Unlocking the Biofuel Potential of Tamarind Shells through Deep Eutectic Solvent Pretreatment

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Abstract. Lignocellulosic biomass is a promising feedstock for bioethanol production, but its recalcitrant structure requires efficient pretreatment to improve sugar release. This study valorized tamarind shells, an abundant agricultural residue, for bioethanol production through pretreatment with two deep eutectic solvents (DESs): a ternary DES containing choline chloride, ethylene glycol, and p-toluenesulfonic acid, and a binary DES containing urea and monoethanolamine. Pretreatment significantly modified biomass composition, with the ternary DES removing hemicellulose completely, while the binary DES reduced lignin and partially solubilized hemicellulose. Enzymatic saccharification increased reducing sugar yields from 31.80 mg/g in untreated shells to 183.94 mg/g and 170.50 mg/g for ternary and binary DES-pretreated biomass, respectively. Fermentation of the hydrolysates produced ethanol concentrations of 91.48 mg/g for the ternary DES, and 85.76 mg/g for the binary DES, representing 6.9- and 6.4-folds increase over untreated shells (13.34 mg/g). These results demonstrate that DES pretreatment effectively enhances sugar release and ethanol production, highlighting tamarind shells as a sustainable feedstock for bioethanol.

1 Introduction

The global population continues to grow rapidly, driving a parallel rise in energy demand, while traditional energy resources are being depleted [1]. The extensive use of these resources has also led to severe environmental consequences, including increased greenhouse gas emissions, air pollution, global warming, and ecosystem degradation [2]. These impacts have

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strengthened global efforts to expand renewable energy solutions, such as water-driven electricity, sunlight-based power, and fuels derived from biological feedstocks [3].

Amid this shift, biomass-based processing systems, known as integrated biomass conversion industries, are emerging as a core pillar of the sustainable bioeconomy [4]. These systems enable the transformation of plant-derived materials into multiple product streams, including clean energy, industrial chemicals, and bio-based materials, while further reducing dependence on petroleum feedstocks [5]. The adoption of such integrated models increases the efficient use of renewable biological resources and supports the transition toward sustainable industrial production [6]. In agricultural nations, large-scale crop production generates substantial quantities of lignocellulosic biomass (LCB), offering a valuable, underutilized resource for clean fuel production rather than conventional waste disposal or open field burning [7].

Among bio-based fuels, ethanol produced through microbial fermentation has become one of the most widely adopted renewable transport fuels, particularly when derived from plant structural biomass [8]. Industrial fuel production commonly relies on the yeast microbe, a globally established fermentation catalyst, to convert extracted glucose into ethanol [9]. However, the rigid and resistant structure of plant fibers limits direct sugar accessibility in LCB, requiring a pretreatment step to enhance digestibility and improve fermentable sugar release [8].

For the pretreatment of LCB, deep eutectic solvents (DESs) have recently gained attention as efficient pretreatment agents due to their unique properties [10]. Compared to conventional solvents, DES offer advantages such as low cost, excellent recyclability, non-volatility, low toxicity, and high biodegradability, making them highly suitable for applications in biomass pretreatment, catalysis, and separation processes [9]. DES are synthesized by combining a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) at moderate temperatures under continuous stirring. The flexibility to select different HBDs and HBAs endows DES with versatile and customizable properties, allowing them to be tailored for specific applications such as cellulose dissolution, delignification, or hemicellulose extraction [10].

Among various agricultural residues, tamarind shells represent a promising feedstock for bioethanol production. The tamarind plant (*Tamarindus indica* L.) is a multipurpose species whose roots, wood, leaves, fruits, and seeds are utilized in food, pharmaceuticals, herbal medicine, fuel, timber, fodder, and textile industries [11]. During fruit processing, seed and shell residues are generated as by-products. Tamarind shells are rich in cellulose and contain smaller amounts of hemicellulose, making them suitable substrates for producing fermentable sugars [12]. However, their substantial lignin content, a complex and hydrophobic polymer, forms a rigid structure that hinders enzymatic hydrolysis and limits direct utilization [9].

Although tamarind shells have been investigated for applications such as nanoparticle synthesis, mineral extraction, and as additives to enhance material properties, their potential for bioethanol production remains unexplored [11], [13]. The objective of this study is to characterize the composition of tamarind shells and to valorize them for bioethanol production through DES pretreatment. This pretreatment is expected to reduce the recalcitrant structure of tamarind shells, enhancing the release of fermentable sugars compared to untreated samples. Accordingly, this study will compare the sugar yield and ethanol yield of two different DES-pretreated tamarind shell samples with those of untreated shells.

