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Analytical assessment of a $ZnCl_2$–Urea deep eutectic solvent for fruit and vegetable waste biorefining: synergistic catalysis, fractionation efficiency, and bioethanol yield enhancement

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Abstract

The global surge in fruit and vegetable (F&V) waste represents a critical challenge, necessitating innovative valorization strategies aligned with the circular bioeconomy. This study developed a novel, low-cost Type IV Deep Eutectic Solvent (DES) composed of zinc chloride and urea ($ZnCl_2$:Urea, 1:4 molar ratio) for the pretreatment of a blended lignocellulosic biomass from banana peel, lemon peel, and cabbage. The synthesized DES was thoroughly characterized using FTIR, NMR, TGA, and physicochemical analyses, confirming its formation via an extensive hydrogen-bonding network and high thermal stability (decomposition onset: 195 °C). The DES pretreatment under mild conditions (80 °C, 5 h) resulted in significant biomass fractionation, achieving 71.9% delignification and a 44.9% reduction in cellulose crystallinity index. This multi-scale deconstruction led to a high enzymatic digestibility, yielding 85.2% glucose after 72 hours. Subsequent fermentation of the hydrolysate, which contained negligible fermentation inhibitors (furfural < 0.1 g/L, HMF < 0.05 g/L), yielded 42.8 g/L of bioethanol with 84% fermentation efficiency. Comparative analysis with conventional 1% H_2SO_4 pretreatment revealed that the DES process, while marginally lower in ethanol titer, offered superior environmental benefits, including minimal inhibitor formation, reduced corrosivity, and excellent solvent recyclability over five consecutive cycles. Collectively, the results demonstrate that $ZnCl_2$:Urea DES is a highly effective, sustainable, and economically viable pretreatment agent, paving the way for its application in advanced biorefineries for F&V waste management and biofuel production.

1. Introduction

The 21st century is characterized by converging crises in waste management, resource depletion, and climate change. The global food system, particularly the fruit and vegetable (F&V) sector, plays a major role in this challenge, with approximately 45% of total production lost as waste [1]. This loss not only poses significant disposal challenges but also represents a substantial waste of embedded resources, including water, land, and energy, while contributing markedly to greenhouse gas emissions from landfill decomposition [2,3]. In agricultural economies such as Nigeria, post-harvest losses are especially severe, underscoring the urgent need for decentralized, cost-effective valorization technologies to mitigate these impacts [4].

The complex lignocellulosic structure of F&V waste, primarily composed of cellulose, hemicellulose, and lignin, positions it as an ideal feedstock for biorefineries [5,6]. However, its inherent recalcitrance necessitates an effective pretreatment step to disrupt the lignin seal, reduce cellulose crystallinity, and increase porosity, making carbohydrates accessible for enzymatic hydrolysis [7,8]. Conventional pretreatment methods (e.g., dilute acid, alkaline) often suffer from significant drawbacks, including high energy consumption, the generation of fermentation inhibitors, equipment corrosion, and environmental pollution from chemical waste streams [9,10]. Therefore, the development of a novel, efficient, and environmentally benign pretreatment technology is paramount for the commercial viability of lignocellulosic biorefineries.

Deep Eutectic Solvents (DESs) have emerged as a revolutionary class of solvents that address many limitations of conventional methods. First reported by Abbott et al. [11], DESs are eutectic mixtures formed between a Hydrogen Bond Acceptor (HBA) and a Hydrogen Bond Donor (HBD). This work focuses on Type IV DESs, composed of a metal salt (e.g., $ZnCl_2$) and an HBD (e.g., Urea), which are particularly attractive due to their very low cost, ease of synthesis, and tunable properties [12]. DESs have demonstrated a remarkable ability to selectively fractionate biomass, solubilizing lignin and hemicellulose while leaving a cellulose-rich solid residue, an approach highly desirable for integrated biorefining [13,14].

While the application of Choline Chloride-based DESs (Type III) in biomass pretreatment has been widely explored, the potential of non-cholinium, Type IV DESs, specifically $ZnCl_2$:Urea, for treating complex, multicomponent F&V waste streams remains relatively underexplored. The synergistic effect of Lewis acidic Zn^{2+} ions, known to catalyze bond cleavage in lignin-carbohydrate complexes, and urea, a potent hydrogen-bond disruptor, presents a compelling mechanistic hypothesis for enhanced pretreatment efficacy. Furthermore, comprehensive studies linking the physicochemical properties of this specific DES to its performance on real-world, mixed-waste feedstocks are scarce.

