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Extraction of Cellulose from Corn Leaves Using Green Solvents: Evaluation of the Properties and Purity of the Obtained Cellulose

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ABSTRACT

The extraction process of biocompounds can be important in completing the value chain of agricultural products. One of the environmentally friendly methods for extracting useful compounds is the use of deep eutectic solvents (DESs). The present study aimed to extract cellulose from corn leaves using a deep eutectic solvent, in which choline chloride (ChCl) was used as a hydrogen bond acceptor and glycerol (Gly) as a hydrogen bond donor. In this study, first, the physicochemical properties of the solvents, including pH and viscosity, were investigated with different molar ratios of 1:1, 1:2, and 1:3 of ChCl to Gly. Then, to determine the most appropriate ratio of solvents during cellulose extraction, the extraction process was performed with three different ratios of ChCl:Gly and indicators such as extraction efficiency, holocellulose, cellulose, lignin, and hemicellulose were evaluated. It was found that there was no significant difference in the pH of the samples by changing the ratio of choline chloride to glycerol in the deep eutectic solvent. The lowest viscosity was for the sample with the middle ratio of ChCl:Gly. It was found that with increasing the molar ratio of glycerol from 1 to 3, the flow behavior index increased from 0.36 to 0.71. The results also showed that in the ratios of 1:1, 1:2 and 1:3, the extraction efficiency was 69.2 ± 0.28, 69.9 ± 0.32 and 70.88 ± 0.17 percent, respectively, and the cellulose content was 44.68 ± 0.45, 46.3 ± 0.63 and 48.28 ± 1.1 percent. Also, the amount of lignin impurity in the cellulose extracted by the solvent with the ratio of 1:3 choline chloride to glycerol was significantly lower than the other two ratios, indicating a higher efficiency of the solvent with this ratio than ChCl:Gly in purifying cellulose.

1- INTRODUCTION

The increasing population has led to an increase in the production of agricultural waste and residues, including leaf, stem, and wood waste of these products. These wastes constitute a major part of agricultural wastes and, due to their lignocellulosic components (cellulose, hemicellulose, and lignin), they become a valuable resource for processing these wastes [1]. Considering this, their processing provides the opportunity to not only reduce waste, but also protect the environment and achieve value-added and efficient products in various industries. [2]. Corn leaves is one of the important agricultural wastes, the role of which is to protect corn grains against pests, moisture, and environmental damage. After the corn grains are consumed, this husk remains and is generally used as animal feed. The components of the corn plant vary depending on its type and species, but in general, it can be noted that to achieve 1 kg of dry corn grains, approximately 0.22 kg of corn leaves is produced as waste [3]. The development of technologies for extracting cellulose from agricultural waste and converting it into value-added products such as nanocellulose, biodegradable films, and purification membranes is one of the solutions for the appropriate use of these wastes [1, 4]. Cellulose is the most abundant natural biopolymer on earth and the main component of plant cell walls. For example, leaves, stems, and corn cobs contain approximately 40, 45, and 36 percent cellulose, respectively [3]. This polymer plays a key role in the strength, stability, and growth of plant cells and has attracted attention in various industries due to its biodegradability, nontoxicity, and renewable properties [5]. Cellulose is a linear polymer composed of β-D-glucose units, and these units are linked together via β1-4 glycosidic bonds [6]. In the cellulose chain, both glucose units rotate 180 degrees relative to each other, and this arrangement forms the repeating unit of cellobiose [7]. Cellulose microfibrils are long and thin, with a diameter of approximately 3–4 nm, and consist of approximately 500–1400 D-glucose units joined together to form them. The degree of polymerization of cellulose varies depending on its source and the cellulose extraction processes [8].

Common solvents used in cellulose extraction include sodium hydroxide (NaOH), acid (such as H2SO4, HCl), and sodium chlorite (NaCLO2), which remove impurities such as lignin and hemicellulose and bleach the resulting cellulose. These chemical solvents are common but are not environmentally friendly and pose risks to humans and the environment [9]. For example, Han and geng (2023) removed impurities such as lignin from olive pomace by treating it with 6% sodium hydroxide at 95°C for 2 hours. They then bleached the sample by treating it with 7.5% hydrogen peroxide and 5% sodium hydroxide for 2 hours at 75°C. The yield of extracted cellulose in this experiment was 20.36% [10]. In another study, Singh and Singh (2013) used corn cob to extract cellulose. They used 8% sodium hydroxide in a ratio of 1:20 (w/v) for 3.5 hours at 100°C. In the next step, they applied a bleaching treatment using 5% sodium hypochlorite for 3 hours at 30°C. Finally, they dried the resulting cellulose at 60°C in a vacuum oven. The yield of their experiment was 20 g of cellulose per 100 g of dry raw material [11]. One of the challenges of using these materials is the high degradation and low yield of cellulose, which justifies the need to use new methods and green solvents.

Nowadays, with the advancements and emergence of green solvents, the desire to use them in various processes including cellulose extraction has expanded. Deep eutectic solvents (DESs) are one of the most important green solvents that can extract cellulose from biological sources with high efficiency and appropriate purity by reducing environmental risks [12]. These solvents are mainly composed of a combination of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), which lead to the formation of these solvents by establishing hydrogen bonds. Common examples of hydrogen bond acceptors include choline chloride, nicotinic acid, and L-aniline; while common substances as hydrogen bond donors include urea, glycerol, oxalic acid, ethylene glycol, and malic acid. The eutectic point represents the chemical composition and temperature at which a mixture of two solids melts completely at the lowest melting point relative to each of the compounds. The first known eutectic mixture consisted of choline chloride with a melting point of 302°C and urea with a melting point of 133°C, with a ratio of 1:2 of choline chloride to urea, which gave a

eutectic point of 12°C. Deep eutectic solvents have been used in the cellulose extraction process in the past [13, 14]. However, research is still ongoing into the composition of deep eutectic solvents and the ratio of components to maximize the efficiency or purity of cellulose. The properties of deep eutectic fluids can be precisely tuned by changing the type and ratio of the constituent components, allowing customization for specific applications [15]. On the other hand, the extensive properties of these solvents, including low melting point, negligible vapor pressure, and non-flammability, have made them environmentally friendly and safe solvents [16].

