. 2020). After DES treatment, the relative content of O1 decreased from 65.0 to 55.2 %, while the O3 fraction rose from 6.4 to 29.2 %. It indicated a decreased presence of lignin in treated fibers, with an increased proportion of hemicellulose and cellulose.

3.3 Micromorphology of isolated lignin by MA-DES method

The microscopic morphology of isolated lignin from bamboo using MA-DES approach is shown in Figure 3a–c. It can be seen that the extracted lignin showed irregular morphology. It displayed a loose, porous, and fluffy structure. The high magnifications showed an agglomeration state for interconnected particles. These particle sizes ranged from 1 to 6 μm . The similar phenomenon was found for the extracted lignin from corn cob using DES containing choline chloride and ethanolamine (Luo et al. 2021).

As shown in Figure 3d–f, the calcium lignosulfonate was smooth and uniform, with scattered and a blocky structure. It differed from the DES extracted lignin owing to the condensation reactions during extraction and preparation

processes for the ready-made calcium lignosulfonate. However, the DES facilitated the fragmentation of chemical bonds, i.g., ester and ether bonds between hemicellulose and lignin. The rigid structure between lignin and hemicellulose was broken by polar molecules under the microwave energy (Yang et al. 2024), resulting in different apparent morphology of lignin.

3.4 Crystal structure of bamboo fibers after MA-DES treatment

The crystal structure of bamboo fibers treated with MA-DES was assessed through XRD technique (Figure 3g). It exhibited a typical type I cellulose crystalline for treated bamboo fibers, closely resembling that of untreated fibers. The peaks observed at 15.6°, 22°, and 34.5° corresponded to the (110), (200), and (004) crystalline planes of cellulose, respectively (Segal et al. 1959). After MA-DES treatment, the intensities of diffraction peaks were obviously increased, indicating the change of relative crystal content in bamboo fibers. The untreated bamboo fiber showed a C_rI at 25.7 %, while it was increased to 46.1 % upon MA-DES treatment. It could be attributed to the removal of amorphous hemicellulose and lignin, resulting in an increased content of crystal cellulose (Liu et al. 2024).

3.5 FTIR analysis of isolated lignin and bamboo fibers

The changes of chemical groups in bamboo fiber were analyzed by FTIR spectroscopy (Figure 3h). The major absorption peaks were observed at 3,400 cm^{-1} , 1,740 cm^{-1} , 1,510 cm^{-1} , 1,375 cm^{-1} , 1,250 cm^{-1} , 1,162 cm^{-1} , and 898 cm^{-1} . The broad spectrum (3,000–3,600 cm^{-1}) corresponds to the stretching vibration of OH bond in polysaccharides (Amnuaycheewa et al. 2016). The absorption peak at 3,400 cm^{-1} shifted towards higher wave numbers after formic acid treatment, indicating the increase in surface hydroxyl groups and the reduction in internal hydrogen bonds. The peak at 1,740 cm^{-1} was attributed to ester bonds, such as the acetate in hemicellulose and γ -acetate in lignin (Kim and Ralph 2010). The absorption peaks at 1,250 cm^{-1} and 1,375 cm^{-1} were corresponded to the C–O and C–H stretching between lignin and hemicelluloses, respectively (Hou et al. 2012). The above peaks were obviously decreased in the intensity, indicating that most hemicellulose were removed. The peaks between 1,423 and 1,655 cm^{-1} were attributed to typical aromatic skeletal vibrations (Guo et al. 2022). Notably, it showed that the intensity of absorption