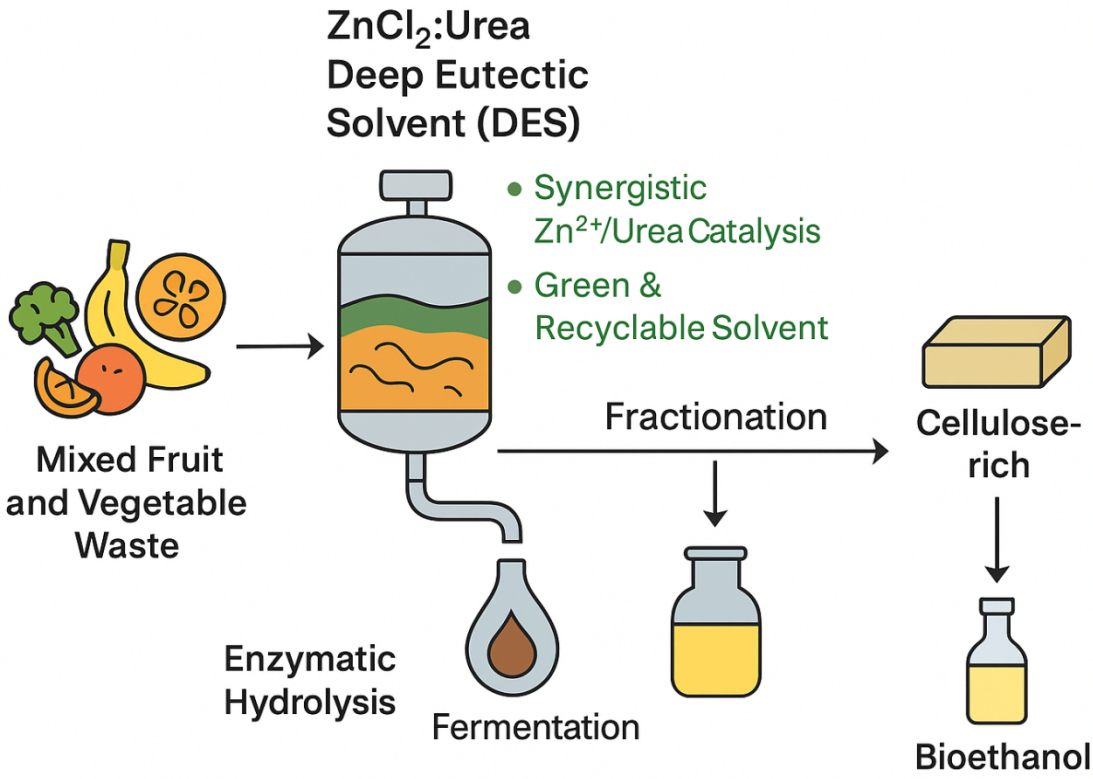

Figure 1. Integrated biorefinery schematic showing $ZnCl_2$:Urea DES pretreatment of fruit and vegetable waste and subsequent conversion of cellulose to bioethanol with lignin valorization.

The overarching aim of this research is to develop and validate a green and efficient pretreatment strategy for F&V waste valorization using a novel $ZnCl_2$:Urea DES to facilitate high-yield bioethanol production. The specific objectives are: (1) To synthesize and comprehensively characterize a Type IV DES from Zinc Chloride and Urea; (2) To evaluate its pretreatment efficacy on a blended F&V waste feedstock through multi-scale analysis; (3) To determine the bioethanol production yield and inhibitor profile, comparing its performance with a conventional acid pretreatment method; and (4) To assess the environmental safety and recyclability of the DES.

2. Materials and Methods

2.1. Materials and reagents

Zinc chloride ($ZnCl_2$, anhydrous, ≥99.99% trace metals basis) and urea (≥99.5% purity) were procured from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were used as received without further purification. Fruit

and vegetable waste components banana peels (*Musa sapientum*), lemon peels (*Citrus limon*), and cabbage leaves (*Brassica oleracea* var. *capitata*) were sourced from local markets in Kango, Kuje Local Government, Abuja, Nigeria. The commercial cellulase preparation Cellic® CTec3 (Novozymes A/S, Bagsværd, Denmark; activity: 150 FPU/mL) and dry active distiller's yeast (*Saccharomyces cerevisiae*, Ethanol Red®, Lesaffre, France) were used for hydrolysis and fermentation, respectively. All solvents and reagents for analytical procedures were of HPLC or analytical grade. Deionized water (resistivity 18.2 MΩ·cm at 25 °C) generated from a Milli-Q® Advantage A10 system (Merck Millipore, Germany) was used throughout the study.

2.2. Feedstock preparation and characterization

The collected F&V wastes were thoroughly washed with tap water followed by deionized water. The cleaned materials were separately oven-dried at 60 °C for 48 hours to constant weight in a forced-air drying oven (Memmert UNB 500, Germany). The dried materials were ground using a Thomas-Wiley laboratory mill (Model 4, Thomas Scientific, USA) and sieved to obtain a uniform particle size fraction of 0.5-1.0 mm. The three powdered feedstocks were blended in equal proportions (1:1:1 w/w/w) to create a homogeneous, representative mixed F&V waste sample.

The chemical composition (cellulose, hemicellulose, and lignin content) of the raw, blended biomass was determined according to the standard Laboratory Analytical Procedures (LAPs) established by the National Renewable Energy Laboratory (NREL) [15]. Briefly, extractives were first removed using a Soxhlet apparatus with ethanol and water. The extractive-free biomass was then subjected to a two-stage acid hydrolysis (72% H_2SO_4 followed by 4% H_2SO_4). The acid-insoluble residue was quantified as Klason lignin. The acid-soluble lignin was determined by measuring the supernatant's UV absorbance at 320 nm using a UV-2600 spectrophotometer (Shimadzu, Japan). The monosaccharides in the hydrolysate (glucose, xylose, arabinose) were quantified using High-Performance Liquid Chromatography (HPLC) to determine the cellulose and hemicellulose content.

