




Article

High Temperature Lignin Separation for Improved Yields in Ethanol Organosolv Pre-Treatment

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Abstract: The full utilization of renewable raw materials is necessary for a sustainable economy. Lignin is an abundant biopolymer, but is currently mainly used for energy production. Ethanol organosolv pre-treatment produces high-quality lignin, but still faces substantial economic challenges. Lignin solubility increases with temperature, and previous studies have shown that it reprecipitates during cooling after the pre-treatment. Thus, a possibility for the optimization of lignin production with this process can be the separation of extract and residual biomass at high temperatures. In this work, lignin was extracted from wheat straw at 180 °C, and the extract was separated from the remaining solids at several temperatures after the pre-treatment. The results show that 10.1 g/kg of lignin and 2.2 g/kg of carbohydrates are dissolved at the pre-treatment temperature of 180 °C, which is reduced to 8.6 g/kg of lignin and 1.2 g/kg of carbohydrates after cooling. The precipitation of lignin separated from the extracts at 180 °C showed that a higher lignin concentration at high temperatures results in a 46% improvement in the yield of solid lignin, while there was no significant impact on the lignin purity.

Keywords: biorefinery; organosolv; lignin; pre-treatment



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1. Introduction

The efficient utilization of renewable resources, such as lignocellulosic biomass, is a highly relevant issue nowadays. Biorefinery processes have been heavily investigated as a method to separate and valorize the major components of lignocellulose, showing both great potential and economic obstacles [1,2]. Lignin is one of the three major compounds of lignocellulose and should play a major role, since it is the most abundant renewable polymer with an aromatic skeleton [3]. Lignin is already produced and available in large quantities as a side-product of pulping processes. However, lignin from conventional pulping processes, such as Kraft- and sulfite pulping, is scarcely used as a material, but is mainly used for energy production, despite it being produced in larger amounts than necessary to cover the internal energy demand of the pulping process [4]. In comparison, ethanol organosolv pre-treatment results in a high-quality lignin that is suitable for various value-added material applications, such as carbon fibers [5,6], food packaging [7], or sunscreens [8], while employing a completely renewable solvent in a sulfur-free process. Such applications could both improve the economic viability of a lignocellulose biorefinery and offer a more sustainable raw material for these products. On the other hand, organosolv pre-treatment has some economic drawbacks, such as the necessity to recover the solvent by distillation [9], and the higher demand on equipment due to high pressures during pre-treatment [10]. To be economically competitive, this technology needs to be optimized towards the production of high-quality lignin at simultaneously high yields, and the sufficient delignification of the residual biomass.

In most studies investigating organosolv pre-treatment, biomass and solvent are mixed in an autoclave, heated to a specified temperature for a certain time, and cooled to

room temperature, followed by separation of solids and liquids, and an analysis of the fractions [11–15]. However, since lignin solubility increases with temperature [16], it is likely that some lignin is solubilized at high temperatures but reprecipitates during the cooling. In fact, several works have reported this lignin re-precipitation during the cooling process. Guo et al. [17] washed the biomass after pre-treatment and compared the lignin in the wash with the lignin dissolved in the liquor. They found very similar structural features in both lignins; however, the molecular weight of the reprecipitated lignin was slightly higher than that in the liquor. Rossberg et al. [18] reported the precipitation of lignin during cooling in a pilot scale operation, which formed deposits in the tanks, but had properties that made it still suitable for material applications. From a process perspective, these depositions are a relevant issue for several reasons, since they indicate an increased delignification, but also necessitate frequent removal. Studies from Weinwurm et al. [19] and Xu et al. [20] showed that some of the reprecipitating lignin also forms deposits on the pre-treated fibers. This shows that reprecipitation during cooling has to be considered in process design, as it potentially reduces both the yield of the resulting lignin and the quality of the residual biomass. On the other hand, this indicates a promising route for optimization by lowering solvent demand, and increasing lignin yield and delignification, which was also suggested by Schulze [21].

While the liquor and residual biomass can be separated at elevated temperatures in pilot-scale organosolv pulping [21], this is generally not performed in lab-scale experiments. As mentioned, previous studies have found that lignin re-precipitates during cooling after the extraction, but a systematic investigation of the temperature dependency of this is still missing. In this work, we investigated the influence of temperature on the solubilization of lignin and other compounds after ethanol organosolv extraction. Samples were taken at different temperatures during cooling after extraction and were analyzed on their composition, with a focus on the lignin content. The lignin in the obtained samples was also analyzed on its molar mass distribution, yield, and purity after precipitation by mixing with water. The results suggest that significantly more lignin is dissolved at high temperatures, and provide information about the solubility of lignin at elevated temperatures. This could lead to a more efficient process for the production of lignin, which is suitable as a sustainable and renewable raw material in value-added applications.