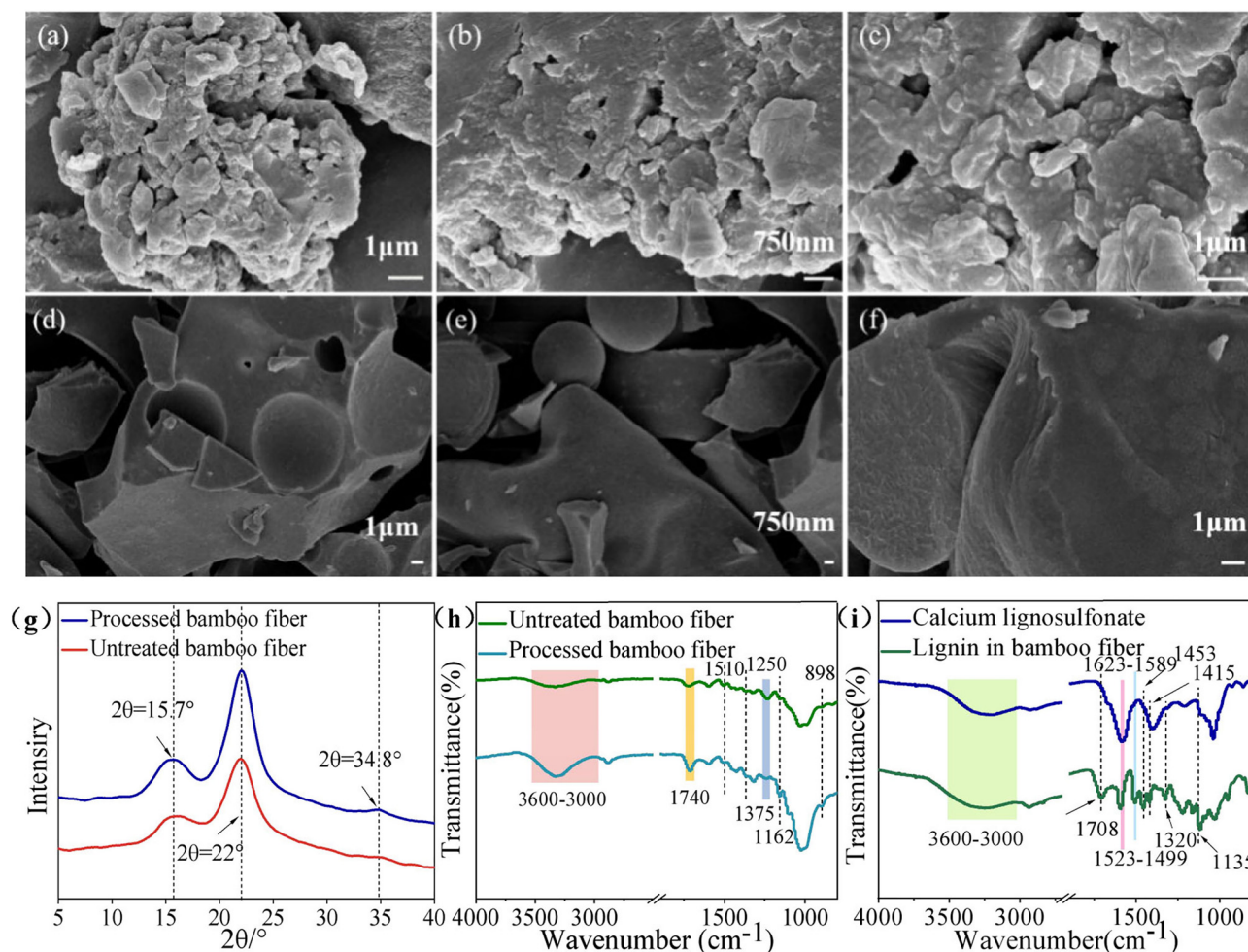


Figure 3: SEM images of (a–c) MA-DES isolated lignin and (d–f) calcium lignosulfonate; (g) X-ray diffraction spectra of bamboo fibers; (h) FTIR spectra of bamboo fibers before and after treatment; (i) FTIR spectra of lignosulfonate and isolated lignin from bamboo fibers.

peak at $1,510\text{ cm}^{-1}$ was evidently decreased after DES treatment, indicating that most lignin molecules were removed. The typical absorption peaks at $1,162\text{ cm}^{-1}$ and 898 cm^{-1} were corresponded to the ether bond stretching vibration and glycosidic linkages related to carbonyl linkage, respectively. Evidently, the representative functional groups of cellulose and hemicellulose were exhibited in the treated sample spectrum.

