Lactic Acid-Proline Solvent Pretreatment of Cowpea Shells: Effects of Process Variables on Glucan, Xylan, and Lignin Composition

ABSTRACT

The aim of this study is to investigate the effectof temperature, duration, and water dilution on the composition of glucan, xylan, and lignin in cowpea shells pretreated with a Lactic acid-proline solvent. The composition of glucan, xylan, and lignin was analyzed using the NREL method. The highest xylan removal (12%) was achieved after a 6-hour pretreatment at 150°C, while 11.35% lignin removal was observed after 5 hours at the same temperature. The most significant increase in glucan content, reaching 78.3 %, was observed after 4 hours at 150°C using a 2.5% (w/w) water-diluted solvent. While neat solvents promoted the dissolution of xylan and lignin, the addition of water preserved glucan from the harshness of the pretreatment. These findings provide a foundation for further investigations into optimizing the entire process.

GRAPHICAL ABSTRACT

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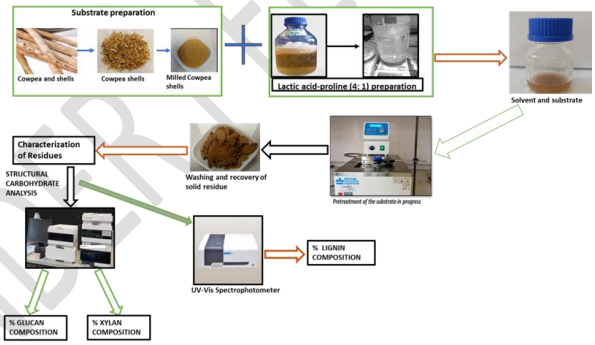

Keywords: Pretreatment, Cowpea shells, Natural deep eutectic solvent, Biomass. Lactic acid, Proline

1. INTRODUCTION

As the global human population continues to grow, there is a concurrent increase in the volume of waste generated by agricultural activities and food processing. According to a research by the United Nations Environmental Programme on waste conversion, it was

estimated that about 140 billion metric tons of agro-waste (biomass) is generated globally on yearly basis [1]. And these wastes are disposed primarily through open-air burning and open-field dumping [2]. This practice raises serious environmental and human health concerns, necessitating the explorationof sustainable management strategies. Many agricultural and post-harvest wastes are rich in polysaccharides, particularly cellulose and hemicellulose, making them promising for conversion into valuable products[3]. To overcome the inherent recalcitrance nature of lignocellulosic biomass, and to achieve an effective biochemical and thermochemical conversion [4]. Pretreatment stands as an important initial step that aids structural modifications in the biomass.

But for sustainability in biomass conversion, it is highly recommended to adopt pretreatment methods that do not threaten the environment and one such approach is the use of Natural deep eutectic solvents (NADES). These solvents are made from substances found in plant cells Choi et al.[5]. NADESs are classified as green solvents [6] and offer many advantages such as minimal toxicity, biodegradability, sustainability, affordability, and straightforward preparation procedure [7]. NADESs are also easily produced by combining hydrogen bond donors (such as lactic acid, malic acid, and oxalic acid) and hydrogen bond acceptors (such as glycine, choline chloride, proline, alanine, and betaine) commonly found in plants in specific molar ratios.

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These solvents have been studied as a chemical route for the pretreatment of different biomass because of their high solubilisation power and tunability [8]. Previous reports have been published on the use of NADES for the pretreatment of agricultural waste such as rice straw [9], corn cob [10], oil palm empty fruit bunch [11], poplar sawdust [12], Eucalyptus waste [13], chestnut [14].

