

Delignification of Hemp Stalk Using a Low Transition Temperature Mixture Composed of Choline Chloride and Lactic Acid

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This work used a literature search to select the most suitable system for the delignification process of annual plants. This process was evaluated in terms of lignin removal from plant biomass. Based on previous experiments, a low transition temperature mixture (LTTM) was used, which was a mixture of choline chloride and lactic acid in a molar ratio of 1:2. Samples of hemp fibers were examined. The delignification of biomass was monitored by changing the content of the main components. After application of the solvent at a temperature of 100, 120, or 140 °C and a delignification time of 2 or 4 h, the lignin content expressed by Kappa number decreased to the level of 22 to 13.7. Yields of hemp fibers sown after LTTM application ranged from 72.5 to 90.6%. The results confirmed that the use of LTTM (choline chloride/lactic acid) is a suitable system for the extraction of lignin from biomass. The findings showed that LTTM was an effective delignifying agent for hemp, as a relatively low Kappa number of 13.7 and a yield of 75.9% were achieved after only 2 h and at 140 °C.

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INTRODUCTION

Replacing commonly used solvents with green solvents is a very wide area of research and development. A low transition temperature mixture (LTTM) is a green solvent. LTTMs are rapidly evolving and are being used as alternative solvent systems for processing lignocellulosic materials. The isolation of fibers from annual plants is currently becoming a crucial topic, given the availability of wood biomass. Several methods have been developed for the pretreatment of lignocellulosic materials (biomass) to obtain a high yield of the desired material. All of these methods must be adapted to the specific source of lignocellulosic biomass (Jablonský *et al.* 2015). Several studies have focused on the influence of LTTM in the biomass pretreatment process and on its final composition. Eutectic mixtures have been used to dissolve parts of the biomass (Škulcová *et al.* 2016). Most studies investigating the dissolution of lignocellulose have used eutectic mixtures based on choline chloride as a hydrogen bond acceptor. The most-commonly used donors in various studies were lactic acid, oxalic acid, and glycerol. Several articles have described the delignification of different types of biomass using deep eutectic solvents (DES)/LTTM, and many applications of DES/LTTM have been published (Vigier *et al.* 2015; Kumar *et al.* 2016a). Delignifications of wheat straw (Jablonský *et al.* 2015; Jablonský *et al.* 2019a; Lou *et al.* 2019; Suopajäri *et al.* 2020) and rice straw (Kumar *et al.* 2018; Gao *et al.* 2020), of

various types of wood (willow, poplar, beech, oak) (Alvarez-Vasco *et al.* 2016; Xia *et al.* 2018; Jablonský *et al.* 2019a; Tian *et al.* 2020), or delignification of corn cob (Xu *et al.* 2018) have been described. In addition to these types of biomass, several research papers from various authors have been added in recent years, focusing on oil palm empty fruit bunch pretreatment, eucalyptus, and pulp delignification (Nor *et al.* 2016; Škulcová *et al.* 2017; Liang *et al.* 2019; Jablonský *et al.* 2019b). Kumar *et al.* (2016a) researched the application of DES to the processing of rice straw. The delignification efficiency ranged from 64 to 75% using the choline chloride/lactic acid (ChCl/LacA) system in different molar ratios (1:2; 1:5; 1:9) (Kumar *et al.* 2016a). Another study focused on optimizing the extraction of lignin from wheat straw using LTTM (choline chloride and lactic acid). The LTTM used could extract high purity lignin (94.8%) in high yield (81.5% from air-dried samples and 85.9% from oven-dried samples) from wheat straw (Lou *et al.* 2019). In research of valorization of lignin from biomass (Alvarez-Vasco *et al.* 2016), soft and hard wood were selected, namely Douglas fir and poplar. Maximum lignin yields were observed at 145 °C from all DES/LTTM. ChCl/LacA was the best LTTM for lignin extraction with 58.2% lignin yield.