2. Materials and Methods

2.1. Materials

The wheat straw used in this work was harvested in Lower Austria in 2019. The particle size was reduced in a cutting mill equipped with a 2 mm round hole sieve, after which the fine particles were removed with a 0.209 mm vibrating screen and the coarse particles were removed with a 0.606 mm vibrating screen. The wheat straw was characterized in a previous work [22] using methods from the National Renewable Energy Laboratory (NREL) for the characterization of lignocellulosic biomass [23–25]. It consisted of 35.31 wt% glucan, 21.94 wt% xylan, 2.13 wt% arabinan, 0.72 wt% mannan, 0.67 wt% galactan, 17.35 wt% lignin, 1.09 wt% ash, and 20.45 wt% extractives. Ethanol (ChemLab 100%, Zedelgem, Belgium) and ultra-pure water (18 M Ω /cm) were used as solvents in the extraction; 2-furaldehyde (furfural, 99%), hydroxymethylfurfural (HMF, 99%), acetic acid (99.7%), arabinose, galactose, glucose, xylose, and mannose were purchased from Merck (Darmstadt, Germany) and used for the analytics.

2.2. Extraction

All extractions were carried out with a 1 L Zirbus autoclave (Bad Grund, Germany). The autoclave was filled with 40 g of wheat straw dry matter and 440 g of 60 wt% aqueous ethanol. The mixture was pretreated for 60 min by heating to 180 °C and holding it at that temperature, which was reached after 50 min. After 60 min of combined heating and holding time, the autoclave temperature was set to the respective sampling temperature and held at that temperature during the sampling by the manual control of cooling and heating.

2.3. Sampling and Lignin Precipitation

The samples of the extract were taken through a submerged metal tube equipped with a 0.5 μm metal sinter filter at set temperatures after pre-treatment, specifically 180, 160, 140, 80, and 20 $^{\circ}\text{C}$. Samples acquired at 20 $^{\circ}\text{C}$ were centrifuged at $24,104\times g$ for 20 min (according to the methods used in previous works [26]) to verify that all the solids were removed with the filter. No sediment was found after the centrifugation, and no significant differences between the dry matter contents of the filtered extracts and the filtered and centrifuged samples were found. From the sampling tube, the sample was led into a sampling bottle through a tube. For samples taken at temperatures above 80 $^{\circ}\text{C}$, approximately 750 mL of ultra-pure water (18 $\text{M}\Omega/\text{cm}$) at an ambient temperature was filled into the sampling bottle to avoid solvent evaporation. The exact mass of the water was noted to calculate the amount of sample in the mixture of water and extract. A schematic of the autoclave including the sampling tube, filter and sampling bottle is depicted in Figure 1. To avoid the clogging of the filter and carry-over of the samples, the sampling tube and filter were disassembled and cleaned between experiments. Three separate experiments were carried out for each sampling temperature, and the reported results are averages of the triplicates and standard errors.

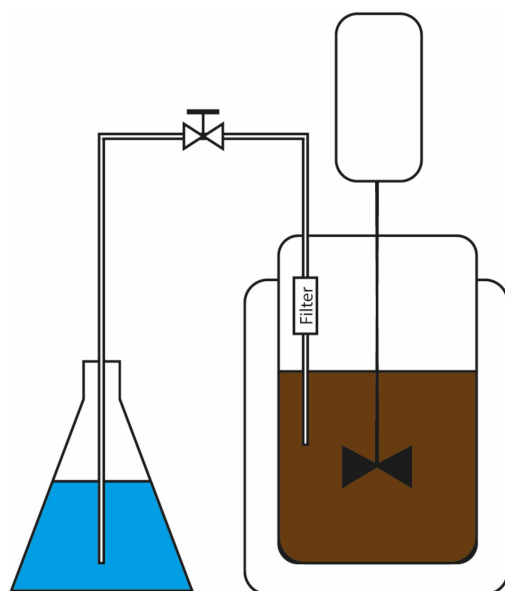


Figure 1. Schematic of autoclave and sampling unit.

After sampling, the autoclave was cooled to room temperature. The remaining extract and straw were collected in a nylon cloth and the extract was pressed from the residual straw using a hydraulic press (Hapa, HPH 2.5, Achern, Germany) at 200 bar. The extract was centrifuged at $24,104\times g$ for 20 min and decanted to remove all solids.