In this study,a natural deep eutectic solvent produced by combining a hydrogen bond donor (lactic acid)and a hydrogen bond acceptor (proline)was used to fractionate cowpea shells. Lactic acid is an organic acid commonly found in sour milk and is classified as a generally safe chemical [15] while Proline is an amino acid found in plants, and it is vital for primary metabolism [16]. These chemicals naturally found in our environment were used for the pretreatment of cowpea shells (CS). Cowpea is one of the major sources of plant protein. According to FAO [17], 8.9 million tonnes of cowpeas were produced globally in 2020, of which Africa accounts for 97 %. Nigeria and the Republic of the Niger as the highest producers: 42 % and 31 %, respectively. Cowpea shells (*Vigna unguiculata* shells*)* a post-harvest waste generated during the deshelling of cowpea pod**.**A study by Kemausuor et al [18] showed that cowpea shells residue has the potential of generating 17 Mega Liters of cellulosic ethanol per year.A study by Jekayinfa et al. [19] estimated that 5285×10^3 tonnes of cowpea shells are generated yearly in Nigeria,therefore these underutilized biomass can serve as feedstock for biorefineries.

In biorefinery factors such as temperature, time, and water dilution have been established as some of the factors that affect the use of NADES [20]. Addition of water to solvents are done for several reasons which includes to reduce viscosity [21], to inhibit the solubility of cellulose. [22] and to reduce the volume of solvent used in a process [20]. The main objective

of this research is to study the effect of temperature, time and water dilution variations on the glucan, xylan and lignin composition of cowpea shells pretreated using Lactic acid-proline solvent, and to the best of the best of our knowledgethis area of research has not beenexplored.

1. MATERIALS

The cowpea shells(*Vignaunguiculatashells*) were locally sourced from in Chanchaga Local Government Area, Minna, Niger State, Nigeria. Lactic acid (90 %) was purchased from Thermo Scientific (USA). Proline (99 %), Sulphuric acid (98 %), D- (+)-glucose and D- (+) -xylose were purchased from Sigma Aldrich Company Ltd, Germany. These chemicals were utilized without any purification process.

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2. METHODS

2.1.Sample andNADESpreparation

The substrate was washed with deionised water and sundried for two days before ball milling with a Retsch PM 100 Planetary ball mill (Haan, Germany) and afterwards sieved with a 425 µm laboratory test sieve before using a Soxhlet extractor and ethanol to remove extractives for 6 h, after which the substrate was air-dried and stored in sealed containers for subsequent use.

Lactic acid (L) and Proline (P) were combined in a molar ratio of 4: 1 based on the findings of Francisco et al [23]stating that this molar ratio formed a clear liquid when combined and remained stable. Lactic acid-proline solvent was prepared based on the protocol described by Dai et al [24]. Briefly, the reagents were measured and added to a tightly capped bottle, followed by incubation in a water bath set at 50 ° C with continuous stirring at 150 rpm for 50 minutes.

2.2. NADES pretreatment

Pretreatment was carried out at 130 ^{0} C, 140 ^{0} C and 150 ^{0} C. The temperature range were selected based initial studies and research findings of Kumar et al. [25] and Procentese&Rehmann[26]. The pretreatment time was varied as follows (2 h, 3 h, 4 h, 5 h and 6 h) using neat solvent and water-diluted solvents. Based on the research findings of these authors, Kumar et al.[27], Hou, Feng, Ye, Huang, & Zhang [28]and Kumar et al. [29], water dilutions varied between 2.5 % (w/w) and 5 % (w/w).Pretreatment was carried out with a solid load of 5 % (w/w), determined by Kumar et al. [29] in a capped Schott bottle placed in a high-temperature bath circulator (Daihan Scientific, Korea). Solid was recovered from the slurry by first washing with water only and afterwards with 5 ml of an anti-solvent (ethanol) before finally rinsing with deionized water.This procedure was adopted from the reported by Procentese et al. [30] and Li et al. [9]. For sustainability in the pretreatment process ethanol used for washing was recovered using a Vacuum rotavapor (BüchiLabortechnik, Switzerland) and reused during the entire process. The solid residue recovered was oven dried at 35 ^{0} C as recommended by the National Renewable Energy Laboratory (NREL)[31]. The NREL method was used for the structural carbohydrate characterization (glucan and xylan) and lignin composition. All experiments were carried out in duplicates.