Shen et al. (2021) reported a delignification rate of 84.4% and a residual cellulose in the solid of 79.2% from the treatment of poplar wood using a deep eutectic solvent consisting of choline chloride and lactic acid with a ratio of 1:10 of choline chloride to lactic acid for 90 minutes at 130°C [17]. In another study, Pan et al. (2017) separated cellulose from rice straw using a deep eutectic solvent consisting of choline chloride and urea with a ratio of 1:2 at 130°C for 6 hours. The results of these researchers showed that the delignification rate using this combination was 43.19% and the residual cellulose in the solid was 25.06% [18]. In another study, Panyamao et al. (2023) extracted cellulose from wheat straw using a deep eutectic solvent consisting of a glycerol and urea mixture in a ratio of 1:2 at a temperature of 90 °C for 3 hours. Their results indicated that the delignification rate in the experiment was 75.6% and the remaining cellulose in the solid was 76.13% [19].

Considering the background of research in the field of cellulose extraction using deep eutectic solvents, studies showed that sufficient information is not available about the extraction of cellulose from corn leaves using a combination of choline chloride and glycerol and determining their optimal ratio. Therefore, in the present study, cellulose extraction from corn husks using this combination was carried out in three ratios of 1:1, 1:2 and 1:3 (fixed ratio of 1 for choline chloride and variable ratio of 1 to 3 for glycerol) to accurately evaluate and compare the efficiency of each of these ratios in separating and extracting cellulose. For this purpose, to determine the appropriate solvent, items such as the amount of holocellulose obtained, lignin and hemicellulose (as impurities) remaining in the extracted celluloses were measured. Also, the characteristics of the different solvents used were evaluated to conduct a more detailed study in the field of selecting the optimal solvent. Finally, Fourier transform infrared spectroscopy analysis was also performed on the optimized cellulose to investigate the functional groups.

2- Materials and Methods

2-1- Materials

Corn leaves waste was obtained from farms in Isfahan province and used in cellulose extraction. Choline chloride, sodium chlorite, and glycerol were obtained from Merck, Germany, and used to make DES. Hydrochloric acid and glacial acetic acid were also obtained from Mojallali Company.

2-2- Preparation of deep eutectic solvent

To make the desired solvent, first, choline chloride (chcl) and glycerol (gly) were combined with molar ratios of 1:1, 1:2, and 1:3 of choline chloride to glycerol, and then subjected to magnetic stirring at 90°C for 2 hours until a completely clear fluid was obtained [13].

2-3- Measuring the properties of prepared solvents

2-3-1- pH measurement

After making deep eutectic solvents consisting of choline chloride (with a ratio of 1) as a hydrogen acceptor and glycerol (with different ratios of 1, 2, and 3), the pH of the deep eutectic solvents made with different ratios was measured using a pH meter model 3330 from Jenway, England, at a temperature of 25°C.

2-3-2- Viscosity measurement

The viscosity of the prepared DES solvents was measured using a Brookfield viscometer model DV-II+ Pro and an S-21 spindle at speeds of 10, 15, 20, 30 and 40 RPM at ambient temperature. Then, nonlinear power model fitting was performed with MATLAB 2023 software using shear rate and shear stress data and the model coefficients were extracted. The power model is given in equation (1). In this model, the coefficient k is the consistency index and n is the flow behavior index.

$$\tau = k\gamma^n \quad (1)$$

2-4- Cellulose extraction from corn leaves using deep eutectic solvent

Cellulose extraction was performed at 90°C for 2 hours using a hot plate and at a solids to solvent ratio of 1:15 with different ratios of choline chloride and glycerol. Three ratios of choline chloride to glycerol were used to prepare the deep eutectic solvent including 1:1, 1:2 and 1:3 to determine the optimal ratio based on the extraction parameters evaluated including yield, lignin, cellulose and hemicellulose. Finally, the resulting celluloses were dried in a Heraeus model fan oven at 55°C for 24 hours [13].

2-5- Extraction yield

To measure the extraction yield performed by DESs, the required calculations were performed according to equation (2).

$$\text{Extraction Yield} = \frac{\text{Weight of Extracted Cellulose (Dry)}}{\text{Weight of Initial leaf } (Dry)} \times 100 \quad (2)$$

2-6- Lignin Measurement

In this step, the residual lignin content in cellulose extracted from corn leaves was examined. In this method, a solids to acid ratio of 15:1 was used with 72% sulfuric acid at 30°C for 2 hours. After the 2-hour treatment, the acid was diluted to a concentration of 3% and heated at 110°C for 4 hours. Subsequently, to remove the acid solution and separate the lignin, the contents were filtered and to ensure complete removal of the acid, the filtered lignin was washed with distilled water. Finally, the lignins were dried in an oven at 55°C and their content was measured according to equation (3) [20, 21].

$$\text{Lignin Content}(\%) = \frac{\text{Weight of Dry Lignin}}{\text{weight of Initial Sample}} \times 100 \quad (3)$$