To determine the comparability of pre-treatment conditions and the impact of the increased time due to sampling, the severity factor (R_0) of the pre-treatments was calculated:

$$R_0 = \int_0^t e^{\frac{T-100}{14.75}} dt \quad (1)$$

where t is the time in minutes, T is the temperature in $^{\circ}\text{C}$, 14.75 is an empirical constant, and 100 is the reference temperature of 100 $^{\circ}\text{C}$ [27].

2.4. Lignin Precipitation

All samples were diluted to the liquor/water ratio of the most dilute sample (1:4.68 wt/wt) with ultra-pure water at an ambient temperature to precipitate the lignin. The resulting suspensions were filtered at an ambient temperature with a cellulose nitrate filter

(Whatman, Maidstone, UK), with a pore size of 0.1 μm . The filtrate was dried at 105 $^{\circ}\text{C}$ until it was at a constant weight, in order to determine the dry matter and calculate the precipitation yield. The solids were removed from the filter, freeze-dried, and analyzed on their lignin and carbohydrate content.

2.5. Analytics

The dry matter content of the liquid samples was determined by drying them in a drying oven at 105 $^{\circ}\text{C}$ until at constant weight. The liquid samples were characterized on their lignin and carbohydrate content, degradation products formed during extraction, ash content, and carbohydrate content according to laboratory analytical procedures from the National Renewable Energy Laboratory (NREL) [23,25,28]. A Thermo Scientific ICS-5000 HPAEC-PAD system with deionized water as eluent was used for the sugar determination. The degradation products were determined with a Shimadzu LC-20A “prominence” HPLC system using 5 mM H_2SO_4 as eluent.

The molecular weight distribution was determined by high performance size exclusion chromatography (HP-SEC) using three TSK-Gel columns in series (PW5000, PW4000, PW3000; TOSOH Bioscience, Darmstadt, Germany) at 40 $^{\circ}\text{C}$ in an Agilent 1200 HPLC system (Agilent, Santa Clara, CA, USA). The samples were freeze-dried and dissolved in 10 mM NaOH (which was also used as eluent in the HP-SEC) at 1 mg/mL. Polystyrene sulfonate reference standards (PSS GmbH, Mainz, Germany), with molar mass peak maxima at 78,400, 33,500, 15,800, 6430, 1670, 891 and 208 Da, were used for calibration.

3. Results and Discussion

Wheat straw was extracted at 180 $^{\circ}\text{C}$ using ethanol organosolv extraction, and samples were taken at different temperatures during cooling. The samples obtained with this method are named hot-sampled extract (HSE), followed by the sampling temperature in $^{\circ}\text{C}$. Figure 2 shows examples of temperature profiles in the autoclave during the extractions. The temperature during sampling was maintained by the manual control of the mantle heating and cooling, resulting in slight temperature fluctuations in some cases (e.g., Sampling at 80 $^{\circ}\text{C}$ in Figure 2). However, these fluctuations can be assumed negligible compared to the differences between the sampling temperatures.

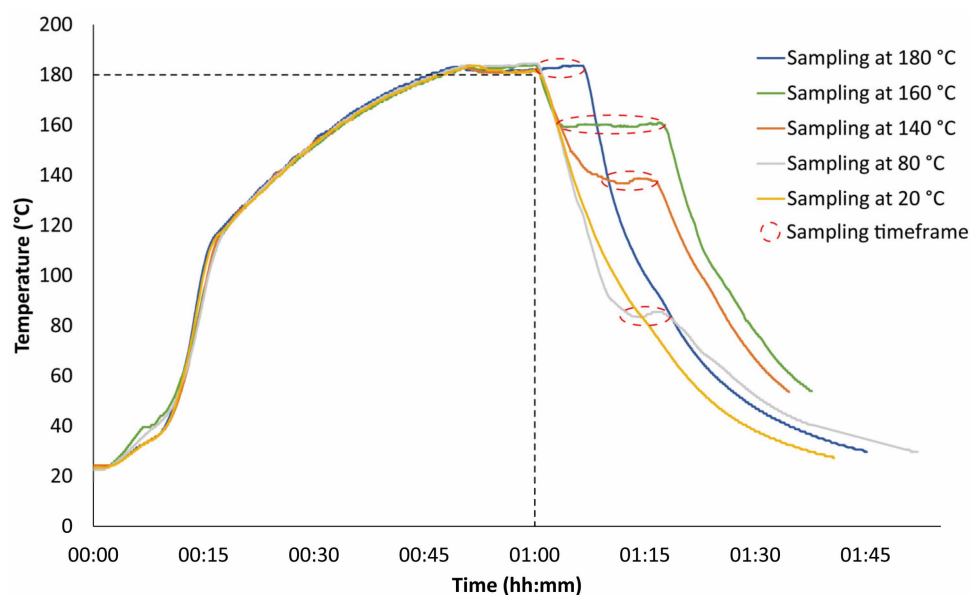


Figure 2. Temperature curves in the autoclave during experiments.