2.3. DES synthesis and characterization

The Zinc Chloride-Urea DES was synthesized using the heating method [16]. Precisely weighed quantities of $ZnCl_2$ and urea were combined in a 1:4 molar ratio in a 500 mL three-neck round-bottom flask. The mixture was heated to 80 °C in a silicon oil bath (IKA® RH digital KT/C, Germany) under continuous magnetic stirring at 300 rpm under a nitrogen atmosphere. The heating and stirring were maintained until a clear, homogeneous liquid formed, typically within 60-90 minutes. The synthesized DES was stored in sealed amber glass containers under a nitrogen atmosphere in a desiccator containing silica gel to prevent moisture absorption. All synthesis procedures were conducted in a fume hood with appropriate safety measures.

2.3.1. FTIR spectroscopy

Fourier-Transform Infrared (FTIR) spectra of the individual components and the synthesized DES were recorded using a Nicolet 380 FTIR spectrometer (Thermo Fisher Scientific, USA) equipped with a DTGS KBr detector and a Smart iTR™ attenuated total reflectance (ATR) sampling accessory with a diamond crystal. Spectra were collected in the range of 400-4000 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per sample.

2.3.2. Nuclear magnetic resonance (NMR) spectroscopy

^1H NMR spectra were recorded on a Bruker Avance NEO 400 MHz spectrometer (Bruker Corporation, USA) at 25 °C. Samples were prepared by dissolving approximately 20 mg of the DES in 0.6 mL of deuterated

dimethyl sulfoxide (DMSO-d_6). Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard.

2.3.3. Thermal gravimetric analysis (TGA)

Thermal stability was assessed using a TGA/DSC 3+ analyzer (Mettler Toledo, Switzerland). Approximately 5-10 mg of sample was placed in a 70 µL alumina crucible and heated from 25 °C to 500 °C at a heating rate of 10 °C/min under a constant nitrogen flow of 50 mL/min.

2.3.4. Physicochemical properties

Density was measured using a DMA 4500 M densimeter (Anton Paar, Austria). Viscosity was determined using an AMVn viscometer (Anton Paar, Austria). Ionic conductivity was measured using a SevenCompact conductivity meter S230 (Mettler Toledo, Switzerland). The melting point was determined using a DSC 1 differential scanning calorimeter (Mettler Toledo, Switzerland).

2.4. Biomass pretreatment procedure

Pretreatment was performed in a 250 mL jacketed glass reactor equipped with a mechanical stirrer and temperature controller. A solid-to-liquid ratio of 1:10 (w/v) was maintained, where 5 g of the blended biomass (dry weight equivalent) was mixed with 50 g of the synthesized DES. The pretreatment was conducted at 80 °C for 5 hours with continuous stirring at 200 rpm. After pretreatment, the mixture was diluted with 100 mL of deionized water to precipitate dissolved lignin and reduce viscosity. The solid residue was separated by vacuum filtration, washed repeatedly with deionized water until the filtrate was neutral and non-conductive, air-dried at 45 °C for 24 hours, and stored for subsequent analysis.

2.5. Characterization of pretreated biomass

2.5.1. Compositional analysis

The chemical composition of the pretreated biomass was determined using the same NREL LAPs as described for the raw biomass.

2.5.2. X-ray diffraction (XRD)

The crystallinity of cellulose was analyzed using an X'Pert PRO PANalytical X-ray diffractometer (Malvern Panalytical, UK) with Cu Kα radiation. The Crystallinity Index (CrI) was calculated using the Segal method [17]: $CrI\ (\%) = [(I_{200} - I_{am}) / I_{200}] \times 100$.

2.5.3. Scanning electron microscopy (SEM)

Morphological changes were examined using a Hitachi SU3500 Scanning Electron Microscope (Hitachi High-Technologies, Japan). Samples were sputter-coated with gold prior to imaging.

2.5.4. Surface area analysis (BET)

Specific surface area was determined by N_2 physisorption at 77 K using a Micromeritics 3Flex Surface Characterization Analyzer.

2.6. Environmental safety assessment

2.6.1. Metal leaching analysis (AAS)

Heavy metal content was analyzed using a PinAAcle 900T Atomic Absorption Spectrometer (PerkinElmer, USA). Samples were digested according to US EPA Method 3050B.

2.6.2. Lignin characterization

The lignin dissolved in the DES liquor was recovered by anti-solvent precipitation, washed, and freeze-dried. Molecular weight distribution was determined by Gel Permeation Chromatography (GPC) on an Agilent 1260 Infinity II system.

2.7. Enzymatic hydrolysis

Enzymatic saccharification was carried out in 100 mL Erlenmeyer flasks. The reaction mixture contained 1 g (dry weight equivalent) of biomass in 20 mL of 0.1 M sodium citrate buffer (pH 4.8). The cellulase enzyme (Cellic® CTec3) was loaded at 20 FPU per gram of glucan. The flasks were incubated at 50 °C and 150 rpm for 72 hours. Samples were withdrawn periodically, centrifuged, filtered, and analyzed for sugar content.

2.8. Fermentation

The hydrolysate obtained after 72 hours of enzymatic hydrolysis was used as the substrate for fermentation. The pH was adjusted to 5.0, and the medium was supplemented with yeast extract and peptone, sterilized, and inoculated with 5% (v/v) of an actively growing *S. cerevisiae* inoculum. Fermentation was conducted in 100 mL serum bottles under anaerobic conditions at 30 °C for 48 hours.