2-7- Holocellulose Measurement

First, 2 g of the sample was weighed and then 150 ml of distilled water was added to it. Then, 5 g of sodium chlorite (NaCLO2) and 1.08 ml of glacial acetic acid were added to the sample and it was subjected to magnetic stirring at 96°C for 90 minutes. The resulting holocellulose was dried in an oven at 55°C for 24 hours. Finally, the percentage of holocellulose was calculated according to equation (4) [22].

$$\text{Holocellulose}(\%) = \frac{\text{Weight of Dry Holocellulose}}{\text{Weight of Initial Sample}} \times 100 \quad (4)$$

2-8- Measurement of Cellulose and Hemicellulose

To measure cellulose, 2 g of holocellulose was weighed, 100 ml of 1% sodium hydroxide was added to it, and it was stirred magnetically for 60 minutes at 100°C. The resulting cellulose was finally dried in an oven at 55°C for 24 hours. The percentage of cellulose and hemicellulose obtained was calculated according to equations (5) and (6) [23].

$$\text{Cellulose}(\%) = \frac{\text{Weight of Dry Cellulose}}{\text{Weight of Initial Sample}} \times 100 \quad (5)$$

$$\text{Hemicellulose}(\%) = \text{Holocellulose}(\%) - \text{Cellulose}(\%) \quad (6)$$

2-9- Fourier Transform Infrared Spectroscopic Analysis (FTIR)

The FTIR test was used to investigate the functional groups present in the selected optimal sample by various chemical tests, including the efficiency and residual impurities such as lignin and hemicellulose. During this test, the selected optimal sample was mixed with potassium bromide and after compression, special tablets were prepared for the device. Measurements were performed in the wavelength range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1} and the functional groups present in the optimal sample were determined [13].

2-10- Statistical Analysis

In order to statistically examine the data obtained from cellulose extraction with deep eutectic solvents and compare the quantitative and qualitative characteristics of the product (including the amount of cellulose, holocellulose, hemicellulose and lignin), a completely randomized design (CRD) was used. All experiments were performed in triplicate and the data were analyzed using one-way analysis of variance. To compare the means and detect significant differences between treatments (different solvent ratios), a post hoc least significant difference (LSD) test was used at a 95% confidence level ($p < 0.05$). All statistical analyses in this study were performed using SPSS software.

3- Results and Discussion

3-1- pH

Measurement of the pH of deep eutectic solvents with molar ratios of 1:1, 1:2 and 1:3 at

ambient temperature showed that this index had a significant difference and its value was 4.39 ± 0.01, 4.09 ± 0.02 and 4.28 ± 0.01, respectively. The obtained numbers are also consistent with the results of Sazali et al. (2023) [24]. This is a result of the relative stability of pH in this range, which indicates the chemical stability of the synthesized solvents and is considered one of the main advantages of DES systems based on choline chloride. This feature allows the extraction process to be carried out under controlled and repeatable conditions. By increasing the ratio of glycerol as a hydrogen bond donor from 1:1 to 1:2, the pH value decreased and then increased again at a ratio of 1:3. These fluctuations in pH can be attributed to changes in the strength of the hydrogen bond network between choline chloride and glycerol. In fact, molecular interactions in different proportions change the availability of free protons, leading to changes in the concentration of hydrogen ions in the environment.

3-2- Viscosity

The viscosity index of deep eutectic solvents with three different ratios was evaluated at five shear rates. The changes in the viscosity of solutions with different ChCl:Gly ratios are shown in Figure 1. It is clear from the results that in all three ratios, with increasing shear rate (from 10 to 40), the viscosity decreased significantly. This behavior indicates the shear thinning or non-Newtonian behavior of these solvents. At the molecular scale, this phenomenon occurs due to the temporary breaking of the hydrogen bond network between choline chloride and glycerol due to the application of shear force, which facilitates the movement of fluid layers over each other.

In general, the viscosity of the solvent with a ratio of 1:3 of choline chloride to glycerol is higher at all measured points than the viscosity of the solvents prepared with the other two ratios of ChCl:Gly. This is due to the presence of a higher amount of glycerol in this ratio; glycerol, due to its high intrinsic viscosity and strong hydrogen network, causes the system to behave similar to pure glycerol in this region [25, 26]. An interesting result is that with increasing the glycerol ratio from 1:2 to 1:3, the viscosity increased. This indicates that the presence of an optimal amount of hydrogen bonding agent is essential for reducing the viscosity. Increasing the glycerol ratio to 1:3 probably led to the formation of a denser network of hydrogen bonds between glycerol molecules, which ultimately increased the internal friction and solvent viscosity. In mixtures with a high glycerol ratio, choline chloride plays a mainly plasticizer role and weakens part of the network, but with further increase in glycerol, the glycerol concentration and the number of hydrogen bonds again become so high that under high shear rate conditions, even after partial orientation and network breakage, the residual structure is still very dense and maintains a higher viscosity than 1:2 and even 1:1 [27-29]. In the solvent with a 1:1 ratio of ChCl:Gly, although the initial viscosity is high, a significant part of this viscosity is due to structural inhomogeneities that are more easily broken under severe shear, as a result, the viscosity curve of the solvent with a 1:1 ratio shows a greater drop than that of 1:3 [27, 29].

Comparing the viscosity of solvents with different ratios of ChCl:Gly shows that the solvent with a ratio of 1:2 of choline chloride to glycerol has the lowest viscosity at all shear rates. This finding is very important from a mass transfer perspective, because the lower viscosity at the ratio of 1:2 facilitates the penetration of the solvent into the dense lignocellulosic structure of corn leaf and leads to improved extraction of impurities and better release of cellulose. However, in the mass transfer phenomenon, in addition to viscosity, other factors also influence this phenomenon, the sum of their effects determines the overall efficiency.