It is evident from Figure 2 that the sampling required a certain amount of time (approximately 10 min), which slightly varied, depending on pressure and filter performance. The severity factor R_0 was calculated for all experiments (Table 1) according to Equation

(1), in order to estimate the impact of the sampling time on pre-treatment severity. Around 250 mL of the extract was taken as a sample in each experiment, and the rest was separated from the straw after complete cooling (termed “extract after cooling”, EAC); this was also analyzed on its composition. The comparison of these extracts from the experiments with samples taken at 180 °C and the experiments at 20 °C (Figure 3) show that the difference between the sampling temperature has a far greater impact on the amount and composition of the extracted compounds than the increased severity caused by the time needed for sampling. This is in agreement with findings from other groups [11,29,30], showing that the time has a relatively small impact on delignification compared to other factors. It can be concluded that the differences between the samples are mainly caused by differences in solubility, rather than increased severity.

Table 1. Severity factor of the experiments.

Experiment	R_0
HSE180	7670 ± 415
HSE160	6397 ± 123
HSE140	6030 ± 82
HSE80	6353 ± 101
HSE20	6120 ± 80
EAC180	8090 ± 274

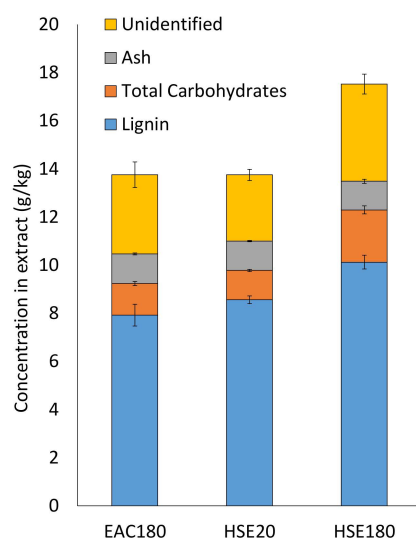


Figure 3. Compositions of the samples EAC180, HSE20, and HSE180.

The samples were also analyzed on the content of HMF, Furfural and Acetic acid (Figure 4). While there is an increase in the concentrations of HMF and Furfural, the standard errors for the higher concentrations are comparatively large. These higher concentrations in some samples could be explained by the slightly higher severities at higher sampling temperatures. However, the results do not indicate a significant correlation of the concentrations with the sampling temperatures for any of the compounds. This further supports the assumption that the changes in severity are negligible and the increase in extract composition are due to the temperature-dependent solubility.

Figure 5 depicts the average dry matter contents and compositions from all experiments. The dry matter content decreases from 17.5 ± 0.4 to 13.6 ± 0.2 g/kg during the cooling process from 180 to 20 °C, while the total lignin content decreases from 10.1 ± 0.3 to 8.6 ± 0.2 g/kg. However, the ratio of lignin in the dry matter decreases with increasing sampling temperatures, from 62.3% at 20 °C to 54.3% at 160 °C, increasing again to 57.8% at 180 °C. There are no significant changes in the extracts' ash content with the sampling temperature, and thus a slight decrease in the ratio of ash in the dry matter is observed.

Consequently, the ratio of carbohydrates and unidentified compounds increases with the sampling temperature. This suggests that the solubility of these compounds is increased more by higher temperatures than that of lignin. Interestingly, the absolute content of lignin in the extract has the largest decrease, from 180 to 160 °C, while the ratio of lignin in the dry matter has the largest decrease, from 80 to 20 °C. The former indicates that the solubility of some lignin fractions at first rapidly decreases during cooling, while the decrease in solubility below 160 °C is comparatively small. On the other hand, the significant decrease in the ratio of lignin in the dry matter from 80 to 20 °C must be due to the different solubility behavior of the other compounds.