To monitor the compositions of extract, the isolated lignin were further analyzed (Figure 3i). The peak appeared at $1,708\text{ cm}^{-1}$ was corresponded to C=O stretching vibration in non-conjugated ketones, esters, and carbonyls (Remy et al. 2023). The peak at $1,623\text{ cm}^{-1}$ assigned to the conjugated carbonyl C=O stretching vibration. These peaks at $1,589\text{ cm}^{-1}$, $1,499\text{ cm}^{-1}$, $1,453\text{ cm}^{-1}$, and $1,415\text{ cm}^{-1}$ were attributed to the vibration of aromatic rings of lignin in the extracted products. The characteristic peak appeared at $1,320\text{ cm}^{-1}$ and $1,135\text{ cm}^{-1}$ corresponding to the C–O and C–H group in lignin,

respectively. Therefore, it is well demonstrated the existence of lignin in isolated extracts.

3.6 Thermal property of isolated lignin and bamboo fibers

The thermal stability of bamboo fibers treated with MA-DES were studied by TGA (Figure 4a and b). The first stage of mass loss occurred between 30 and 105°C was due to the water evaporation. The second stage at $200\text{--}600^\circ\text{C}$ was corresponding to the decomposition of lignocellulose. Typically, ascribed to the presence of acetyl groups, the decomposition temperature of hemicellulose ($200\text{--}300^\circ\text{C}$) was lower than that of lignin ($200\text{--}600^\circ\text{C}$) and cellulose ($275\text{--}400^\circ\text{C}$) (Ullah et al. 2019). The DTG peak between 296 and 330°C was appeared in untreated bamboo fibers, and but not detected in the treated fibers, which was mainly ascribed to the

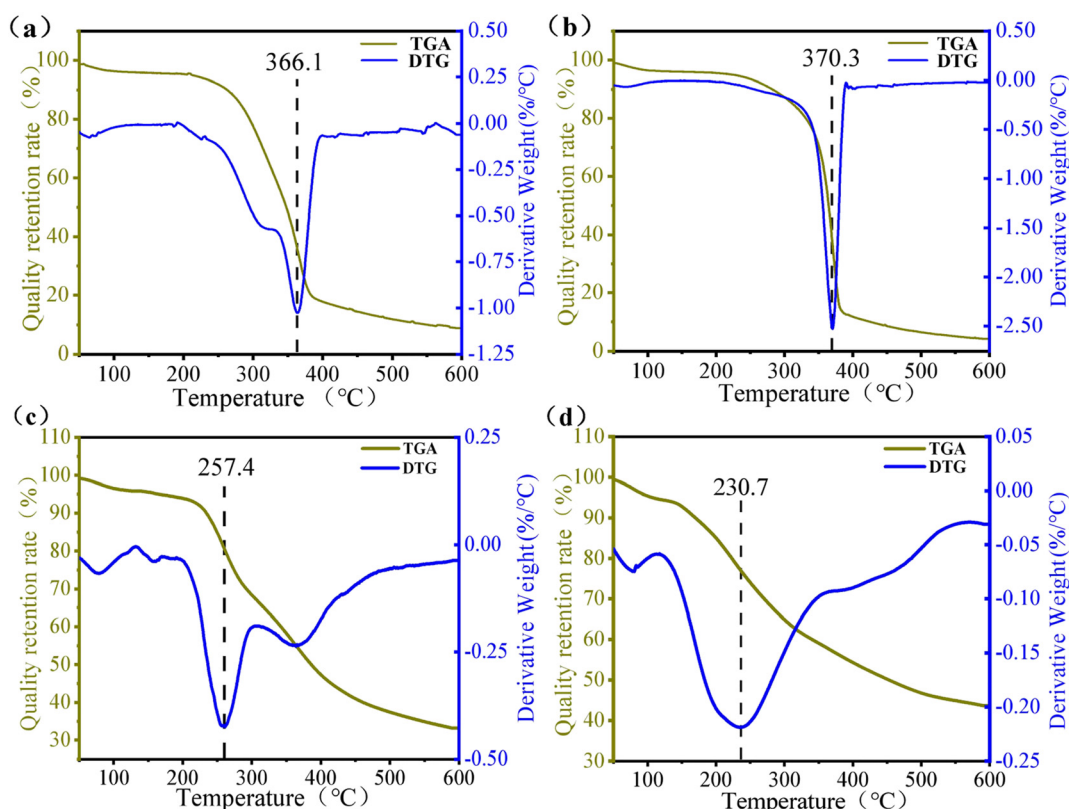


Figure 4: TGA and DTG curves (a) untreated and (b) DES treated bamboo fibers; TGA and DTG curves of (c) isolated lignin and (d) calcium lignosulfonate.

selective cleavage of β -O-4 and $C\beta$ -O bonds (Ma et al. 2024). Compared to untreated fibers, the treated fibers were increased in the maximum degradation rate, indicating that the volatiles derived from pyrolysis were easily released.