Based on the results of the power model fitting given in Table 1, the power model has high R^2 and low RMSE in all samples, which indicates the suitability of the power model for predicting the rheological behavior of the solvent during stirring. In all ratios of choline chloride to glycerol, the value of $n < 1$ indicates pseudoplastic or non-Newtonian behavior. The solvent with a ratio of 1:1 showed the lowest value of the flow behavior index ($n = 0.3617$). Therefore, the highest non-Newtonian behavior among the samples is related to this sample. With increasing shear rate, the structure of this solvent is rapidly destroyed and its resistance to flow decreases. This is usually due to the presence of very dense and intertwined hydrogen networks in high concentrations of choline chloride, which easily change direction when stress is applied. Also, in the solvent with

a ratio of 1:3, compared to other samples, the flow behavior index is closer to 1. In fact, the presence of high-ratio glycerol causes the solvent structure to act more uniformly and reduces the sensitivity of viscosity to shear rate, which contributes to the uniformity and predictability of the solvent behavior during the extraction process. Also, the highest consistency index is related to the sample with the highest amount of glycerol.

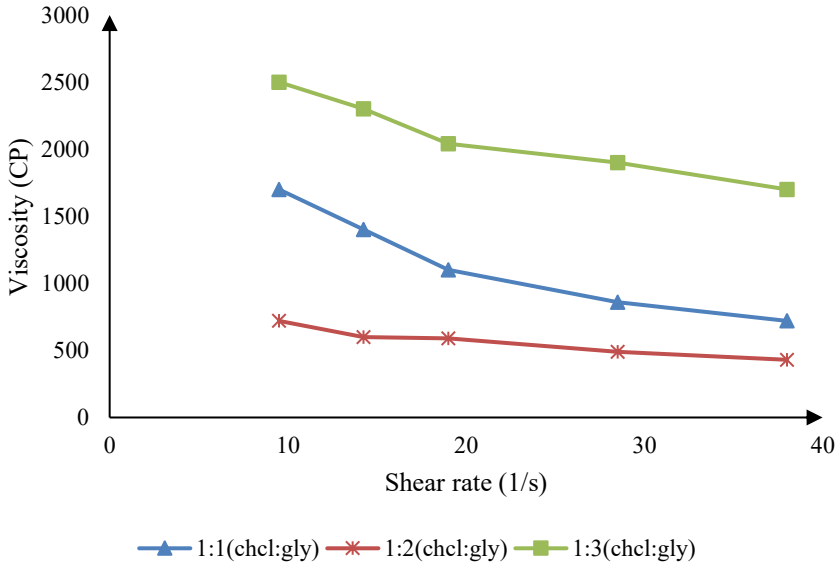

Figure 1- The viscosity of 3 different ratios of DES composed of choline chloride and glycerol at 4 different shear rates (All tests were performed in 3 replicates with 95% level of significance)

Table 1- Results of the solvent power-law model

chcl:gly	k	n	R^2	RMSE
1:1	7.327	0.3617	0.93	0.98
1:2	1.689	0.6273	0.98	0.49
1:3	4.786	0.7178	0.99	0.90

3-3- Results from cellulose extraction with 3 different deep eutectic solvent ratios

3-3-1- Extraction yield

The cellulose extraction yield is shown in Figure 2. As is clear, the cellulose extraction yield using solvents with ratios of 1:1 and 1:2 did not differ significantly ($p<0.05$). On the other hand, the cellulose extraction yield obtained from the solvent with a ratio of 1:3 choline chloride to glycerol was significantly ($p<0.05$) higher than the extraction yield calculated using solvents with the other two ratios of choline chloride to glycerol. This significant improvement in the extraction efficiency using the solvent with a ratio of 1:3 choline chloride to glycerol is probably due to the fact that the concentration of hydroxyl groups (-OH) in the solvent increases with the increase in the ratio of glycerol (hydrogen donor) from 1 to 3. These groups have a high ability to hydrogenate with oxygens present in cellulose and lignin. This reinforced cross-linking network allows the solvent to have a greater ability to penetrate into the fibers and dissolve the cellular matrix [12].

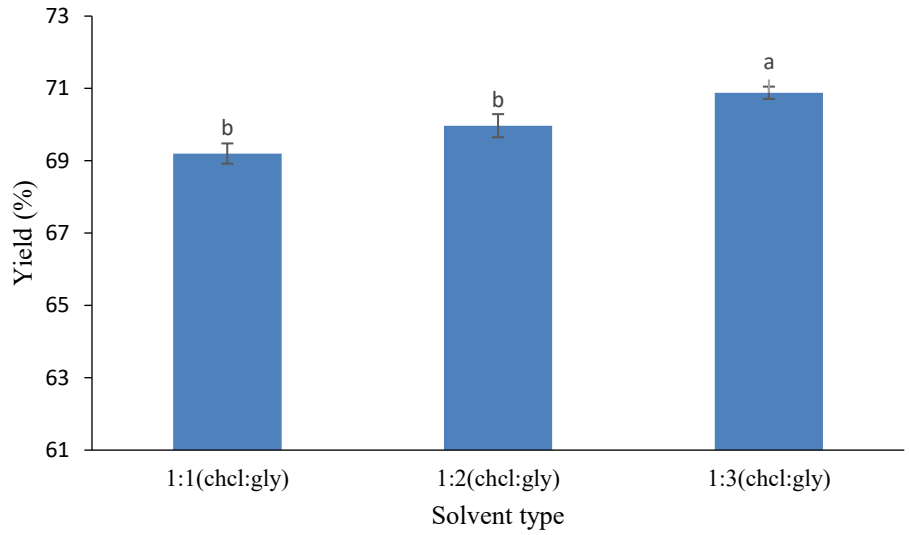

Figure 2- The cellulose extraction yield (All tests were performed in 3 replicates with 95% level of significance)