Various processes of delignification of poplar wood using LTTM based on lactic acid and various hydrogen bond acceptors (choline chloride, glycine) were studied. The extraction process was temperature and time dependent for both DESs/LTTMs. LacA/ChCl treatment represented approximately 90.4% lignin removal and lignin yield from poplar 78.5% (Li *et al.* 2019). This study showed that the solubility of poplar wood flour in ChCl/LacA is higher than in other LTTM, representing more than 50%, indicating that ChCl/LacA is the optimal LTTM for dissolving wood flour (Chen *et al.* 2019). Li *et al.* (2017) showed that ChCl/LacA treatment had a significant effect on lignin isolation. DES/LTTM types, molar ratio of ChCl to hydrogen bond donors, extraction temperature and yield time of fractionated DES-lignin showed that the optimal yield of LTTM lignin (91.8% by weight, based on the initial lignin in the willow) with a high purity of 94.5 % was achieved at a ChCl/LacA molar ratio (1:10), an extraction temperature of 120 °C and a time of 12 h. In the case of ChCl and LacA (weak organic acid $K_a = 1.34 \times 10^{-4}$), extraction of hemicelluloses and lignin may also occur, as documented by other studies where this combination is used to remove lignin from wood or annual plants (Alvarez-Vasco *et al.* 2016; Chen and Wang 2018; Jablonský *et al.* 2015, 2019; Zhang *et al.* 2016; Li *et al.* 2017).

In recent years, raw materials such as rice, corn, straw (Suopajärvi *et al.* 2020; Zhong *et al.* 2022) have been delignified, using LTTM composed of ChCl/Lactic acid. LTTMs can provide a mild acid-base catalytic mechanism that will initiate the controlled cleavage of labile ether bonds between phenylpropane units (Alvarez-Vasco 2016; Chen *et al.* 2019; Da Costa Lopes *et al.* 2020; Francisco *et al.* 2012; Ji *et al.* 2021; Li *et al.* 2021). The results of several recent studies (Alvarez-Vasco 2016; Chen *et al.* 2019; Da Costa Lopes *et al.* 2020; Francisco *et al.* 2012; Ji *et al.* 2021; Li *et al.* 2021) suggest that LTTMSs containing choline chloride and lactic acid may have a unique ability to dissolve phenolic substances because of the effect of protolytic reactions. When applying the choline chloride and lactic acid system to extract lignin from biomass, it has been shown that recycling of this system is possible using water and even more advantageous if ethanol is used to recover lignin and to recycle the solvent system (Smink *et al.* 2020). The mentioned results suggested that a system for recycling and recovering lignin may be economically advantageous. In the screening of various LTTM based on choline chloride, the lactic acid and choline chloride system extracts the highest amount of lignin (Jablonský and Šima 2019; Fernandes *et al.* 2020). Based on the literature search and results from various studies that

showed increased efficiency in lignin extraction from plant biomass compared to other LTTM used, the LTTM system based on ChCl/LacA was used for the delignification of hemp stalks. This process was evaluated in terms of lignin removal from plant biomass.

EXPERIMENTAL

Materials and Chemicals

Hemp stalk from OP papirna s.r.o., Olšany, Czech Republic were used as raw material. All reagents were of analytical grade. Choline chloride (ChCl) ($\geq 98.0\%$) was purchased from Sigma-Aldrich (Taufkirchen, Germany). A 90.0% lactic acid solution (LacA) was obtained from VWR International (Bratislava, Slovakia). Choline chloride was dried in vacuum.

Prior to the individual delignification processes, the properties of the original raw materials were determined on a sample of fibers of annual plants (hemp stalk). The resulting average values of holocellulose content (Wise *et al.* 1946), dry weight (%) (TAPPI T264 cm-97, 1996), and lignin content (TAPPI Method T222, 1998) in the samples of hemp stalks are given in Table 1.

Table 1. Average Values of Original Hemp Raw Material

Sample	Dry Weight (%)	Holocellulose (%)	Klason Lignin (%)
Hemp	94.3 \pm 0.2	87.0 \pm 1.9	6.4 \pm 1.2

Preparation of DES

The solvent (LTTM) consisted of choline chloride and lactic acid in a molar ratio of 1:2; water content was 5.4% by weight. LTTM were prepared by mixing the mixture at max 80 °C for 30 min in a flask on a vacuum evaporator (without vacuum, until the choline chloride dissolved), resulting in a clear homogeneous liquid (Jablonský and Šima 2019).

Pulping Conditions

Samples of hemp stalks (21.2 g hemp stalks, (20 g absolutely dry hemp stalks, size 1.0 to 1.5 cm) were combined with 200 g LTTM (ChCl/LacA, 1:2), *i.e.*, the ratio of absolutely dry fibers to LTTM was 1:10. The prepared LTTM with the weighed hemp stalks was placed in a digester and the batch was started. During delignification, the temperature was monitored and recorded. After reaching the required temperature, the holding time was tracked according to the parameters of individual batches. The batch of hemp stalks was carried out in 1 L autoclaves placed in a rotary digester with an oil bath at the time of delignification (2 and 4 h) at a maximum temperature of 100, 120 and 140 °C with a lye to fiber ratio of 10:1. The heating up of batch/samples to the cooking temperature was done as follows: for 100 °C heating time was 70 min, for 120 °C it was 90 min, for 140 °C it was 110 min. At the end of the batch time, the digester was cooled, and the (LTTM) liquors were filtered on a Büchner funnel. After delignification, the fibers were washed with water until they reached neutral pH. They were pulped with water in a mixer and washed again with water using a sachet. The filter cake was air dried.