2.3.Characterization of untreated and pretreated CS

According to NREL method, 0.3 g of the substrates were mixed with 3 ml of 72% sulfuric acid and incubated for 1 hour at 30 ° C in a water bath. The acid concentration was then adjusted to 4% by adding 84 ml of deionized water, followed by autoclaving at 121 ° C and 1.3 bar for 1 hour using a Hirayama autoclave (HV-110, Japan). The resulting filtrate from the dilute acid hydrolysis was further diluted, and the pH was adjusted using calcium carbonate. Subsequently, the neutralized filtrates were filtered through 0.45 µm nylon syringe filters and filled into HPLC autosampler vials. The composition of glucose and xylose was analysed using an Agilent 1260 Infinity HPLC system equipped with a refractive index detector. The analysis was carried out at 55 ° C, using an Aminex HPX-87C column (300 x 7.8 mm, Bio-Rad Laboratories, Hercules, California, USA) at 60 ° C. The column temperature was maintained at 60 °C, and the mobile phase consisted of 0.005 M H_2SO_4 at a flow rate of 0.6 ml/min. Glucose and xylose values obtained were converted to the glucan and xylan equivalent using equations developed by NREL[31].

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The acid-insoluble lignin (AIL) content was determined using the residue obtained from the acid hydrolysis step. The residue was placed in a crucible and dried at 105 ° C for 4 hours. Afterwards it was placed in a muffle furnace at 575 ° C for 4 hours. The weight loss of the acid-insoluble residue after ashing was measured gravimetrically to determine the acid-insoluble lignin content (AIL). The acid-soluble lignin (ASL) composition in the filtrates was evaluated using the Agilent Technologies Cary 60 UV-vis spectrophotometer at the absorbance wavelength of 288 nm.Based on the absorbance value obtained the acid-soluble lignin content was calculated using the equation developed by NREL [31]. The lignin content is determined by the sum of AIL and ASL, yielding the total lignin content.

3. RESULTS AND DISCUSSIONS

3.1.Effect of water dilution on the composition of pretreated CS

The chemical composition of the untreated cowpea shell was analyzed, showing glucan at 21.32 % (± 0.39), xylan at 21.46 % (± 0.29), and a total lignin content of 37%. The glucan and lignin composition is similar to coffee silverskin[26] and the biomass has been studied for pretreatment using deep eutectic solvent.

For effective conversion of cowpea shells, delignification of the sample and preservation of cellulose is essential. However, it has been established that the high viscosity of natural deep eutectic solvents obstructs effective mass transfer thereby affecting their applications in the field of biomass pretreatment[20].And application of high temperature pretreatment solubilizes cellulose[32]. Nevertheless, these challenges can be addressed by diluting the solvents with water.

Xylan and lignin removal of 5% and 28%, respectively, were achieved at 130°C with the neat solvent after 6 hours of pretreatment(refer to Figure 1). The introduction of water dilutions at 2.5% and 5% (w/w) to the natural deep eutectic solvents (NADES) at 130°C did not result in improvements in delignification or xylan removal within the sample, consequently leading to

a lower glucan yield.At 140°C (see Figure 2), using the neat solvent resulted in higher xylan and lignin removal compared to the 2.5% and 5% (w/w) water dilution. However, a higher glucan content of 41.1% was obtained at 2.5% water dilution after 5 hours of pretreatment, indicating the stability of glucan due to reduced pretreatment severity.

The highest xylanremoval of 12 % was obtained with the neat solvent at 150 ^{0} C after 6 hours. The xylan removal obtained in this study is lower than previous published data on deep eutectic solvents. Procentese&Rehmann[26] obtained 49 % xylan removal from coffee silvershin after 3 h at 150 ^{0} C using choline chloride -glycerol solvent. Another study by Su et al. [12] reported 76 % xylan removal from polar sawdust, using lactic acid-choline chloride (10: 1) at 130 ^{0} C for 90 mins. The result obtained in this work therefore shows xylan is relatively stable in lactic acid -proline solvents even at high temperature. From the results obtained (refer to Figure 3), applying 2.5% water at 150°C after 4 hours preserved glucan to achieve a 41% composition, while using the undiluted solvent resulted in 38% glucan composition. Lignin removal was also highest (11.35 %) with the neat solvent at 150 ^{0} C after 5 hours the 2.5 and 5 % water diluted solvents did not improve delignification when compared to the neat solvents.