3-3-2- Lignin content

The results of the residual lignin content in celluloses obtained from different solvents (different ChCl:Gly ratios) are given in Figure 3. The lignin content in the ChCl:Gly ratios is 1:2>1:1>1:3 and they were significantly different from each other ($p<0.05$). Therefore, it is clear that increasing the amount of HBD (glycerol) in addition to increasing the yield led to more lignin removal. Therefore, this result shows the appropriate selectivity of this group of green solvents in cellulose extraction. Therefore, with increasing the HBD (glycerol), a significant decrease in lignin was observed. Therefore, the breaking power of hydrogen bonds between lignin and cellulose increased. The selectivity of deep eutectic solvents in extraction has been previously reported [30]. The amount of residual lignin in cellulose extracted with different molar ratios of ChCl:Gly was as follows: the ratio of 1:2 had the highest amount of residual lignin, then the ratio of 1:1 and finally the ratio of 1:3 had the lowest amount of lignin. This observation can be justified based on the changes in the viscosity and surface properties of the green solvent with the change in the molar ratio of its two components. The reason for the results of this study in the lignin section seems to be related to the different efficiency of the solvent in different molar ratios of these two components. As previously proven in the study of Firouzi et al. (2025), the yield of lignin removal does not always increase with increasing HBD ratio, but depends on the optimal solvent ratio [13]. Therefore, it is likely that the pattern observed in the present study has similar reasons. Therefore, a simple increase in glycerol does not always lead to greater lignin removal, but the optimal solvent ratio needs to be determined to maximize lignin removal [31]. On the other hand, according to some researchers, due to the formation of a complex between lignin and other carbohydrates, there is a possibility that lignin is not completely removed and remains in the solid material [32, 33]. Firouzi et al. (2025) showed in a study that with an increase in the molar ratio of glycerol to choline chloride in the deep eutectic solvents made, the ratio of cellulose to lignin increases [13]. On the other hand, Shen et al. (2019) also reported that the separation of lignin from poplar wood by deep eutectic solvents consisting of choline chloride and lactic acid in a ratio of 1:10 was very high and had a separation rate of 93.2% [34]. Chen et al. (2019) mentioned the reason for the delignification of deep eutectic solvents as the breaking of most of the b-O-4 bonds between lignin and carbohydrates. In addition, due to the interaction principles of HBA and HBD, the ether bonds between phenylpropane units are easily broken and delignification is carried out. It should be noted that the lignin separation may be due to the acid catalysis behavior of HBD [35].

Amran et al. (2022) also delignified palm leaves and clusters using choline chloride and glycerol in different ratios. Their results also indicated that the ratio of 1:3 had the highest delignification rate, and its rates for fruit clusters and palm leaves were 27.6 and 16.1

percent, respectively. They also mentioned that the presence of chloride ions in ChCl, which target β-O-4 bonds and successfully break the ether bonds in the lignin-carbohydrate bond, was one of the reasons for the success in lignin separation [36]. Nisa et al. (2025) used a 1:3 ratio of choline chloride and glycerol to separate lignin from sugarcane bagasse. They achieved a separation rate of 80.14 percent at a temperature of 100°C and a solid-to-liquid ratio of 1:20. On the other hand, their cellulose content increased significantly, introducing this solvent as a suitable alternative to hazardous solvents [37].

Hong et al. (2022) used a 1:1 mixture of choline chloride and oxalic acid to extract cellulose from non-woody waste of loofah sponge. Their test temperature and time were 90°C and 150 min. The resulting cellulose content was 76.4 and the residual lignin was 10.7 [38]. Suriyachai et al. (2024) used a 1:1 mixture of choline chloride-glycerol and choline chloride-lactic acid for delignification of sugarcane bagasse. The lignin separation rate of these two mixtures was about 62 and 70%, respectively [39]. These results indicate that the type of HBD has a significant effect on lignin separation and the reason for the lower delignification of glycerol compared to lactic acid and oxalic acid, as explained by Amran et al. (2022), is due to the weak hydrogen bonding between the chloride ion and the hydroxyl group of DES and also due to the low acidity of DES [36]. However, it should be noted that although acidic DES may lead to greater removal of impurities including lignin and hemicellulose, ChCl-Gly offers a greener and less corrosive alternative. On the other hand, the results mentioned suggest that reducing the amount of HBD has reduced the yield of cellulose obtained and lignin separation. Also, in the 1:3 ratio, there are more hydroxyl groups and according to the theory of Zhang et al. (2016), the free hydroxyl groups of polyols interact with the free hydroxyl and ether groups of lignin, which allows the breaking of the lignin-carbohydrate complex bonds and the separation of lignin [40]. In general, the residual lignin content in the cellulose fraction decreases with increasing glycerol ratio, but its complete removal is rarely achieved without acid additions or further processing [39].