The TGA curves of isolated lignin and lignosulfonate were shown in Figure 4c and d. They exhibited three major degradation stages: below 150 °C (stage I), 150–600 °C (stage II), above 600 °C (stage III). In stage I, the weight loss was attributed to the evaporation of moisture and small molecular impurities (Li et al. 2022). The enormous degradation of isolated lignin occurred in stage II, generating substantial volatiles. The most weight loss peak occurred in 150–400 °C, which was ascribed to the fracture and degradation of aromatic skeleton (Zhang et al. 2012). The maximum degradation rate were at 257.4 °C and 230.7 °C, respectively, for isolated lignin and lignin sulfonate. As the temperature above 600 °C (stage III), the bio-char became the primary product rather than volatiles. It was worth noted that the isolated lignin showed lower residue content (33.2 %) compared that (43.6 %) of lignin sulfonate, indicating that DES treatment helped to inhibit the formation of coke from lignin pyrolysis.

3.7 Optimization process parameters for maximizing lignin yield

As illustrated in Figure 5a, the choline chloride/formic acid (ChFA), choline chloride/oxalic acid (ChOA), and choline chloride/urea (ChU) were synthesized, respectively, for the lignin extraction from bamboo fibers. The ChFA showed the best lignin yield at 54.2 %, followed by ChOA at 44.9 % and by ChU at 35.0 % (Figure 5b). The lignin yield was increased with the reduction in the molar ratio of ChFA (Figure 5c). As the molar ratio changed from 2:1 to 1:6, the lignin yield improved from 44.5 to 78.0 %, indicating that a small ratio of ChCl played an essential role in the removal of lignin (Hong et al. 2020). It was due to the increase of acid dosage enhancing the cracking of lignin from bamboo fibers.

The effect of MA time on the lignin yield was shown in Figure 5d. The lignin rate increased with reaction time in 5–10 min. The extension of treatment time improved the accessibility of DES into bamboo fibers. The lignin yield reached 54.2 % in 10 min. The prolonged treatment time could result in the condensation of lignin fragment on the fiber surface and reduce the yield (Wang et al. 2017). The