Pulp Characterization

Holocellulose was quantified by sodium chlorite treatment (Wise *et al.* 1946). The

Kappa sample number determined according to T236 cm-85 (1996) was used to estimate the lignin content. The yield (Y) was determined after individual delignification processes of annual plant fibers and was calculated according to Eq. 1,

$$Y (\%) = m / n \times 100 \quad (1)$$

where n is the amount of absolutely dry fibers fed to the digester, and m is the amount of absolutely dry fibers obtained after cooking.

RESULTS AND DISCUSSION

In the work of Jablonský and Šima (2019), the results of the use and efficiency of DESs/LTTMs for the delignification of different types of biomass were summarized. According to this quantification of efficiency of lignin removal, regardless of the type of biomass and delignification conditions (molar ratio, time, solid/liquid ratio, and applied technique), ChCl/LacA is the most effective in lignin removal. The summarized studies show that to achieve effective delignification and to shorten the delignification time (less than 3 hours) it is necessary to increase the delignification temperature, and a temperature of 100 to 140 °C seems to be suitable (Alvarez-Vasco *et al.* 2016; Kumar *et al.* 2018; Li *et al.* 2017). Therefore, in this work, we have chosen this range of operating temperatures. The work evaluated the delignification process in terms of the efficiency of lignin removal from plant biomass using solvents (Kumar *et al.* 2016b; Li *et al.* 2017; Chen *et al.* 2019) and used in the delignification process of annual plants (hemp fiber). The main part of the experiment was delignification processes, which took place at three temperatures (100, 120, 140 °C) and in 2-h and 4-h time intervals. Delignifications were performed on a sample of hemp sieved with LTTM, which was a 1:2 molar ratio of ChCl/LacA. Sample 1 was delignified at 100 °C, for 2 or 4 h. The Kappa number was determined after each batch and reached 22 ± 0.3 (2 h) and 21.5 ± 0.5 (4 h) after delignification. The holocellulose content and yield were determined after each delignification process, and the results are shown in Table 2. At 120 °C, 2 and 4 h, sample 2 was delignified. The kappa number decreased to 16.3 (2 h) and 17.2 (4 h). As the temperature increased, the Kappa number decreased, and based on the holocellulose content, there was less loss of holocellulose than for delignification at 100 °C. At the highest temperature, at 140 °C, the delignification of sample 3 took place, which was conducted in the same way at 2 and 4 h. At 140 °C and 2 h, a relatively low Kappa number of 13.7 and a yield of 75.9% were achieved. A higher content of holocellulose was recorded, which means that a larger amount of lignin was removed from the given samples of hemp fibers.

Table 2. Kappa Number, Yield, and Holocellulose Content of Hemp after Delignification

Sample	Temperature and Time of DES Delignification	Kappa Number	Holocellulose (%)	Yield (%)
Sample 1	100 °C, 2 h	22.0 ± 0.3	90.6 ± 0.2	84.6
	100 °C, 4 h	21.5 ± 0.5	90.8 ± 0.2	84.3
Sample 2	120 °C, 2 h	16.3 ± 0.5	90.3 ± 0.9	90.6
	120 °C, 4 h	17.2 ± 0.7	92.5 ± 0.9	83.2
Sample 3	140 °C, 2 h	13.7 ± 0.6	94.6 ± 0.1	75.9
	140 °C, 4 h	14.5 ± 0.8	94.9 ± 0.1	72.5