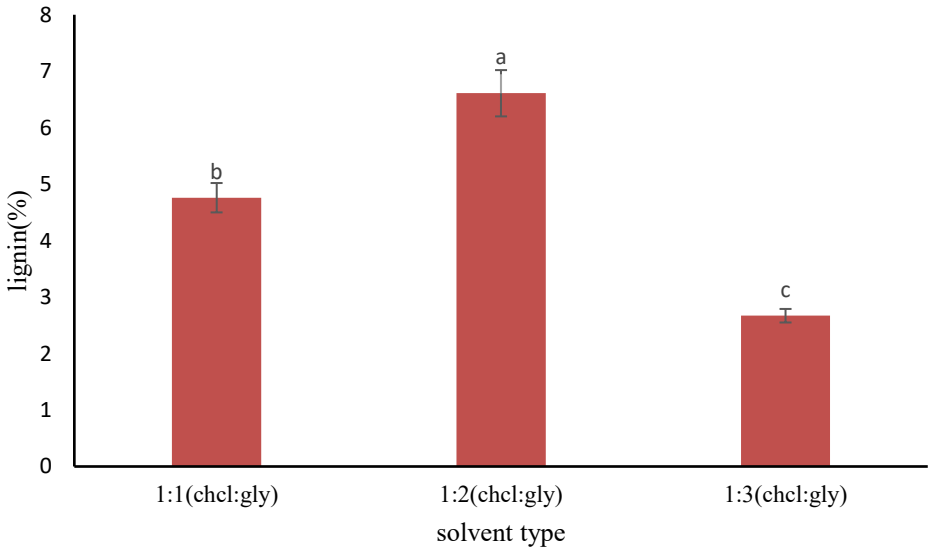

Figure 3- Amount of remaining lignin (All tests were performed in 3 replicates with 95% level of significance)

3-3-3- Holocellulose, cellulose and hemicellulose content

The results of this section are shown in Figure 4. According to the results, the amount of cellulose was 1:3>1:2>1:1. On the other hand, the amount of holocellulose obtained from the extraction using a solvent with a ratio of 1:3 ChCl:Gly was significantly different from the ratio of 1:1, and its hemicellulose was significantly lower than the ratio of 1:1 ChCl:Gly. This also confirms the higher amount of cellulose obtained from the ratio of 1:3 because holocellulose is a combination of cellulose and hemicellulose, which, given the lower amount of hemicellulose, indicates that the cellulose obtained from the ratio of 1:3 is higher. Increasing the amount of hydrogen bond donor (glycerol) leads to greater cellulose preservation and increased pure cellulose, and

this result indicates that the cellulose structure is less damaged and the final purity is higher. This result is also consistent with the research of Nissa et al. (2025) [37]. Firouzi et al. also reported that a deep eutectic solvent consisting of choline chloride and glycerol increased the yield and purity of cellulose. In one of their treatments, they used a 1:4 ratio of ChCl:Gly, and the resulting cellulose crystallinity was reported to be 81.4%, an increase of 5.2% compared to untreated hemp [13].

Hossein et al. (2021) reported in a study using the deep eutectic solvent chcl:Gly (with a molar ratio of 1:2) that by performing the reaction in a ratio of 1:20 of solid to solvent at a temperature of 150°C for 15 hours, 52% by weight of the initial lignin present in rice straw was removed, while more than 91% of glucan (cellulose) remained in the solid. This high level of glucan retention indicates the appropriate yield of this solvent in cellulose extraction and selective lignin removal. One of the reasons for the presence of very high glucan resulting from their treatment could be due to their high temperature and long time (150°C and 15 hours). On the other hand, the removal of 29.6% of xylan could also indicate the effective removal of hemicellulose [31]. Rodrigues et al. (2024) used a 1:5 ratio of choline chloride and lactic acid to treat corncobs and succeeded in reducing the amount of hemicellulose from 37.26 to 18.60 percent and increasing the amount of cellulose from 38.69 to 68.89 percent [41]. Zhang et al. (2016) also treated corncobs using a 1:2 ratio of choline chloride to glycerol, and their results indicated an increase in glucose content compared to the untreated sample, and this increase in glucose itself could be a reason for the increase in the amount and purity of cellulose [40].

It can be stated that increasing the glycerol ratio (from 1:1 to 1:3) generally increases the yield and purity of cellulose but may decrease the solubility and yield of lignin [36]. In view of this, at ratios of 1:2 or 1:3 of choline chloride to glycerol, higher cellulose purity can be achieved by reducing hemicellulose and lignin contamination. In particular, it can be noted that the ratio of 1:3 causes significant removal of lignin and hemicellulose, leading to an increase in the yield and purity of cellulose [13, 36].

According to the results of the aforementioned research and as shown in Figure 4, the ratios of 1:2 and 1:3 lead to the removal of a greater amount of hemicellulose, which is also consistent with the results mentioned above. Based on these results, deep eutectic solvents with a ratio of 1:3 of choline chloride to glycerol were more selective because they were able to dissolve hemicellulose more easily and also retain cellulose better.

From the results, it can be inferred that, considering all extraction conditions being the same and the difference in molar ratios made from deep eutectic solvents, the ratio of 1:3 significantly increased the extraction yield, increased cellulose, and reduced impurities such as lignin and hemicellulose, and was a more suitable ratio for extracting cellulose from corn leaves.