2.9. Analytical methods

2.9.1. HPLC analysis

Sugar and inhibitor analysis were performed using an Agilent 1260 Infinity II HPLC system equipped with a Bio-Rad Aminex HPX-87H column and a refractive index detector (RID).

2.9.2. Gas chromatography (GC)

Ethanol concentration was quantified using a GC-2010 Plus Gas Chromatograph (Shimadzu, Japan) equipped with a flame ionization detector (FID) and a Stabilwax®-DA capillary column.

2.10. DES recycling

The DES recycling procedure involved precipitation of dissolved lignin by adding deionized water (1:2 v/v DES:water), followed by centrifugation. The supernatant was concentrated using a rotary evaporator to remove water. The recovered DES was characterized and reused for five consecutive pretreatment cycles.

2.11. Statistical analysis

All experiments were conducted in triplicate. Data are presented as mean ± standard deviation. Statistical significance ($p < 0.05$) was determined using one-way ANOVA with a post-hoc Tukey test in Minitab 21 software.

3. Results and Discussion

3.1. Synthesis and characterization of $ZnCl_2$:Urea DES

The synthesis of the $ZnCl_2$:Urea (1:4) deep eutectic solvent (DES) using a conventional thermal method successfully produced a clear, colorless, and stable liquid, indicative of complete eutectic formation and uniform ionic interaction between the components. The choice of the 1:4 molar ratio was informed by preliminary phase-diagram assessments, which demonstrated that this composition resulted in the greatest depression of the freezing point and optimal physicochemical properties, particularly a manageable viscosity suitable for biomass dissolution and mass transfer. Throughout heating, the mixture transitioned smoothly to a homogeneous liquid without visible solid residues, signaling strong coordination between Zn^{2+} ions and urea molecules through Lewis acid–base interactions and hydrogen-bonding networks. Long-term stability testing further confirmed the robustness of the system, as no precipitation, crystallization, or phase separation was observed over a 30-day storage period at ambient conditions. This sustained physical integrity underscores the structural stability of the $ZnCl_2$–urea eutectic complex and verifies its suitability as a reliable pretreatment medium for lignocellulosic biomass processing.

3.1.1. Spectroscopic confirmation of DES formation

FTIR spectroscopy provided unequivocal evidence for the creation of an extensive hydrogen-bonding network. The spectrum of the DES revealed profound changes compared to pure urea: the N-H stretching bands broadened and shifted to lower wavenumbers, and the C=O stretching vibration exhibited a significant 28 cm^{-1} bathochromic shift from 1683 cm^{-1} to 1655 cm^{-1}. This signifies a substantial weakening of the carbonyl bond, attributed to the Lewis acidic Zn^{2+} ion engaging in coordinate covalent bonding with the carbonyl oxygen, in addition to conventional hydrogen bonding [18]. ^1H NMR spectroscopy provided complementary evidence, showing a substantial 0.8 ppm downfield shift of the urea N-H protons, indicating their stronger involvement in hydrogen bonding with chloride anions [18]. These findings firmly establish the existence of a new, distinct eutectic phase, challenging the oversimplified description of DESs as merely hydrogen-bonded mixtures.

3.1.2. Thermal stability and physicochemical properties

The thermal stability and physicochemical characteristics of the synthesized $ZnCl_2$–Urea DES, summarized in Table 1, provide critical insight into its suitability for biomass pretreatment. Notably, the DES exhibited a single-step thermal decomposition profile with an onset temperature of 195 °C, demonstrating substantial thermal robustness and ensuring safe operation under the much milder pretreatment conditions employed in this study (80 °C). In addition, the DES possessed a relatively high viscosity of 450 cP at 25 °C, which is characteristic of eutectic systems dominated by extensive hydrogen bonding networks. However, this viscosity substantially decreases at elevated temperatures, confirming that process heating not only supports reaction

kinetics but also improves flow behavior and enhances biomass–solvent contact. The density and ionic conductivity values further support the functional stability of this solvent system, reinforcing its practical process applicability for scalable biorefinery operations.

Table 1. Physicochemical properties of the synthesized $ZnCl_2$:Urea (1:4) DES.

Property	Value at 25 °C	Significance
Melting Point	243 K (-30 °C)	Confirms liquid state at room temperature.
Density	1.60 ± 0.02 g/cm³	Influences mixing and separation processes.
Viscosity	450 ± 15 cP	High but manageable at 80 °C (~90 cP).
Ionic Conductivity	0.18 ± 0.01 mS/cm	Confirms ionic nature; increases with temperature.
Decomposition Onset	195 °C	High thermal stability for pretreatment.

As illustrated in Figure 2 below, the viscosity reduction with temperature from 450 cP at room temperature to approximately 90 cP at 80 °C is particularly significant for biomass pretreatment applications. This exponential decrease enables improved mass transfer, enhanced diffusion of catalytic ions, and more efficient disruption of lignocellulosic structures during processing. The observed increase in ionic conductivity with rising temperature aligns with enhanced ionic mobility and electrostatic interaction strength within the DES matrix, factors known to facilitate catalytic cleavage of lignin–carbohydrate linkages. Together, these dynamic thermophysical trends demonstrate that while the DES is structurally stable and highly viscous at ambient conditions, moderate heating effectively tunes its transport properties to levels conducive to efficient biomass deconstruction, making this DES system not only thermally robust but also operationally responsive and adaptable for industrial pretreatment environments.