Hemp is a suitable raw material for fiber production. Various delignifying agents and processes have been utilized to remove lignin and obtain fibers for the paper industry (Tjeerdsma *et al.* 1994; Barberà *et al.* 2011). Barberà *et al.* (2011) used the sulphate chemical process (kraft process) and the semi-chemical process (soda-anthraquinone) on hemp raw material, the obtained yields ranging from 72 to 40% for the chemical process (kraft process) and from 79 to 43% for the semi-chemical process. The Kappa number ranged from 72 to 15 for the chemical process and from 168 to 21 for the semi-chemical treatment. In another study involving organosolv delignification (Tjeerdsma *et al.* 1994), which is a process for pre-treating biomass with organic solvents, an ethanol/water mixture was used to delignify cannabis. Yields ranged from 43.4 to 54.4%, and the Kappa number decreased linearly with increasing temperature from 185 °C (Kappa number 51.6) to 205 °C (Kappa number 19.2). The hemp fiber yield in this work ranged from 72.5 to 90.6% after LTTM application. The Kappa number decreased with increasing temperature and processing time from 22 to 13.7. The yields and Kappa numbers of hemp in different delignification processes are shown in Fig. 1. The goal of the experiment was to obtain the lowest possible Kappa number, which represents the greatest possible removal of lignin from the samples of annual plants and to achieve a high yield. The results showed that the LTTM system is an effective delignifying agent. A relatively low Kappa number of 13.7 with a yield of 75.9% were achieved at 2 h and 140 °C. Delignification with green solvents (LTTM) achieved relatively low Kappa numbers and higher yields such as in organosolv delignification, *i.e.*, the supported delignification process based on LTTM shows, in addition to the economic potential, also a higher efficiency of lignin removal from plant biomass than in other processes.

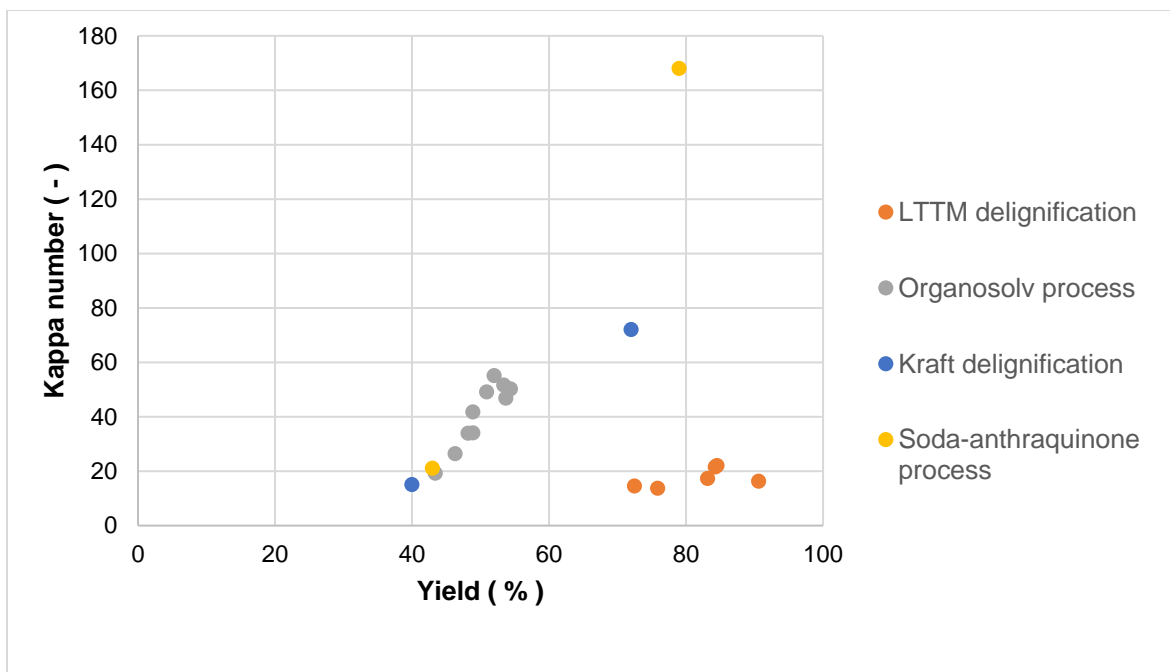


Fig. 1. Comparison of yields and Kappa number of hemp in different delignification process

CONCLUSIONS

1. Choline chloride and lactic acid-based low transition temperature mixtures (LTTMs) are effective in extracting and removing lignin from woody plants and plants. The LTTM system based on ChCl/LacA was used to delignify hemp stalks.
2. A LTTM was used to delignify the hemp. It was a mixture of choline chloride and lactic acid in a molar ratio of 1:2. By application of LTTM, a relatively high removal of lignin was achieved and the Kappa number after delignification was at the level of 22 to 13.7, while the pulp yield was in the range of 90.6 to 72.5%. The most suitable delignification conditions were at a temperature of 140 °C and a delignification time of 2 h, the resulting Kappa pulp number being 13.7 and the yield being 75.9%.
3. LTTM delignification was more efficient than other delignification processes (kraft delignification, soda-anthraquinone process, and organosolv process).

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