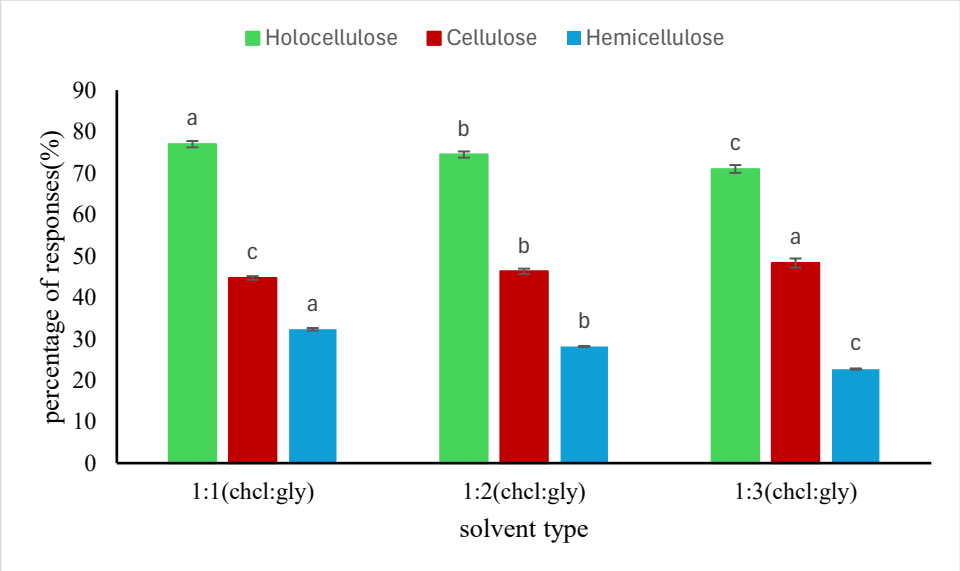

Figure 4- Amount of holocellulose, cellulose and remaining hemicellulose (All tests were performed in 3 replicates with 95% level of significance)

3-3-4- FTIR result of optimized cellulose

Considering that the cellulose obtained from the treatment with a deep eutectic solvent consisting of choline chloride and glycerol in a ratio of 1:3 had the least impurities and the highest purity among the other samples, for this reason it was selected as the selected sample for the FTIR test to examine its functional groups. According to Figure 5, the results of the FTIR spectrum of the sample in question are as follows: at the wavenumber 897 cm^{-1}, a peak related to the C-H bond and the stretching of the C-O-C bond of the β-(1→4) glycosidic linkages is seen, which is related to cellulose. At the wavenumber 1050 cm^{-1}, stretching vibrations related to the C-O and C-C groups related to cellulose are observed. The wavenumber 1163 cm^{-1} indicates the vibrations of the O-C-O glycosidic bond. This peak can indicate the cellulose present in the cell wall. The wavenumber 1249 cm^{-1} is related to the stretching of acetyl groups in lignin and hemicellulose, and the wavenumber 1474 cm^{-1} is related to the stretching of C-H in lignin and hemicellulose. On the other hand, the wavenumber 1637 cm^{-1} indicates the vibration of the aromatic C=C bond related to lignin, and the wavenumber 1735 cm^{-1} indicates the ester bond between lignin and hemicellulose. All these cases indicate the presence of some impurities in the sample, which is also consistent with the reported chemical analyses. The wavenumber 1375 cm^{-1} also indicates cellulose, and the wavenumber 1427 cm^{-1} also indicates the CH_2 bond and the symmetrical bending of this group, which is related to cellulose. The wavenumber 2920 cm^{-1} indicates the stretching vibration of the C-H bond, which is related to cellulose. Also, the wavenumber 3430 cm^{-1} is the stretching vibration of the O-H bond. The aforementioned results are all consistent with the research of Firouzi et al. (2025) and the research conducted by An et al. (2025) and confirm the pattern of the resulting peaks [3, 13, 23, 42].

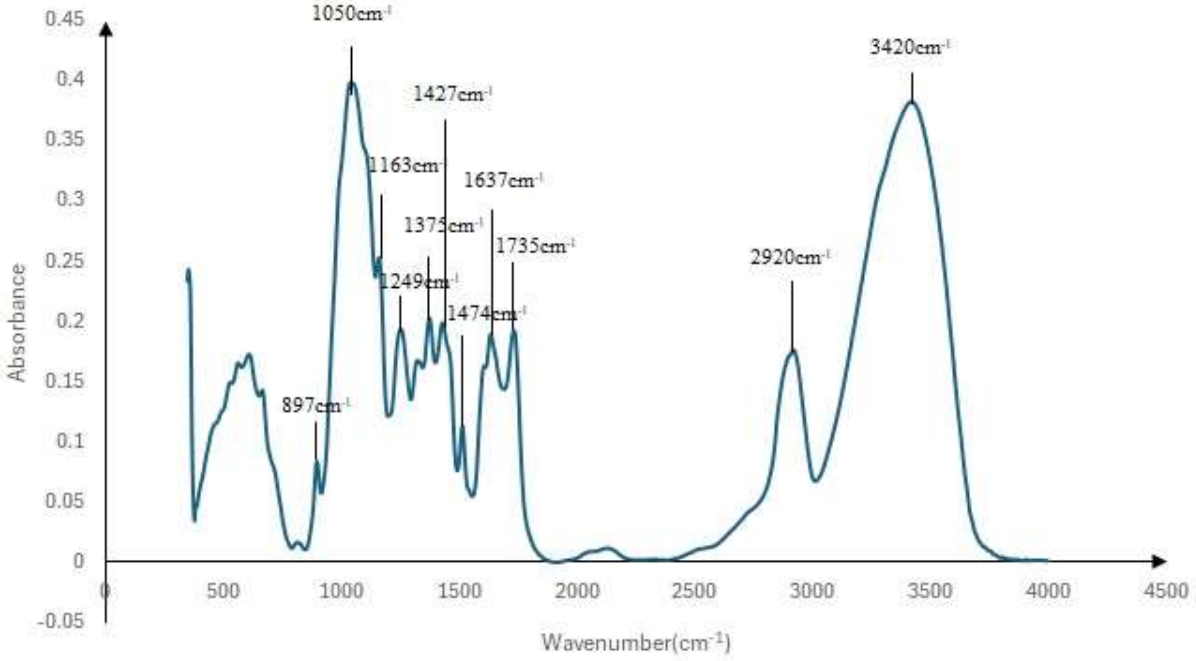

Figure 5- FTIR spectra of the optimized sample (cellulose obtained from the solvent with a 1:3 ratio (chcl:Gly))