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The trend of higher lignin and xylan removal with the undiluted solvent in comparison to diluted solvents, along with the preservation of glucan in the diluted solvent, was also documented by Kumar et al[29]. They attributed the reduced delignification when using diluted solvents to the decreased acidity resulting from the addition of water. This decrease in the delignification capacity of solvents due to water addition and a reduction in cellulose loss were also reported by Hou et al. [28]. Interestingly the reduced delignification capacity of diluted solvent was not the case in another study by New et. al [20]. In their study, addition of water to choline chloride-urea solvent improved delignification of oil palm fronds by 17.11 % after 4 hours of pretreatment at 120 ^{0} C. therefore the impact of water addition on lignin removal from biomass is dependent on the type of solvent applied.

The general pattern of improved glucan content observed at a 2.5% water dilution can be ascribed to the competitive hydrogen bonding of water molecules to cellulose fibers, thereby inhibiting the dissolution of glucan [22].

The pretreatment with neat solvent for the temperature and time range resulted to a lower solid recovery with the lowest 45 % obtained at 150 ^{0} C after 6 hours. Applying 2.5 and 5 % water dilution increased solid recovery for each temperature studied and this is due to less acidity of the solvents hence lower solubilization of substrate.

From the results obtained, addition of water to solvents across the temperatures reduced acidity of the solvent hence reducing their capacity to removal lignin and xylan when compared to the neat solvent, however the water molecules preserved glucan dissolution at high temperatures.

3.2.Effect of temperature and time variation on the composition of pretreated CS.

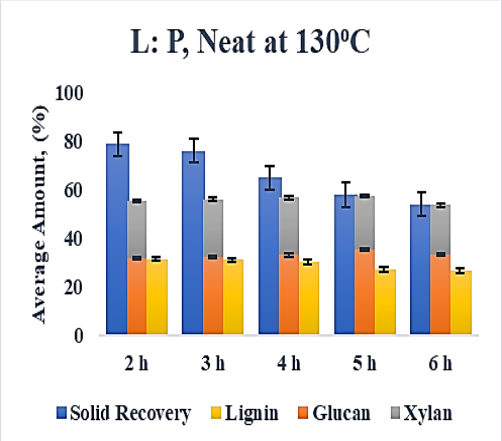
Increasing the pretreatment temperatures from 130 to 150°C and pretreatment time from 2 to 6 hours consistently resulted in increased removal of xylan. The removal of xylan and lignin generally resulted in low solid recovery as temperatures and pretreatment time increased.

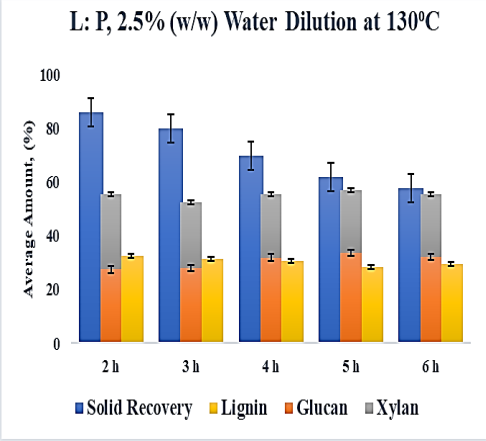
It is noteworthy that there was a slight increase in lignin composition (0.32% to 0.8%) at 150°C for both neat and diluted solvents when the duration increased from 5 to 6 hours. This marginal increase can be attributed to the reabsorption and redeposition of lignin. According to a study by Chen et al. . [33], the dissolved lignin in the bulk phase of a pretreatment medium at high temperatures and longer pretreatment times is redeposited on the biomass surface during the cooling down process.