2 Materials and methods

2.1 Materials and reagents

In this study, tamarind shells were sourced from the waste collector site of a local vendor in Thailand. The shells were ground using a food processor to obtain a particle size of approximately 1.5–2 mm, after which the material was sieved through a 2 mm mesh. The chemicals used included choline chloride (Loba Chemicals, 98%), ethylene glycol (DEAJUNG Chemicals and Metals, ≥ 99%), p-toluenesulfonic acid (CARLO ERBA Reagents), urea (KEMAUS), and monoethanolamine (Sigma-Aldrich, ≥ 99%). All additional reagents applied in the experiments were of analytical grade.

2.2 Deep eutectic solvent (DES) preparation

In this study, two deep eutectic solvents (DESs) were synthesized. The first DES consisted of choline chloride (ChCl) as the hydrogen bond acceptor (HBA) and ethylene glycol (EG) with p-toluenesulfonic acid (pTSA) as the hydrogen bond donors (HBDs). The second DES was prepared using urea (U) as the HBA and monoethanolamine (MEA) as the HBD. The ternary DES, ChCl: EG: pTSA, was formulated in a 1:2:1 molar ratio, while the binary DES, U: MEA, was prepared in a 1:2 molar ratio. In both cases, the components were mixed according to the specified molar ratios and heated at 80 °C with continuous stirring at 200 rpm until clear, homogeneous DESs were obtained. The resulting DESs were stored in a desiccator containing silica gel until further use.

2.3 DES pretreatment of tamarind shells

Tamarind shells were pretreated with ternary DES (ChCl: EG: pTSA) and binary DES (U: MEA) at a solid-to-liquid ratio (SLR) of 1:10 w/w. The pretreatment was conducted in a hot-air oven (SH Scientific, SH-DO-100FG) preheated to the desired temperature. For the ternary DES, the reaction was carried out at 120 °C for 20 min, whereas the binary DES pretreatment was performed at 80 °C for 3 h. After completion, the reaction mixtures were allowed to cool to room temperature and subsequently separated into solid and liquid fractions. The solid residues were washed five times with 50 mL of deionized (DI) water using vacuum filtration. The washed solids were then dried overnight in a hot-air oven at 60 °C, cooled to room temperature by placing in a vacuum desiccator to minimize moisture uptake, and finally stored in sealed plastic bags for subsequent use.

2.4 Biomass composition analysis

The composition analysis of tamarind shells was performed on both untreated and pretreated samples to quantify key components, including cellulose, hemicellulose, and lignin, following standard protocols from the National Renewable Energy Laboratory (NREL). 0.3g of biomass samples were taken in triplicate and combined with 3 mL of 72% H_2SO_4 in a 100 mL pressure vessel and incubated at 30°C for 1 hour, with manual shaking for 1 min at 150 rpm every 10 min. The acid concentration was subsequently reduced to 4% by adding 84 mL of DI water, followed by autoclaving at 121°C for 1 h. After cooling to room temperature, the samples were separated into solid and liquid fractions.

The liquid fraction was split into two portions: one was used to determine acid-soluble lignin (ASL) by using spectrophotometry at 320 nm, and for the second portion, the pH was adjusted to 6–7 by adding $CaCO_3$. After pH adjustment, the supernatant was collected to be analyzed for cellulose and hemicellulose using high-pressure liquid chromatography refractive index detectors (HPLC-RID). The solid fraction was used to quantify acid-insoluble lignin (AIL). For this purpose, the solid fraction was washed twice with 50 mL of

DI water, oven-dried at 105 °C for 24 h, and then combusted in a muffle furnace at 575 °C for 6 h. The weight of the crucibles containing the solid fraction was recorded at each step to calculate the AIL [3].

2.5 Enzymatic hydrolysis

The enzymatic digestibility of cellulose was evaluated for the untreated control (tamarind shells washed with DI water), the ChCl:EG:pTSA DES-pretreated sample, and the U: MEA DES-pretreated sample. For the hydrolysis assay, 0.3 g of dried biomass was suspended in 11.99 mL of 50 mM citrate buffer (pH 4.8) in a 15 mL Falcon tube. Commercial cellulase enzyme CTec2 was added at an activity loading of 20 FPU/g biomass, corresponding to 12.4 µL of enzyme solution. The tubes were incubated at 50 °C under continuous shaking at 150 rpm for 72 h in a shaking incubator (JSR Inc., JSSI-100). Upon completion, hydrolysis was terminated by heating the reaction mixture in a 95 °C water bath for 10 min. The liquid hydrolysate was separated from the residual solids by centrifugation at 6,000 rpm for 10 min, and the supernatant was carefully collected. The released reducing sugars were quantified using the 3,5-dinitrosalicylic acid (DNS) assay, following Miller's method, with glucose employed as the calibration standard [14]. All measurements were performed in triplicate, and the results are presented as mean standard deviation in all the figures and tables. The complete experimental procedure of this study is summarized in Figure 1.