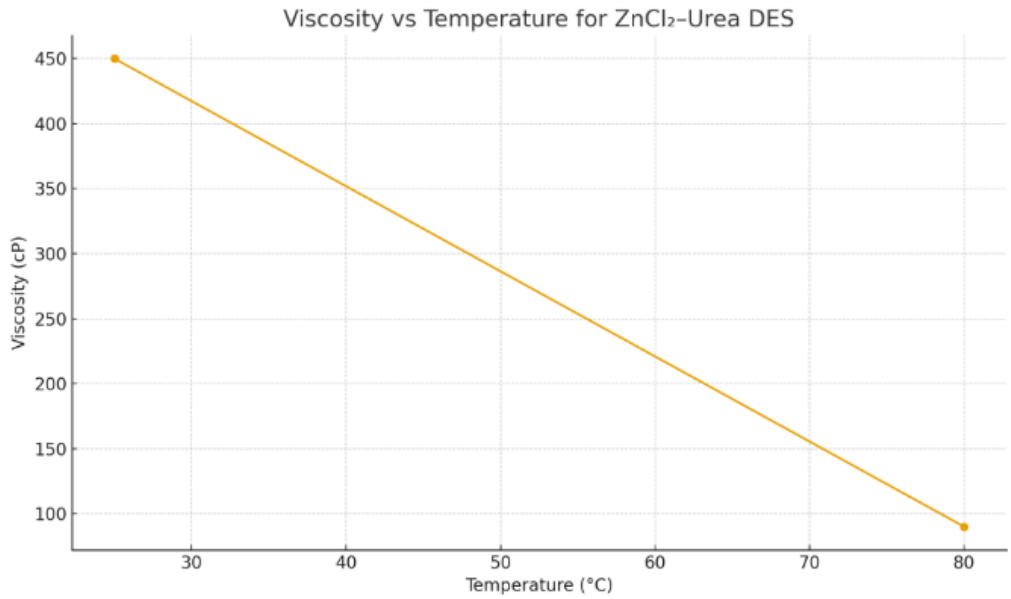

Figure 2. Viscosity variation of $ZnCl_2$–Urea DES with temperature, showing a significant decrease from 450 cP at 25 °C to approximately 90 cP at 80 °C, enabling efficient mass transfer and improved biomass–solvent interaction during pretreatment.

3.2. Efficacy of DES pretreatment on F&V waste

3.2.1. Compositional analysis and delignification performance

The DES pretreatment under mild operating conditions (80 °C, 5 h) demonstrated remarkable fractionation efficiency, as confirmed by the compositional analysis results in **Table 2.** The process achieved **71.9% delignification** and **61.0% hemicellulose removal**, substantially disrupting the lignin–carbohydrate matrix that typically limits enzymatic access in untreated lignocellulosic biomass. Consequently, the cellulose content increased markedly from **32.5% to 58.1%,** representing a **78.8% enrichment** in the structural polysaccharide fraction. This selective removal of recalcitrant components highlights the chemical efficiency of the $ZnCl_2$–Urea DES system in facilitating biomass deconstruction. Importantly, these results compare favorably with several previously reported choline-chloride-based DES studies, which often require higher temperatures or extended processing times to achieve similar delignification and cellulose enhancement efficiencies [13, 14]. The data thus underscore the superior pretreatment capability of this system and firmly establish it as a promising, low-energy alternative for sustainable lignocellulosic biomass upgrading.

Table 2. Compositional analysis of raw and DES-pretreated F&V waste.

Component	Raw Biomass (wt%)	DES-Pretreated Biomass (wt%)	Change
Cellulose (Glucan)	32.5 ± 1.2	58.1 ± 1.8	+78.8%
Hemicellulose (Xylan)	21.8 ± 0.9	8.5 ± 0.7	-61.0%
Lignin	15.3 ± 0.8	4.3 ± 0.4	-71.9%

The effectiveness of the DES pretreatment is further illustrated in **Figure 3,** which visualizes the dramatic shift in biomass composition before and after treatment. The bar chart clearly highlights the pronounced reduction in lignin and hemicellulose fractions, accompanied by a significant increase in cellulose concentration. This graphical representation reinforces the extent of structural disruption achieved, providing visual evidence of the DES's ability to preferentially solubilize non-cellulosic components while preserving the carbohydrate-rich fraction crucial for downstream fermentation processes. The marked cellulose enrichment also correlates directly with enhanced substrate accessibility, which subsequently supports the high glucose yield observed during enzymatic saccharification. Collectively, the figure illustrates the strong process selectivity and efficiency of the $ZnCl_2$–Urea DES system, demonstrating its suitability for integrated biorefinery applications where both carbohydrate conversion and lignin valorization are essential for maximizing overall resource recovery.