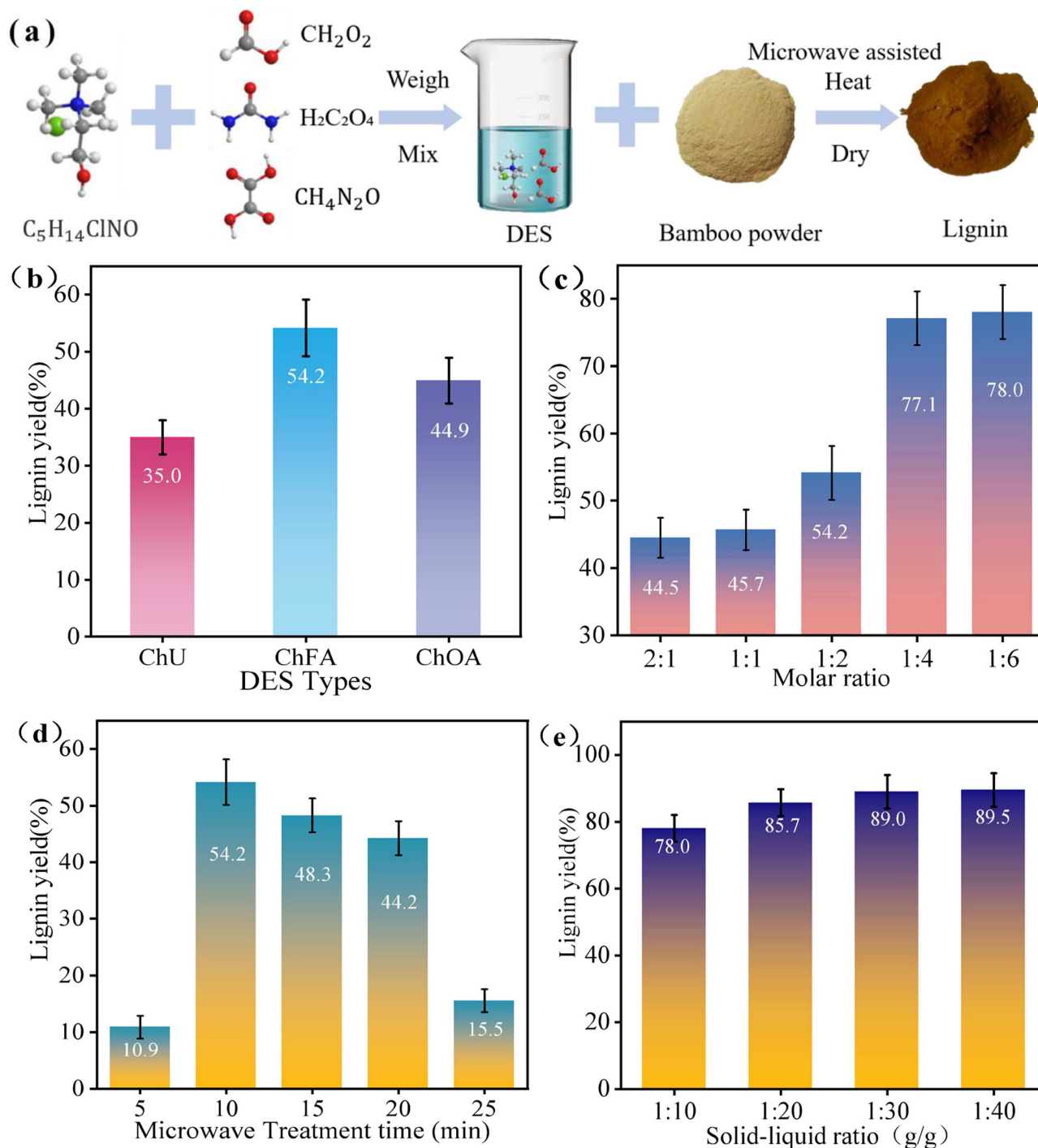


Figure 5: Lignin extraction and yield: (a) process diagram; effects (b) DES types, ChFA molar ratios, (d) MA treatment time, and (e) solid-liquid on the lignin yield rate.

reaction time continued to be prolonged would also degrade cellulose (Zhong et al. 2021).

Figure 5e showed the change of lignin yield with solid-liquid ratio of bamboo fiber to ChFA. The reduction of solid-liquid ratio slightly increased the lignin yield. This is mainly due to that as the increased amount of liquid promoted the

contact of bamboo fibers with DES. As the solid-liquid ratio changed from 1:20 to 1:40, the lignin yield only showed 3.8 % in increment. Considering the solvent consumption, the solid-liquid ratio of 1:20 was considered as the optimal conditions for good lignin extraction rate (85.7 %). Under the same conditions, the lignin rate was only 11.6 % for the

control group. Thus, it was concluded that the MA-DES method has advantages in fast extraction of high-yield lignin from bamboo fibers.

3.8 The superiority of MA-DES extraction of lignin

In bamboo fibers, the lignin binds together with hemicellulose and cellulose (Figure 6a). The fiber cell wall is hardened through a network formed by the interconnection between hemicellulose and cellulose, in which lignin acts as a filler (Yang et al. 2020). The microwave energy causes the movement of polar molecules during the MA-DES treatment, affecting the permeability of DES. The DES can be able to cleavage the ether bonds between the phenylpropene units in lignin by the acid-base catalysis (Chourasia et al. 2021). The DES is selective for the cleavage of aryl-ether bonds catalyzed by hydrochloric acid.

As shown in Figure 6b, the dried bamboo fibers were uniformly mixed with DES and subjected to the MA treatment. After completion, the mixture was filtered for solid-liquid separation. The solid obtained was a cellulose-rich

solid. The liquid was a mixture of lignin and DES. The liquid was added into deionized water to precipitate the regenerated lignin, which was then separated from DES. The processed bamboo fibers were washed and dried. The obtained cellulose-rich solid contained 84.5 % of total holocellulose and 4.5 % of lignin, demonstrating that the lignin was obviously decreased after MA-DES processing.