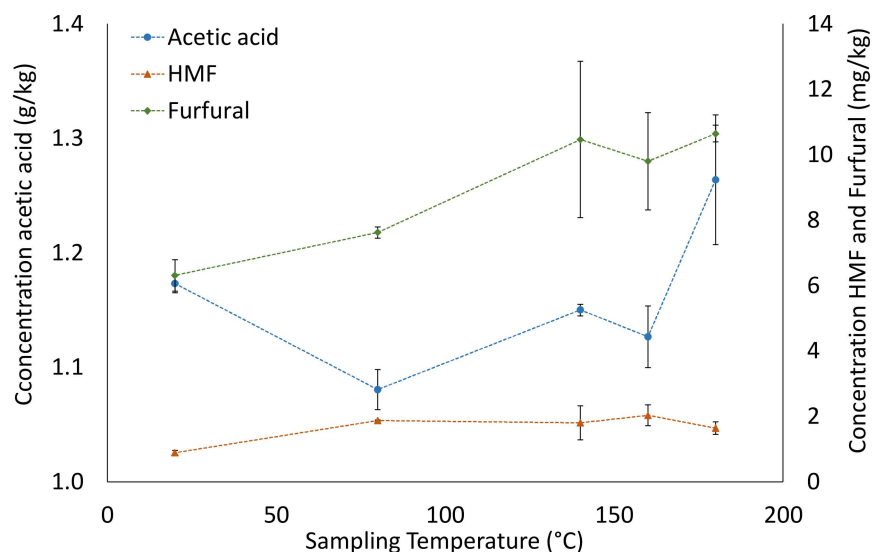


Figure 4. Content of acetic acid, HMF and Furfural at different sampling temperatures.

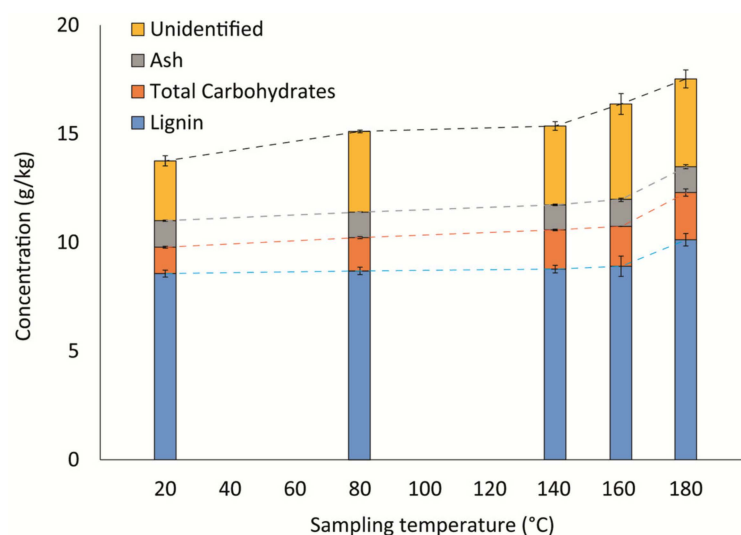


Figure 5. Composition of the dry matter in samples obtained at different temperatures.

A more detailed analysis of the carbohydrates in the samples shows that not all of the carbohydrates present in the extract behave in the same way. Figure 6 shows that there is only a small increase in the concentration of glucan, which is assumed to be mostly derived from cellulose. On the other hand, the sugars derived exclusively from hemicellulose (xylose, galactose, arabinose, and mannose) have significantly increased concentrations at higher sampling temperatures. This seems logical, since hemicellulose is usually subjected to more depolymerization than cellulose in organosolv pre-treatment [10], which is corroborated by the significantly lower concentration of glucan in the samples compared

to the hemicellulose-derived carbohydrates. These results partially explain the change in the lignin ratio of the dry matter with temperature, since lignin solubility shows a modest increase at low temperatures and a sharp increase at high temperatures, while the solubility of carbohydrates has a near-linear increase over the whole temperature range investigated. Comparison of the monomeric and oligomeric carbohydrates in the extracts further reveals that this decrease in solubility is mostly caused by the oligomeric carbohydrates. This suggests that the correlation of sugar concentration and temperature is mostly caused by the solubility limit of oligomeric carbohydrates derived from hemicellulose.