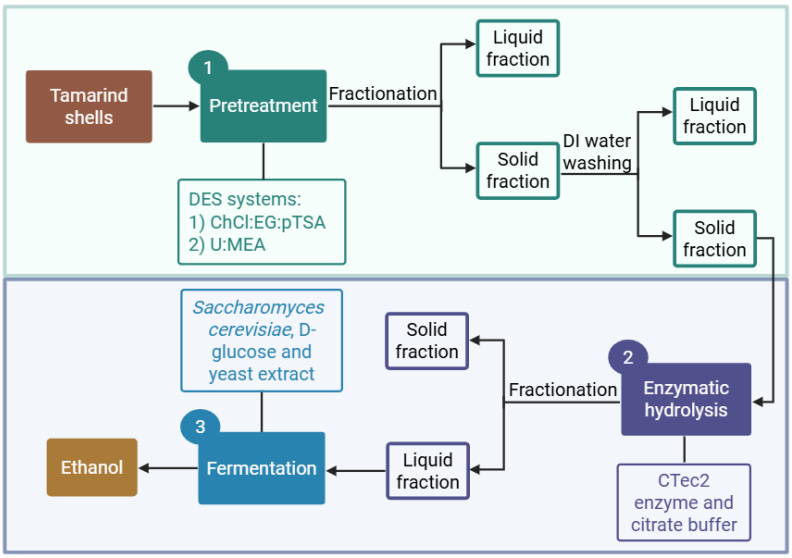

Fig. 1. Process flow of valorization of tamarind shells to ethanol

2.6 Fermentation of hydrolysate

After enzymatic hydrolysis, the collected hydrolysate was fermented using *Saccharomyces cerevisiae* to assess ethanol production. The fermentation medium with pH 5.0 contained 1% w/v D-glucose, 1% w/v yeast extract, and 5 mL of the hydrolysate, and was inoculated with 263 µL of 5% yeast culture. Positive and negative controls were also prepared in parallel: the positive control consisted of 5 mL of yeast broth with 263 µL of yeast inoculum, while the negative control contained 5 mL of yeast broth only. Both the controls were also supplemented with 1% w/v D-glucose and 1% w/v yeast extract. All fermentation setups

were incubated at 30°C for 72 h in a rotary shaker at 150 rpm. After incubation, the fermentation broths were centrifuged at 6000 rpm for 10 min, and the supernatant was collected for ethanol quantification.

2.7 Determination of ethanol concentration

For the determination of ethanol in the fermentation broth, ethanol was first extracted by mixing 1 mL of tri-*n*-butyl phosphate (TBP) with 1 mL of the sample, followed by vortexing for 1 min to facilitate phase transfer. The mixture was then centrifuged at 6,000 rpm for 5 min, yielding two immiscible layers: a clear TBP phase (upper) and an aqueous phase (lower). Subsequently, 700 µL of the clear TBP layer was transferred into a new microtube and reacted with 700 µL of dichromate reagent (10% w/v $K_2Cr_2O_7$ prepared in 5 M H_2SO_4). The solution was vortexed for 1 min and allowed to stand at room temperature for 10 min to complete oxidation, during which the reacted lower phase turned blue-green, indicating ethanol detection. A 100 µL aliquot of the colored lower phase was collected in triplicate and diluted with 900 µL of DI water for absorbance measurement using a UV–Vis spectrophotometer (SpectraMax ABS plus, Molecular Devices, LLC., USA) at 595 nm. Ethanol concentration was determined from an external calibration curve constructed using analytical ethanol standards.

3 Results and discussion

3.1 Effect of pretreatment on tamarind shells composition

In this study, tamarind shells were first milled into fine powder and subsequently subjected to three treatments: (i) a control sample involving washing with DI water, (ii) pretreatment using a ternary DES composed of ChCl:EG:pTSA (1:2:1) at 120 °C for 20 minutes, and (iii) pretreatment with a binary DES, U: MEA (1:2), at 80 °C for 3 hours. The appearance of the untreated (control) and DES-pretreated samples is shown in Figure 2.