Figure 6c shows that the DES as a new generation of ecological solvents is low-cost, sustainable, and highly selective in lignin extraction. It can destroy the macromolecular structure and cleave the linkages between carbohydrates and lignin. It ultimately achieves the dissolution of lignin (Dharmaraja et al. 2022). In addition, DES has the advantages of nontoxicity, low volatility, and biodegradability. Hence, the usage of DES has emerged as a new strategy for biomass refining.

In bamboo treatment process, the usage of microwaves can facilitate the transformation of electromagnetic energy into thermal energy. It reduced the process time and energy consumption (Figure 6d). The DES has high viscosity, causing the heat and mass transfer limitation (Patil and Rathod 2023), which can be addressed by microwave assistance. In comparison with conventional heating ways, the microwave way

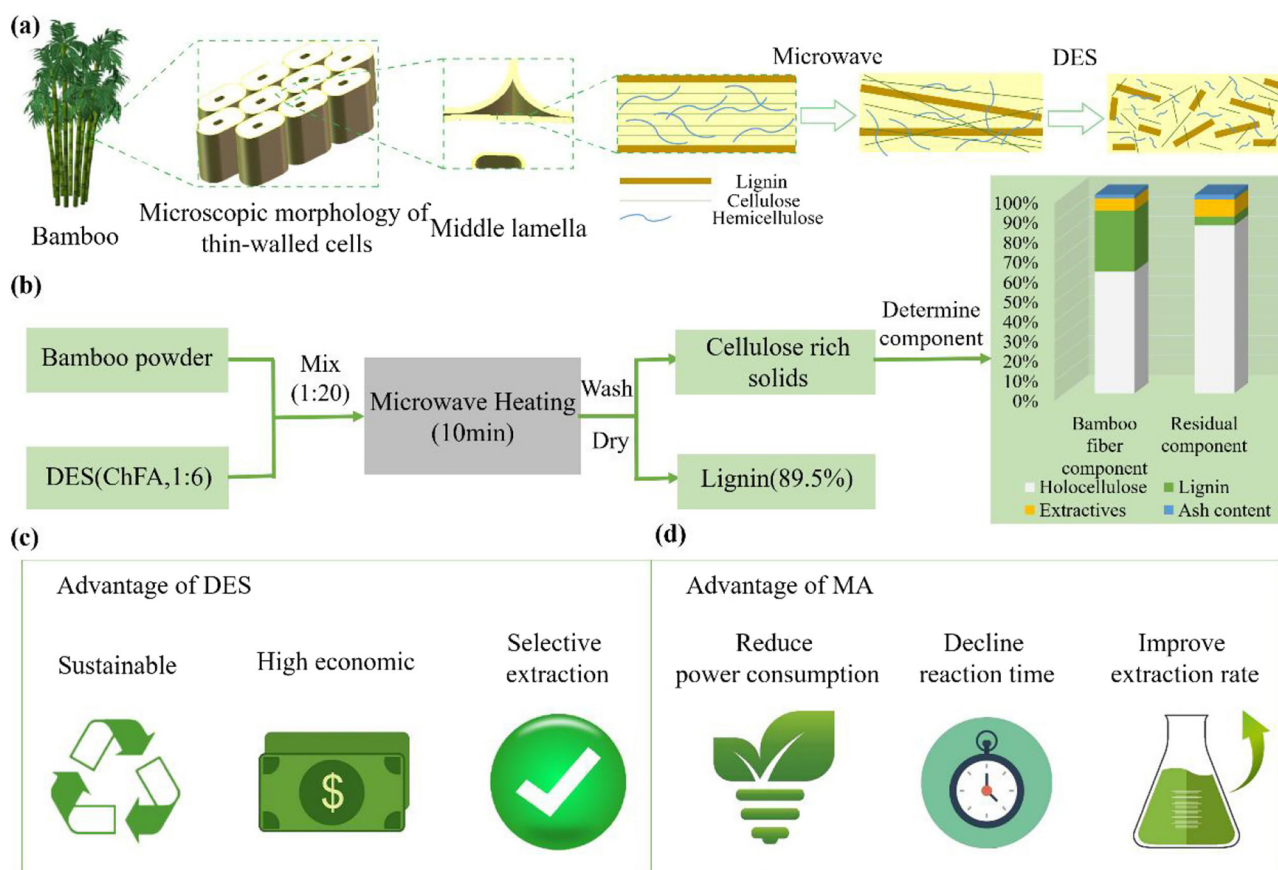


Figure 6: Analysis of extraction technology: (a) multiscale structure of bamboo fibers; (b) lignin extraction process; advantages of (c) DES and (d) MA.