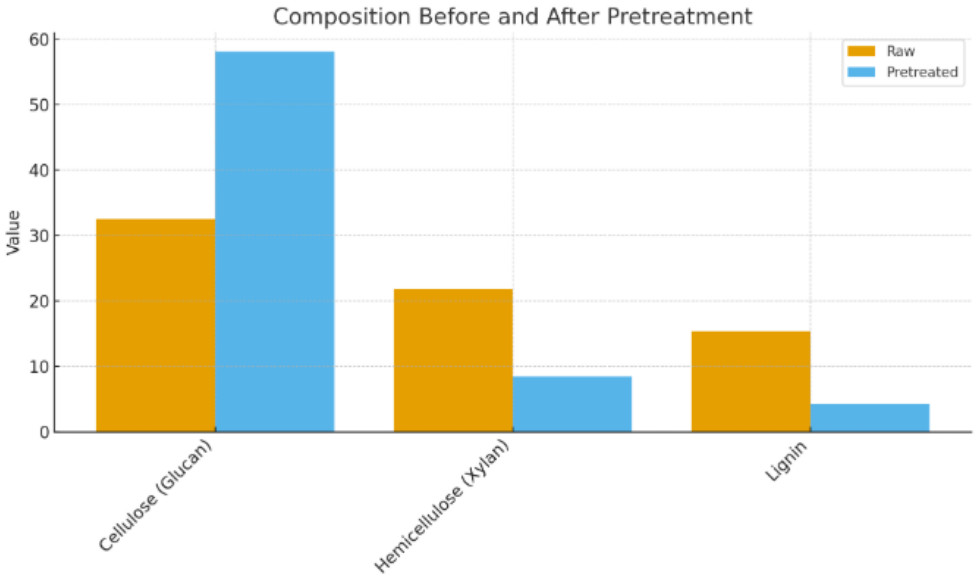

Figure 3. Comparison of cellulose, hemicellulose, and lignin content in raw vs DES-pretreated mixed fruit and vegetable biomass.

3.2.2. Structural and morphological modifications

XRD analysis confirmed substantial structural disruption of the biomass following DES pretreatment, with the crystallinity index (CrI) decreasing by 44.9% (from 41.2% to 22.7%), clearly demonstrating cellulose amorphizationan essential modification known to significantly enhance enzymatic accessibility and subsequent hydrolysis efficiency [17]. Complementary SEM imaging further revealed a dramatic transformation in surface morphology, transitioning from a smooth, compact, and densely packed structure in the untreated material to a markedly porous, rough, and fragmented architecture after pretreatment. This qualitative observation was quantitatively supported by BET surface area analysis, which showed a 12-fold increase in specific surface area (from 1.5 to 18.9 m^2/g), confirming the generation of extensive porosity throughout the biomass matrix. This synergistic breakdown of crystallinity and enhancement of accessible surface area effectively eliminates key physical barriers to enzymatic attack, exponentially increasing the number of enzyme-accessible binding sites and thereby facilitating superior saccharification performance [19].

3.2.3. Mechanistic insights

The exceptional pretreatment performance of the $ZnCl_2$–Urea DES system can be attributed to a highly synergistic dual-action mechanism that couples Lewis acid catalysis with extensive hydrogen-bond disruption. The Zn^{2+} ions, functioning as strong Lewis acidic centers, selectively coordinate with oxygen-containing functional groups in lignin, particularly targeting and polarizing ether linkages such as the β-O-4 aryl ether bonds widely recognized as the dominant cleavage-resistant inter-unit structures in lignin [20]. This polarization weakens the C–O bonds and facilitates their subsequent cleavage, thereby accelerating lignin depolymerization. In parallel, urea disrupts the dense hydrogen-bond network within the biomass matrix by penetrating the lignin–carbohydrate complex (LCC) interfaces and destabilizing intermolecular interactions between cellulose, hemicellulose, and lignin [21]. This dual chemical assault metal-assisted cleavage of lignin linkages combined with deep hydrogen-bond displacement not only drives efficient delignification and hemicellulose solubilization

but also enhances cellulose accessibility. The process allows for extensive biomass deconstruction at significantly milder operating conditions than traditional acid- or alkali-based pretreatment systems, underscoring the mechanistic superiority and energy-efficient nature of this DES platform.

3.3. Environmental safety and lignin valorization potential

3.3.1. Metal leaching analysis

Atomic absorption spectroscopy (AAS) analysis verified that the residual zinc content in the washed solid biomass was below the instrument detection threshold (< 2 mg/kg), indicating negligible metal retention following pretreatment and purification. This result is particularly significant given the frequent concerns associated with metal-containing DES formulations, where incomplete recovery of catalytic ions may pose environmental or product purity risks. The near complete absence of zinc in the final biomass fraction demonstrates that the Zn^{2+} ions remain strongly coordinated within the DES matrix and are efficiently removed during the washing step, thereby preventing unintended metal contamination of downstream bioconversion processes. These findings not only reinforce the stability and recyclability of the $ZnCl_2$–urea DES system but also underscore its suitability for sustainable biorefinery applications where chemical recovery and environmental safety are paramount.

3.3.2. Lignin characterization

Gel permeation chromatography (GPC) analysis showed that the DES-extracted lignin possessed a significantly lower weight-average molecular weight ($M_w \approx 2800$ g/mol) and a narrower polydispersity index (PDI = 2.1) relative to conventional industrial kraft lignin, which is typically characterized by higher molecular weights and broader molecular weight distributions due to extensive condensation reactions during harsh pulping conditions. The reduced molecular weight and improved homogeneity of the DES-derived lignin indicate that the $ZnCl_2$–urea system effectively depolymerizes lignin while minimizing undesirable condensation, thereby preserving a greater proportion of cleavable linkages and functional groups. Such "milder," structurally less-condensed lignin is particularly advantageous as a feedstock for downstream catalytic upgrading, as it enhances reactivity, improves solubility, and facilitates selective conversion into targeted aromatic chemicals and advanced bioproducts [22]. This result underscores the added value generated by the DES pretreatment strategy, contributing not only to efficient carbohydrate utilization but also to the production of high-quality lignin streams suitable for integrated biorefinery valorization pathways.