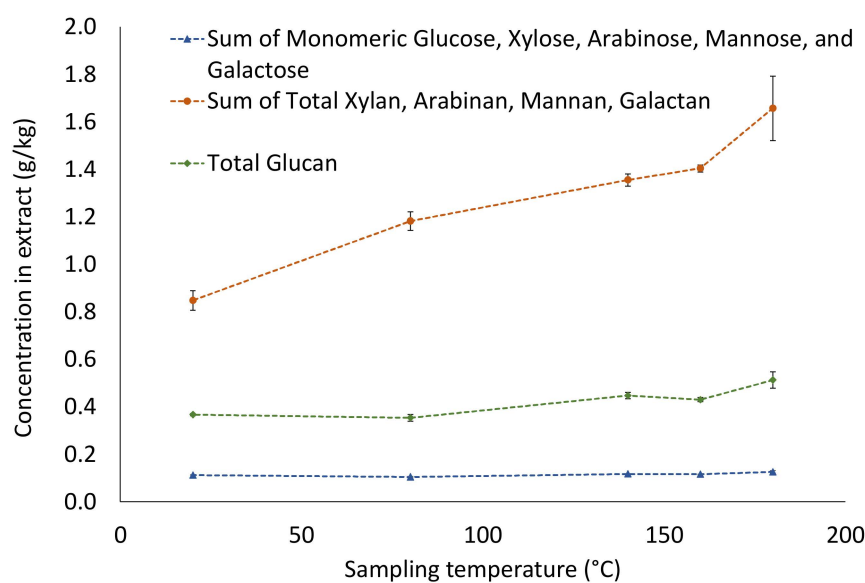


Figure 6. Content of total cellulose- and hemicellulose-based carbohydrates, and sum of monomeric carbohydrates in the extracts at different sampling temperatures.

The trend in lignin solubility could be explained by the polydisperse nature of the solubilized lignin polymers. The molecular weight distribution of the lignin in the samples was measured to determine differences between lignins collected at different temperatures. Both the mass (Mw) and number averaged molecular weights (Mn) showed only slight changes with the sampling temperature (see Table 2). In comparison, Rossberg et al. [18] found that the molecular weight of organosolv lignin spontaneously precipitating during cooling was much higher than that of lignin precipitated by the dilution of the extract. In contrast to this work, they analyzed the re-precipitating lignin separately from the rest. Results more comparable to this work were obtained by Guo et al. [17], who found that the molecular weight of lignin in washing liquor was only slightly higher than that in the original liquor.

Table 2. Average Mw, Mn, and PDI of lignins obtained at different temperatures.

Experiment	Mw (Da)	Mn (Da)	PDI
HSE180	1349 ± 18	722 ± 0.5	1.87 ± 0.02
HSE160	1520 ± 35	720 ± 3.2	2.11 ± 0.05
HSE140	1358 ± 42	740 ± 6	1.83 ± 0.04
HSE80	1313 ± 9	709 ± 3.2	1.85 ± 0.01
HSE20	1244 ± 31	713 ± 4.9	1.74 ± 0.03

Several previous studies [18,26,31] have shown that lignin solubility is connected with molecular weight, and specifically that smaller lignin fragments have a higher solubility than larger molecules. Figure 7 depicts the molecular weight distributions of lignin samples obtained at different temperatures. While there are only small differences in the absorbance-based distributions, the mass-weighted distributions show that there might

be a lignin fraction at the upper limit of the measuring range. These high-mass lignin fractions are significantly lower when the extracts are separated at 20 °C, compared to all other temperatures (see Figure 7b). This corroborates the fact that, during the cooling, predominantly large lignin fractions become insoluble and precipitate.