Table 2: Different research using working conditions and their comparison this work.

Author(s)	Working conditions				The lignin yield (%)
	Used reagent	Feed stock	Heating method	Time (min)	
Li et al. (2012)	Organic acid aqueous solution	Bamboo stem	Microwave-assisted	60	18.0
Zhang et al. (2024)	DES (glycerin/choline chloride)	Poplar powder	Water-bath heating	120–240	49.0
Das and Mohanty (2023)	Solution of acidic aqueous ethanol	Bamboo powder	Ultrasonic bath	55	68.2
Xu et al. (2025)	DES (choline chloride/lactic acid)	Spruce heartwood	Microwave-assisted	2	80.0
Zhou et al. (2024)	L-cysteine/lactic acid	Sugarcane bagasse	Sand-bath heating	360	82.6
Bécsy-Jakab et al. (2024)	NaOH	Corn stover	Oil-bath heating	60	84.0
Balasubramanian and Venkatachalam (2023)	DES (choline chloride/toluene sulfonic acid)	Rice husk	Direct heating	60	87.8
This work	DES (choline chloride/formic acid)	Bamboo powder	Microwave-assisted	10	89.5

can make water molecules acquire a boost in reactivity. Its high kinetic energy accelerates the mass transferring rate (Luo et al. 2017). Researches demonstrated that the MA could enhance the isolation of compounds from biomass (Mustapa et al. 2015). It facilitated the interaction between microwave energy and polar biomass, thereby improving the lignin extraction.

Table 2 shows the comparisons on lignin yield for different extraction methods. Zhang et al. (2024) spent 2–4 h obtaining 49 % of lignin by mixing the sifted poplar powder with DES (i.e., glycerin and choline chloride combined in a molar ratio of 2:1). Bécsy-Jakab et al. (2024) used 1 h getting 84 % of lignin from air-dried cake by sodium hydroxide method. By comparison, it can be seen that the MA-DES method not only shortened the reaction time (10 min) but also improve the lignin rate (89.5 %). It has also received widespread attention in the rapid and efficient extraction of lignin, exhibiting broad prospects.

The lignin can be used for valuable products, e.g., activated carbon, adhesives, and packaging materials. Different separation methods affects not only lignin yield, but also structural and chemical properties (Ma et al. 2023). The surface functional groups influence the adsorption capacity of lignin (Guo et al. 2023). The lignin-derived active carbon can be used for efficiently remove dye molecules, organic pollutants, heavy metal ions, and other impurities from wastewater. The extracted lignin with benzene rings can be used as a phenol substitute in adhesives, such as soybased adhesives (Sivasankarapillai et al. 2019). The lignin is rich in groups including phenolic units and ketones, enlarging applications for UV shielding. It makes lignin as excellent

biopolymers for packaging with strong water resistance and UV blocking (Ma et al. 2022).

4 Conclusions

A microwave-assisted deep eutectic solvent (MA-DES) method was developed for the efficient extraction of lignin from lignocellulose. It confirmed that the microwave heating were an effective approach to destroy the compact fiber structure, facilitating the DES accessibility into bamboo fibers, advantaging for enhancing lignin extraction. The DES displayed a considerable selectivity removal for lignin, while retained a large amount of cellulose. The processed bamboo fibers underwent changes compared to untreated ones, including decreased thermal stability, loose and porous surface, and increased proportion of cellulose by structural characterization. The influence of various treatment parameters (i.e., DES types, MA time, molar ratio and solid-liquid ratio) on the extraction yield of lignin were meticulously examined. The optimum yield of 89.5 % lignin was achieved by ChFA at 10 min, 1:6 (molar ratio) and 1:40 (solid-liquid ratio), demonstrating efficient lignin extraction achieved by adjusting reaction time, molar ratio, and solid-liquid ratio. By comparison, the MA-DES method not only shortened the reaction time but also improve the lignin extraction rate. The aforesaid advantageous effects possessed by the MA-DES method made the same a strong candidate for future large-scale, low-cost production of lignin and open new avenues for efficient lignin utilization.

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