In contrast, the glucan composition in the pretreated cowpea shell (CS) showed a significant increase as the temperature was raised from 130 to 150°C in comparison to the untreated substrate. At 130°C, increase in glucan content, reaching 66%, was observed after 5 hours of treatment with a neat solvent. while, at 140°C and 150°C, the maximum increment in glucan content was achieved using 2.5 % water diluted solvent.A 75% increase was obtained after 5 hours at 140°C and 78.3% increase after 4 hours at 150°C.

However, once the maximum glucan composition was attained, further pretreatment led to a decline in glucan content, indicating glucan dissolution as the severity of the treatment increased. A similar result was reported byYerizam et. al[34], in which coconut husk pretreated with betaine-lactic acid with molar ratios: 1: 4. 1: 6, 1:8 at 121 ^{0} C for 2, 4, 6, and 8 hours. The 1:4 and 1: 8 NADESs reached optimum cellulose yield after 4 hours before declining while 1:6 attained optimum after 6 hours. Yu et al. [32] also observed a similar trend in their study.

Higher pretreatment temperatures and time resulted to decline in solid yield and this is in line with previous reports by Yu et al. [32], Procentese&Rehmann[26] and Shen et al. [13].





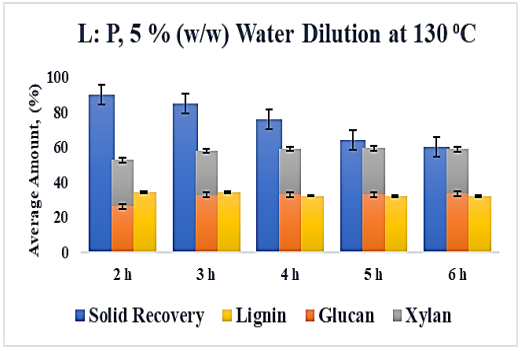
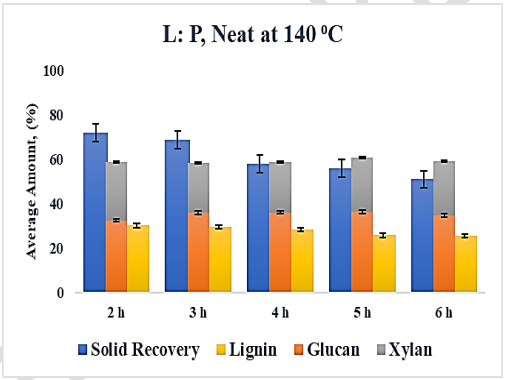
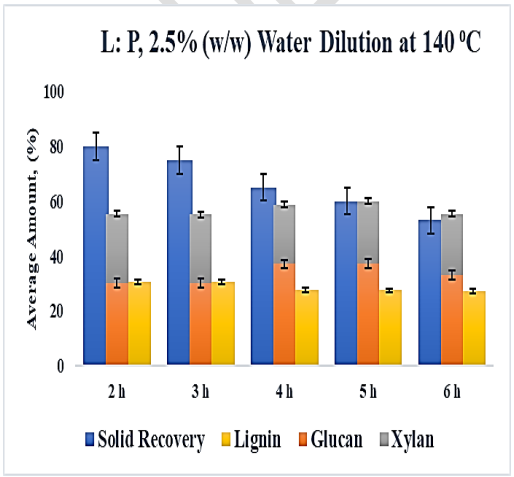


Figure 1: Compositional analysis of cowpea shells treated with Neat and Diluted-L: P solvent at 130^0 C.