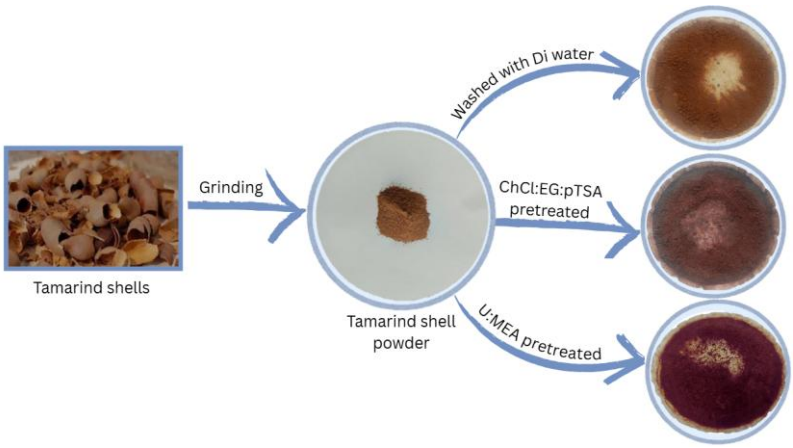

Fig. 2. Visual representation of tamarind shells after washing with DI water and pretreatment with DESs

Following pretreatment, both untreated and pretreated samples underwent compositional analysis. The recovery rates and compositional analysis of the samples indicate the impact of pretreatment on biomass fractionation, as shown in Table 1. The untreated sample exhibited

the highest recovery rate (79.71%) and contained 30.24% cellulose, 8.48% hemicellulose, and 33.79% lignin. This reflects the native structure of the biomass, with most of the components retained.

Table 1. Composition analysis of the biomass

Samples	Recovery rate (%)	Dry biomass composition(%)		
		Cellulose	Hemicellulose	Lignin
Untreated	79.71	30.24±0.9	8.48±1.9	33.79±0.5
ChCl: EG: pTSA pretreated	46.13	42.23±1.9	ND	36.22±0.8
U: MEA pretreated	58.78	42.54±0.8	4.14±0.2	30.26±0.5

ND, not detected

Noticeable compositional changes occurred after DES pretreatment. Following pretreatment, the ChCl:EG:pTSA-treated sample showed a substantial reduction in recovery (46.13%), suggesting significant solubilization of biomass constituents. The ChCl:EG:pTSA DES, being acidic in nature, led to substantial hemicellulose degradation, resulting in no detectable hemicellulose (ND) in the pretreated sample. This pretreatment also increased cellulose content from 30.24% to 42.23%. The slight increase in lignin content (33.79% to 36.22%) can be attributed to lignin repolymerization, a phenomenon commonly observed during acidic pretreatment [5].

In contrast, the U: MEA-pretreated sample exhibited an intermediate recovery rate of 58.78%. The U: MEA DES, which is alkaline, facilitated partial delignification, reducing lignin content from 33.79% to 30.26%. Hemicellulose content also decreased from 8.48% to 4.14%, consistent with alkaline-induced hemicellulose solubilization. As a result of these removals, cellulose content increased significantly from 30.24% to 42.54% [15].

Compared to ChCl:EG:pTSA, U: MEA pretreatment appears milder, selectively removing hemicellulose while retaining more of the biomass, including lignin. Overall, the results demonstrate that pretreatment significantly alters the biomass composition, with ChCl: EG: pTSA providing a more aggressive fractionation and U: MEA offering a moderate, selective modification.

3.2 Effect of DES pretreatment on sugar yield

Enzymatic hydrolysis was performed on the DI-water-washed control sample and the two DES-pretreated samples (ChCl:EG:pTSA and U: MEA). Following hydrolysis, the concentration of reducing sugars in each hydrolysate was quantified and expressed as milligrams of sugar per gram of pretreated biomass. Pretreatment had a pronounced effect on sugar release from tamarind shells, as illustrated in Figure 3.

The acidic DES (ChCl:EG:pTSA) led to the greatest improvement in sugar yield, increasing from 31.80 mg/g (untreated) to 183.94 mg/g pretreated biomass. This substantial enhancement is attributed to the complete removal of hemicellulose and disruption of lignin, which together improve cellulose accessibility. Acidic conditions promote hemicellulose hydrolysis, fiber swelling, and structural loosening, enabling enzymes to hydrolyze cellulose more effectively into fermentable sugars [10]. Although some lignin repolymerization occurred, the overall structural deconstruction still resulted in markedly improved enzymatic digestibility.