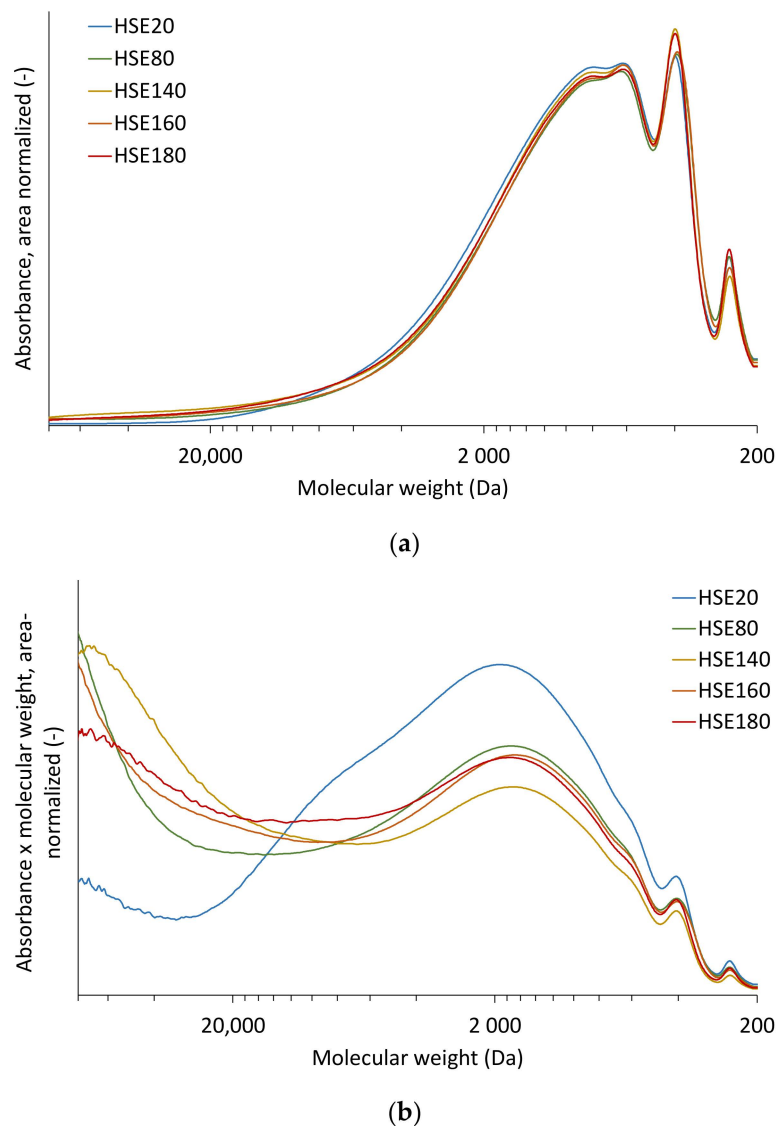


Figure 7. (a) Number- and (b) mass-based molecular weight distributions of lignin from different sampling temperatures.

From a process perspective, the yield of solid lignin product is highly relevant. Lignin is commonly precipitated from organosolv liquors by the addition of water as an antisolvent [32–34], which was also performed in this work. Three different yields were calculated and are shown in Figure 8: The extraction yield was calculated as the mass of lignin in the extract compared to the lignin present in the untreated wheat straw, the precipitation yield was calculated as the mass of solid lignin after precipitation compared to the lignin present in the extract, and the total lignin yield was calculated as the mass of solid lignin after precipitation compared to the lignin present in the original wheat straw. All yields were calculated under the assumption of complete extract separation. Figure 8 shows that there is a significant increase in the lignin yield with the sampling temperature, from $33.6 \pm 1.7\%$ at 20 °C to $48.9 \pm 0.9\%$ at 180 °C. This means that the overall lignin yield can be increased by up to 45.5% if solids and liquids are separated at higher temperatures. In previous works, the solvent-shifting precipitation of lignin from organosolv extracts

resulted in an average yield of 48.2% for the precipitation step [35], which is in the same order of magnitude as the yield for extraction and precipitation combined at the highest sampling temperature.

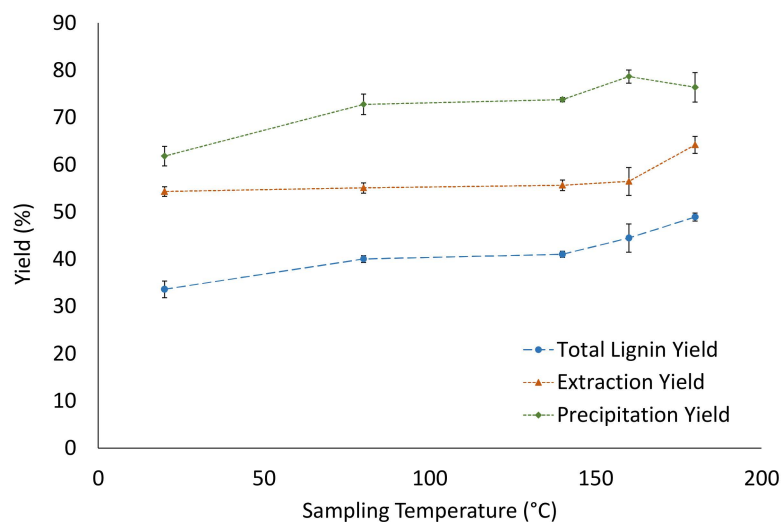


Figure 8. Yield of precipitated lignin for different sampling temperatures.

As can be seen in Figure 8, the increase in the overall yield with the sampling temperature is relatively steady. On the one hand, this increase can be explained by the generally higher lignin content of the extracts separated at higher temperatures, and, on the other hand, by the lower solubility of the lignin separated at high temperatures. Interestingly, there are slight differences between the correlations of the extraction yield and the precipitation yield with the temperature. While the extraction yield has the most significant change from 160 to 180 °C, the precipitation yield has a strong increase from 20 to 80 °C, which then flattens from 80 to 140 °C, and then reaches a maximum or plateau around 160 °C. While the correlation with the extraction yield is likely caused by a decreasing lignin solubility at lower temperatures, the trend in the precipitation yield suggests structural differences in the solubilized lignin, which affect the solubility at lower ethanol concentrations. For example, the average extraction yields for the 20 and 80 °C sampling temperatures are $54.3 \pm 1.0\%$ and $55.1 \pm 1.1\%$, while the precipitation yield significantly increases from $61.8 \pm 2.0\%$ to $72.7 \pm 2.2\%$. This suggests structural changes in the solubilized lignin during the cooling, resulting in more lignin being soluble after the addition of water when sampling at 20 °C compared to 80 °C; this agrees with the much higher amount of smaller molecular weight lignin that is found at the 20 °C sampling temperature (see Figure 7b).