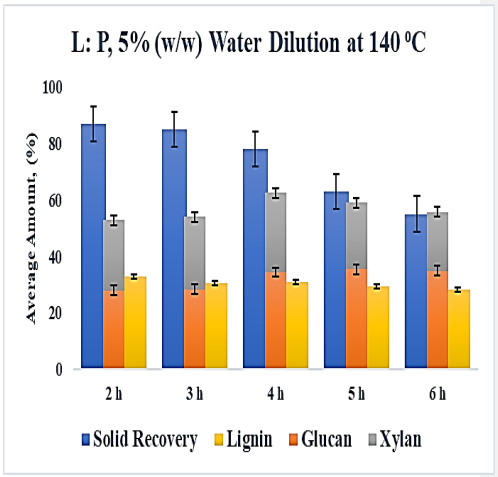


Figure 2: Compositional analysis of cowpea shells treated with Neat and Diluted-L: P solvent at 140^0 C.

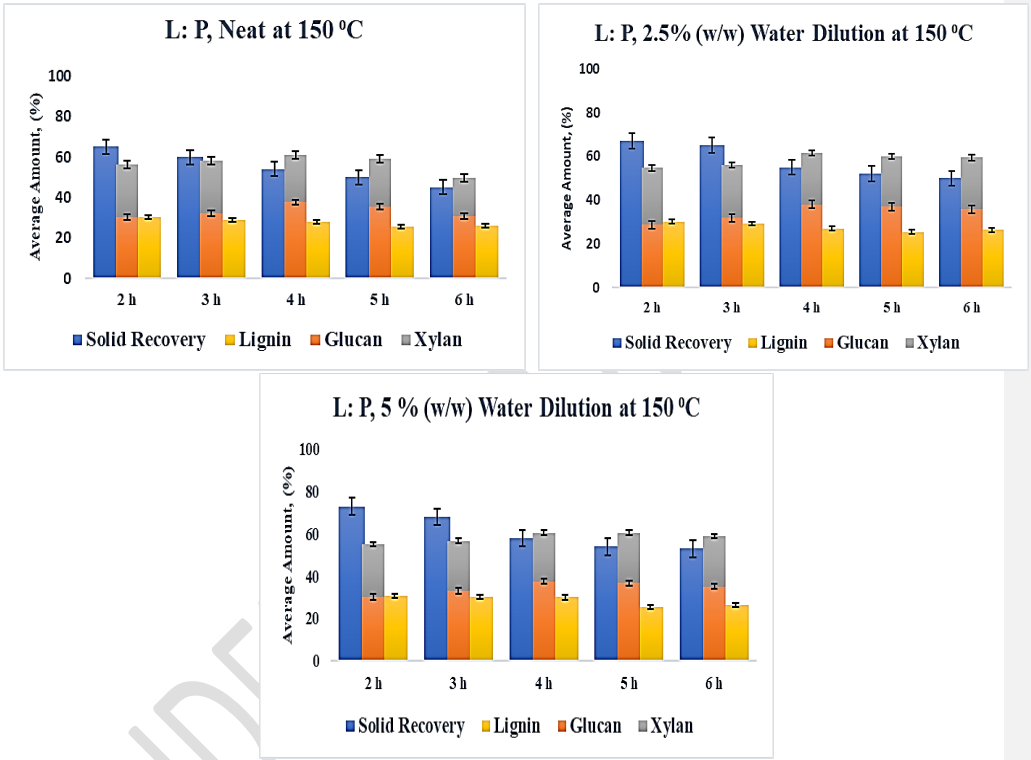


Figure 3: Compositional analysis of cowpea shells treated with Neat and Diluted-L: P solvent at 150^0 C.

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4. CONCLUSION

The study conducted an analysis of cowpea shell composition after pretreatment with NADES (Natural Deep Eutectic Solvents) to investigate the impact of temperature, time, and water dilution variations. The composition analysis was conducted following the NREL protocol. The results revealed a significantincrease in glucan content (78.3%), resulting in a carbohydrate-rich material. These pretreated cowpea shells hold promise for conversion into biofuels and other biochemicals. However, further research is necessary to determine the optimal values for glucan, xylan, and lignin in this context.

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Comment [MF7]: The percentage of recentness is 35.2 ,good

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