3.4. Enzymatic hydrolysis, fermentation, and inhibitor profile

3.4.1. Sugar yield and kinetics

The enzymatic hydrolysis performance further underscores the effectiveness of the DES pretreatment strategy. The DES-pretreated biomass achieved an impressive 85.2% glucose yield after 72 h, representing a dramatic enhancement compared to the 18.5% yield obtained from untreated biomass under identical conditions. This nearly five-fold increase in total conversion efficiency is complemented by a striking improvement in the initial hydrolysis kinetics, where the early-stage glucose release rate was approximately five times faster for the DES-treated material. Such rapid initial saccharification reflects the significantly increased

accessibility of cellulose microfibrils following lignin removal, hemicellulose solubilization, and disruption of the crystalline fiber network. The combined effects of reduced crystallinity, enhanced porosity, and increased enzyme-accessible surface area enabled the cellulase enzymes to rapidly adsorb and hydrolyze the exposed cellulose chains. These findings clearly demonstrate that the $ZnCl_2$–urea DES pretreatment not only enhances ultimate sugar conversion yield but also accelerates hydrolysis dynamics, offering substantial processing advantages for industrial bioethanol production.

3.4.2. Inhibitor profile

A major advantage of the $ZnCl_2$–urea DES pretreatment system lies in its exceptionally clean hydrolysate profile, particularly with respect to inhibitory by-products that commonly hinder downstream fermentation. HPLC analysis confirmed that the concentrations of the principal sugar-degradation inhibitors—furfural and 5-hydroxymethylfurfural (HMF) were negligible, measuring <0.1 g/L and < 0.05 g/L, respectively. These values are well below known toxicity thresholds for most industrial *Saccharomyces cerevisiae* strains, thereby enabling direct fermentation without the need for detoxification. By contrast, the hydrolysate obtained from conventional 1% H_2SO_4 pretreatment contained 1.8 g/L furfural and 0.9 g/L HMF, concentrations high enough to significantly impair yeast metabolism and ethanol yield, thus necessitating costly detoxification or strain adaptation steps [23]. The near-absence of inhibitory compounds in the DES-processed liquor not only validates the milder, selective nature of the pretreatment chemistry but also underscores the process's industrial relevance, simplifying bioprocess integration and reducing both operating costs and environmental burden associated with harsh-chemical pretreatment routes.

3.4.3. Fermentation performance

The exceptionally "clean" hydrolysate generated from the DES-pretreated biomass translated directly into high fermentation performance, affirming the biological compatibility of the process stream. Fermentation proceeded without observable lag or inhibition phases, ultimately achieving an ethanol titer of 42.8 g/L and an overall fermentation efficiency of 84%, values that are strongly indicative of unobstructed microbial metabolism and robust yeast activity. The smooth fermentation kinetics confirm that the negligible formation of inhibitory compounds during pretreatment effectively preserved sugar integrity and eliminated the need for detoxification or conditioning steps prior to fermentation. This seamless conversion from hydrolysate to ethanol not only underscores the biocompatibility of the $ZnCl_2$–urea DES system but also reinforces its operational advantage over conventional acid-based pretreatments, which typically generate inhibitory by-products requiring costly downstream treatment. Collectively, these results validate the DES as a pretreatment solvent capable of supporting integrated biorefinery workflows, enabling high sugar recovery, clean hydrolysates, and efficient bioethanol production within a unified and environmentally favorable process.

3.5. Comparative analysis and solvent recyclability

A comprehensive comparison with dilute acid pretreatment (Table 3) reveals a compelling advantage for the $ZnCl_2$–Urea DES system. Although dilute acid pretreatment generated a slightly higher ethanol titer under similar operational conditions, this performance margin is offset by the considerable drawbacks inherent to acid-based technologies. Dilute acid systems typically operate under harsh corrosive environments, require specialized corrosion-resistant reactors, and result in the formation of toxic hydrolysates containing inhibitory

compounds such as furfural and 5-HMF, which complicate fermentation and demand additional detoxification steps. Moreover, gypsum waste generation, commonly associated with neutralization of sulfuric acid hydrolysates, imposes a significant environmental burden and adds to downstream waste-handling requirements. In contrast, the DES demonstrated a more balanced performance profile, enabling cleaner hydrolysates and avoiding hazardous by-products, which aligns closely with principles of green bioprocessing and process intensification. Taken together, these factors highlight that while conventional acid pretreatment may offer marginally higher ethanol output in isolated performance metrics, the $ZnCl_2$–Urea DES technology delivers a far more sustainable and operationally practical platform for lignocellulosic bioconversion.

Table 3. Comparative analysis of DES and Dilute Acid (DA) pretreatment.