4- Conclusion

The use of deep eutectic solvents such as the combination of choline chloride and glycerol can be considered as an environmentally friendly method for the effective extraction of cellulose from corn waste. In the resulting study, the ratio of 1:3 of choline chloride to glycerol was chosen as the preferred ratio for the extraction of cellulose from corn leaves because it was superior in terms of yield and purity of the resulting cellulose compared to the other two ratios and showed better performance. Increasing the concentration of the hydrogen bond donor (glycerol) to a ratio of 1:3 led to a more effective breaking of hydrogen bonds between cellulose and lignin and hemicellulose. The relationship between the molar ratio of DES solvents and the yield of

cellulose extraction is directly related to the ability of these solvents to disrupt the internal hydrogen bonds of plant fibers. The improved performance, despite the higher viscosity, is due to the higher concentration of the HBD (glycerol), which has a greater tendency to break the lignin-cellulose structure. Although the 1:2 ratio is the most optimal in terms of rheology, the 1:3 ratio is chemically superior and is recommended as the final solvent for cellulose extraction from corn leaves. The reason for this choice is the significantly higher yield of the 1:3 ratio (70.88±0.17) compared to solvents with a ratio of 1:2 (69.97±0.32) and 1:1 (69.2±0.28), as well as better removal of impurities including lignin and hemicellulose and less of these impurities remaining in the cellulose obtained from the 1:3 ratio of choline chloride to glycerol compared to other ratios (the amount of remaining lignin in the cellulose obtained from the 1:3 ratio is 2.67±0.12 and the remaining lignin in the cellulose obtained from the 1:2 and 1:1 ratios is 6.61±0.41 and 4.76±0.26, respectively. Also, the residual hemicellulose in the cellulose obtained from the ratio of 1:3, 1:2 and 1:1 of choline chloride to glycerol was 22.7±0.15, 28.16±0.12 and 32.29±0.31, respectively), showing the superiority of the 1:3 ratio. Also, the amount of cellulose obtained in this ratio was 48.28±1.1, which was significantly higher than the cellulose obtained from the ratio of 1:2 (46.3±0.63) and 1:1 (44.68±0.45).

Data Availability

Research data are included in the text and will not be shared outside of the rules of this journal.

Conflict of Interest

The authors declare that there is no financial or personal conflict of interest in relation to this research.

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Author Contributions

It is hereby confirmed that all authors have actively participated in the design, analysis, writing and approval of the final version of this article.

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مقاله علمی_پژوهشی

استخراج سلولز از پوست ذرت با استفاده از حلال‌های سبز، ارزیابی ویژگی‌ها و خلوص سلولزحاصل

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چکیده

فرآیند استخراج زیست ترکیبات می‌تواند در تکمیل زنجیره ارزش محصولات کشاورزی با اهمیت باشد. یکی از روش‌های سازگار با محیط زیست برای استخراج ترکیبات مفید، استفاده از حلال‌های یوتکتیک عمیق (DESs) است. پژوهش حاضر با هدف استخراج سلولز از پوست ذرت به کمک حلال یوتکتیک عمیق انجام شد که در آن، کولین کلراید (ChCl) به عنوان پذیرنده پیوند هیدروژنی و گلیسرول (Gly) به عنوان دهنده پیوند هیدروژنی مورد استفاده قرار گرفت. در این پژوهش، ابتدا ویژگی‌های فیزیکوشیمیایی حلال‌ها، شامل pH و گرانروی، با نسبت‌های مولی متفاوت ۱به۱، ۱به۲ و ۱به۳ ازChCl بهGly بررسی شد. سپس برای تعیین مناسب‌ترین نسبت حلال‌ها طی استخراج سلولز، فرآیند استخراج با سه نسبت متفاوت ChCl:Gly انجام و شاخص‌هایی همچون بازده استخراج، میزان هولوسلولز، سلولز، لیگنین و همی‌سلولز ارزیابی گردید. مشخص شد که با تغییر نسبت کولین کلراید به گلیسرول در حلال یوتکتیک، اختلاف معناداری در pH نمونه‌ها وجود ندارد. کمترین ویسکوزیته مربوط به نمونه با نسبت حد وسط ChCl:Gly بود. مشخص شد که با افزایش نسبت مولی گلیسرول از ۱ به ۳، شاخص رفتار جریان از ۰/۳۶ به۰/۷۱ افزایش می‌یابد. همچنین نتایج نشان داد که در نسبت‌های ۱به۱، ۱به۲ و ۱به۳، بازده استخراج به ترتیب ۰/۲۸±۶۹/۲، ۰/۳۲±۶۹/۰ و ۰/۱۷±۷۰/۸۸ درصد و محتوای سلولز ۰/۴۵±۴۴/۶۸، ۰/۶۳±۴۶/۳ و ۱/۱±۴۸/۲۸ درصد می‌باشد. همچنین میزان ناخالصی لیگنین در سلولز استخراج شده بوسیله حلال دارای نسبت ۱به۳ کولین کلراید به گلیسرول، به‌طور معناداری کمتر از دو نسبت دیگر بود که نشان‌دهنده کارایی بالاتر حلال دارای این نسبت از ChCl:Gly در خالص‌سازی سلولز است.