Since the increase in yield with the sampling temperature coincides with an increase in oligomeric carbohydrates in the extracts (see Figure 6), it is possible that the increased total lignin yields at higher sampling temperatures are caused by carbohydrates covalently bound to the precipitating lignin; this would lower the purity, and thus the product quality. However, an analysis of the lignin and carbohydrate content of the precipitated lignin (Figure 9) shows that both the purity and the concentration of carbohydrate contamination have no significant correlation with the sampling temperature. This suggests that the additional oligomeric carbohydrates found at higher sampling temperatures are not bound to the lignin, and even though they become insoluble during cooling, they become soluble again at lower ethanol concentrations when lignin is precipitated. Therefore, the lignin yield and delignification can be increased by the separation of residual biomass and extract at high temperatures, without a negative impact on the purity of the resulting lignin. At the same time, more oligomeric, hemicellulose-derived sugars are solubilized in the liquor and stay dissolved after lignin precipitation, making them available for further use, and increasing the cellulose content of the residual biomass.

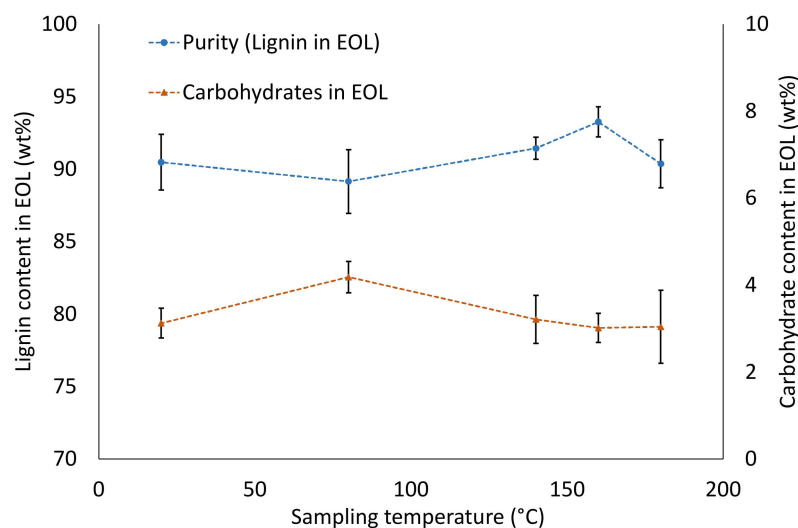


Figure 9. Purity of precipitated lignin for different sampling temperatures.

4. Conclusions

In this work, the composition of organosolv extract at several temperatures after pre-treatment was investigated. The results show that the content of solubilized lignin and oligomeric carbohydrates derived from hemicellulose decreases during the cooling of the extract, with the largest decrease in lignin while cooling occurring from 180 to 160 °C; meanwhile the carbohydrate content decreased almost linearly. This behavior was explained by the correlation of temperature and the solubility of lignin and oligomeric carbohydrates. The higher lignin content at elevated sampling temperatures resulted in a significantly higher yield of precipitated lignin. Based on the lignin present in the untreated wheat straw, a total lignin yield of 49% was reached when sampling at 180 °C, compared to 34% at 20 °C. Concerning the lignin properties, lignin in extracts separated at temperatures above 20 °C had a higher molecular weight, indicating lower solubility of larger lignin molecules. There was no significant trend in the extracts' content of ash, degradation products, or monomeric carbohydrates, suggesting that these compounds do not reach their solubility limit during extraction.

These results indicate that the separation temperature has a significant influence on the process efficiency of organosolv pre-treatment. While the highest lignin contents in the extracts and total lignin yields were achieved by separation at 180 °C, even the second lowest separation temperature of 80 °C resulted in a significant increase in the yield of precipitated lignin (40%), compared to separation at 20 °C (34%). Separation at an elevated temperature should also improve the quality of the resulting residual biomass, since higher amounts of lignin and hemicellulose are removed. Lastly, the purity of the lignin was not affected by the separation temperature. This means that the relatively simple measure of separating extract and residual biomass at elevated temperatures results in increased delignification and the removal of hemicellulose-derived carbohydrates from the biomass, with no significant influence on the purity of the produced lignin. All of these factors combined would constitute an improvement in the efficiency, the subsequent economic viability of a lignocellulose biorefinery, and enable lignin to be used as a sustainable raw material in value-added applications.

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Conflicts of Interest: The authors declare no conflict of interest.

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