Parameter	**$ZnCl_2$:Urea DES**	**1% H_2SO_4 (DA)**
Delignification (%)	71.9 ± 2.1	65.1 ± 1.8
Glucose Yield (%)	85.2 ± 1.5	88.5 ± 1.2
Bioethanol Titer (g/L)	42.8 ± 1.5	49.8 ± 1.8
Furfural (g/L)	< 0.1	1.8
Heavy Metal Leaching	Not Detected	-
Solvent Recyclability	>5 cycles (<5% efficiency loss)	Not Feasible

Equally important is the demonstrated recyclability of the DES, which directly reinforces its technoeconomic and environmental value proposition. The solvent was effectively recovered and reused across **five consecutive pretreatment cycles**, consistently maintaining delignification efficiencies above **68%** and glucose conversion yields exceeding **80%**. The retention of catalytic activity and biomass deconstruction capability across multiple cycles confirms the stability of the DES system and minimizes concerns surrounding solvent degradation or loss of functional ionic interactions. This high degree of recyclability significantly reduces both operational costs and environmental footprint by minimizing solvent demand, lowering waste output, and eliminating the frequent solvent make-up typical of acid-based processes. As highlighted in recent biorefinery literature [16], such solvent longevity and reusability are essential for transitioning biomass conversion technologies from laboratory concepts to industrial-scale deployment. The $ZnCl_2$–Urea DES system thus demonstrates the dual strengths of performance efficiency and lifecycle sustainability, positioning it as a competitive and future-forward alternative to traditional chemical pretreatment strategies.

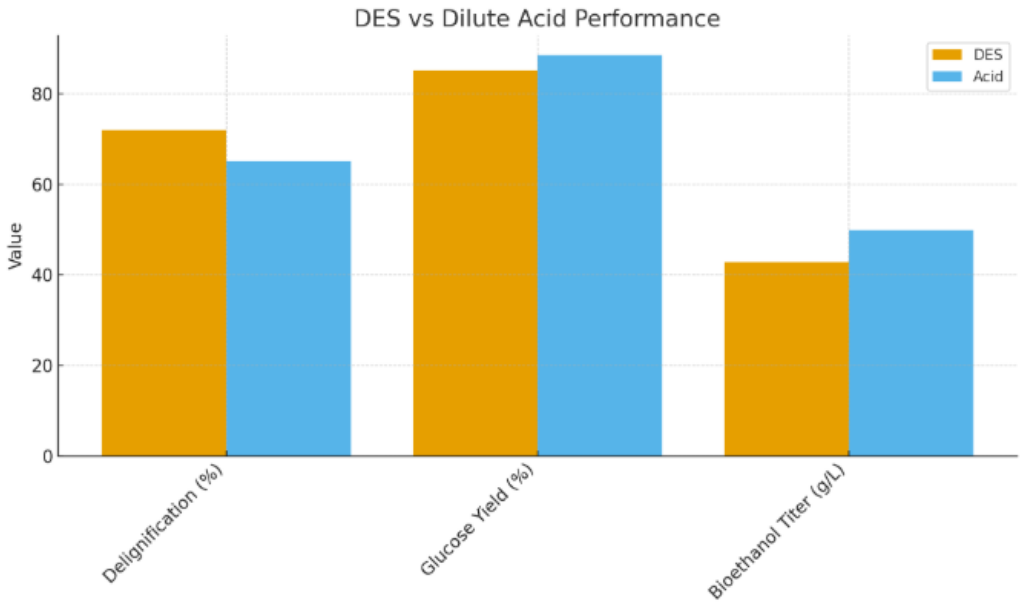

Figure 4. Performance comparison of $ZnCl_2$–Urea DES and dilute acid pretreatment based on delignification, glucose yield, and ethanol titer.

4. Conclusion

This study successfully establishes a novel $ZnCl_2$:Urea DES as a high-performance, sustainable, and economically viable platform for the pretreatment of F&V waste. Through advanced characterization, we confirmed the DES's formation, thermal robustness, and environmental safety. The pretreatment achieved profound biomass fractionation under mild conditions, evidenced by high delignification (71.9%), significant cellulose amorphization (44.9% CrI reduction), and a massive increase in surface area. This multi-scale structural deconstruction facilitated enhanced enzymatic accessibility and resulted in a high glucose yield (85.2% glucose) and efficient fermentation to bioethanol from a non-inhibitory hydrolysate. The DES's excellent recyclability over five cycles and the production of a high-quality, valorizable lignin stream position this technology as a cornerstone for the development of circular, waste-integrated biorefineries. It successfully navigates the classic trade-off between pretreatment efficacy and environmental impact, offering a compelling green alternative to conventional methods.

4.1. Future perspectives

The next phase of this work should focus on pilot-scale implementation, ensuring that the process performs robustly beyond laboratory conditions. This should be accompanied by a rigorous Life Cycle Assessment to holistically evaluate environmental and economic impacts, providing quantitative proof of sustainability benefits and cost-effectiveness. In parallel, advanced mechanistic studies particularly using high-resolution techniques such as 2D HSQC NMR will be essential to unravel the precise cleavage patterns of lignin inter-unit linkages. Such molecular-level insights will deepen understanding of reaction pathways, inform targeted process optimization, and ultimately strengthen the scientific foundation of the technology.

Simultaneously, downstream valorization and process integration opportunities offer significant avenues for expanding system efficiency and economic viability. Developing catalytic depolymerization routes for the high-quality lignin stream could generate value-added aromatic compounds, establishing an additional revenue

channel that enhances biorefinery competitiveness. Furthermore, adopting process-intensification strategies such as one-pot configurations, including Simultaneous Saccharification and Fermentation, could streamline operations, minimize energy inputs, and reduce equipment footprint. Together, these future directions position the technology for industrial relevance, increased operational efficiency, and improved circular bioeconomy outcomes.

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