The alkaline DES (U: MEA) also significantly enhanced sugar production, increasing from 31.80 mg/g to 170.50 mg/g pretreated biomass. This improvement arises from effective delignification and partial hemicellulose removal, which reduces biomass recalcitrance and creates a more open, porous structure conducive to enzyme penetration [9]. Alkaline

pretreatment is particularly efficient at cleaving ester linkages within lignin–carbohydrate complexes, and the observed reduction in lignin content directly contributed to the increased sugar yield.

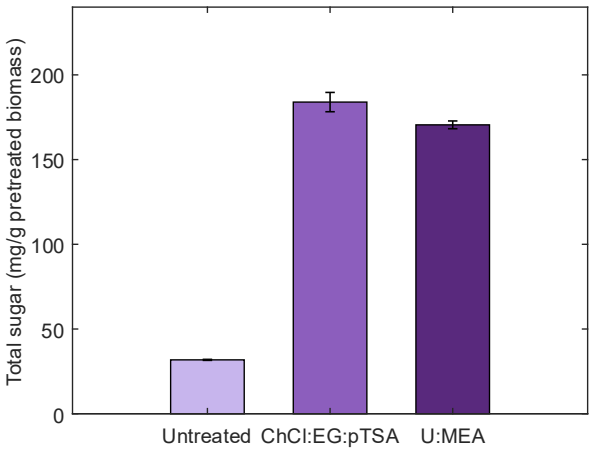

Fig. 3. Sugar yield obtained from untreated and DESs pretreated tamarind shells after enzymatic hydrolysis

3.3 Effect of DES pretreatment on ethanol yield

Following enzymatic hydrolysis, the hydrolysates were fermented, and the resulting ethanol concentrations were analyzed. As shown in Figure 4, the untreated control yielded only 13.34 mg/g of ethanol, indicating a limited availability of fermentable sugars. Pretreatment with the ternary DES (ChCl:EG:pTSA) markedly increased ethanol production to 91.48 mg/g, representing a 6.9-folds improvement over the control. Similarly, the binary DES (U: MEA) pretreatment enhanced ethanol yield to 85.76 mg/g, a 6.4-folds increase compared to the untreated sample. These findings indicate that both DES pretreatments effectively disrupted the biomass structure, facilitating greater sugar release and higher ethanol production, with ChCl:EG:pTSA showing slightly superior performance compared with U: MEA.

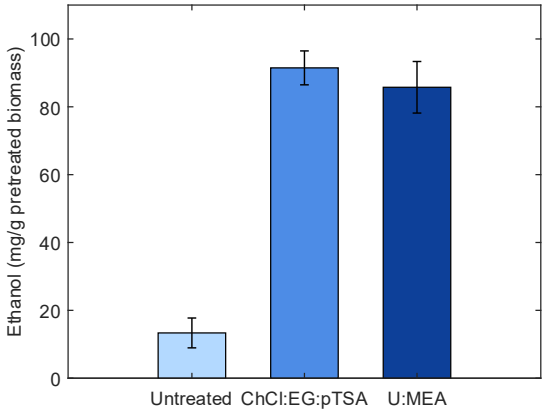

Fig. 4. Ethanol yield obtained after fermentation of the hydrolysates of untreated and pretreated tamarind shells

4 Conclusion

This study demonstrated the successful valorization of tamarind shells for bioethanol production through DES pretreatment. Both the acidic ternary DES (ChCl:EG:pTSA) and the alkaline binary DES (U: MEA) significantly altered biomass composition. ChCl:EG:pTSA completely removed hemicellulose, while U: MEA reduced lignin from 33.79% to 30.26% and hemicellulose from 8.48% to 4.14%, increasing cellulose content to 42.23% and 42.54%, respectively. These compositional changes enhanced enzymatic digestibility, raising reducing sugar yields from 31.80 mg/g in untreated shells to 183.94 mg/g and 170.50 mg/g for ChCl:EG:pTSA and U: MEA-pretreated biomass. Subsequent fermentation confirmed substantial improvements in ethanol production, increasing from 13.34 mg/g (untreated) to 91.48 mg/g with ChCl:EG:pTSA and 85.76 mg/g with U: MEA. Future research should focus on the scalability of DES pretreatment, including solvent recyclability and techno-economic feasibility. Additionally, exploring the valorization of tamarind shells into diverse products and the application of synergistic pretreatment strategies could further enhance their potential for sustainable biorefinery applications.

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