The effect of using different acids to catalyze the prehydrolysis process on the organosolv delignification of beech wood in two-stage process

Ibrahim, Q. [a, b*]; Arauzo, P.J. [a] and Kruse, A. [a]

[a] Department of Conversion Technologies of Biobased Resources, Institute of Agricultural Engineering, University of Hohenheim, Garbenstrasse 9, 70599 Stuttgart, Germany

[b] Fraunhofer Center for Chemical-Biotechnological Process CBP, Am Haupttor (Tor 12, Bau 1251), 06237 Leuna, Germany

* Correspondence author: Qusay Ibrahim

Email Address: qusay.ibrahim@uni-hohenheim.de

Abstract

In this study, beechwood was fractionated in lab-scale rector by one-stage and two-stage processes. The two-stage processes of the prehydrolysis with no catalyst (only water) and catalyst (sulfuric, phosphoric, oxalic acids), and the subsequent effect on the organosolv process were studied. A one-stage organosolv delignification of beech wood (control experiment) was conducted at 155 ºC for 160 min in 1:1 ethanol/water mixture with 100 mM sulfuric acid, while the two-stage processes were conducted starting from the uncatalyzed and catalyzed (20-200 mM) prehydrolysis of beech wood at 175 ºC for 60 min. The results indicate that sulfuric acid enhanced the removal of xylan from beechwood during the prehydrolysis stage. Moreover, the effectiveness of the delignification of beech wood using a one-stage process is more than the two-stage, as well as the enzymatic digestibility of the pulp, which was better than those of the two-stage processes. However, the enzymatic digestibility results of the pulp obtained after sulfuric acid-catalyzed prehydrolysis followed by organosolv delignification shown better results than the other two-stage processes. Additionally, high klason content, low sugar content, low M_w, and high phenolic hydroxyl groups of lignin was recovered by a one-stage organosolv delignification.

Keywords: organosolv, prehydrolysis, pretreatment, enzymatic hydrolysis, lignin

1. Introduction

The increment of energy consumption and the resultant environmental issues, like the emission of greenhouse gases, forces the world to look for alternative renewable energy sources [1, 2, 3, 4]. For these reasons, the approach of the biorefinery is a promising alternative solution. The biorefinery is based on the utilizing of renewable lignocellulosic biomass with the combination of second-generation fuel (e.g., ethanol), and a wide range of the added high-value of chemical products [1]. Two facts for using lignocellulose as a starting material. Firstly, it is the most abundant biomass species on Earth. Secondly, it has great potential for producing similar products to those currently produced in a petroleum refinery, making it a rival feedstock to replace fossil fuels in the future [5, 6]. Lignocellulosic biomass primarily consists of lignin, cellulose, and hemicellulose, as well as other minor components like extractives, water, minerals and protein [7]. The challenge in a biorefinery lies in being able to fractionate the lignocellulosic biomass efficiently into its components to maximize the potential product value obtainable from the biomass. During the lignocellulose fractionation, the typical challenges are the difficult separation of both cellulose and lignin giving impure products. In the case of bioethanol production, the formation of compounds that inhibit fermentation, such as acetic acid from hydrolysis of acetyl groups of hemicellulose, furfural, and 5-HMF from carbohydrate degradation [8] and phenolic compounds from lignin fractions are aimed [9, 10, 11]. Moreover, the current fractionation processes require high amounts of energy and/or chemicals and these processes produce waste. Thus, it is important to find an effective and feasible pretreatment process to fractionate lignocellulosic biomass into high pure and usable fractions.

The conversion of lignocelluloses consists of pretreatment and fractionation. These processes have many goals. For example, to increase the yield of reducing sugars easing enzymes' work by improving the accessibility of lignocellulosic biomass during enzymatic hydrolysis [12]. These could also be either acidic or alkaline, one-stage or two-stage processes [13, 14]. In the acidic pretreatment processes, lignocellulosic biomass is treated with dilute acids like sulfuric acid and oxalic acid at high temperatures, which leads to the formation of inhibitory compounds like furfurals and 5-(Hydroxymethyl)furfural (5-HMF) [15]. H_2SO_4 is the most used inorganic acid for the catalysis of biomass [15]; however, the high corrosivity reduces the cycle life of the industrial installations force to use an alternative acid as H_3PO_4 [16]. $C_2H_2O_4$ is an alternative organic acid with lower corrosivity than H_2SO_4 that enhances the control of the biodegradability of biomass. Furthermore, it is produced from bio-based materials and renewable sources [17]. Contrary to this is the alkaline pretreatment processes in which the alkaline solution modifies lignin structure by degrading the side chains of esters and glycosides, which causes hemicellulose solvation [18, 19, 20].

Typical one-stage processes like Kraft and the sulfite pulping process produces a high pure pulp and yield lignin; however, sulfur-containing compounds are released to the environment [21]. Besides, the organosolv delignification is a one-stage pulping process; wherein delignification is achieved using organic solvents (generally ethanol) mixed with water at temperatures in the range of 130 – 200 ˚C with or without using catalysts [22, 23, 24]. Despite the advantages of the organosolv delignification like the recovery of sulfur-free lignin, it requires high temperatures and concentrated acid catalysts. Then, the price of organosolv lignin is higher than those obtained by the sulfite process. To overcome this

drawback a two-stage process was proposed. Therein, in the first stage, the hemicellulose is separated minimizing the degradation into furans. The furans play a role as an enzyme and fermentation inhibitor. Subsequently, organosolv delignification produces the separation of the cellulose from lignin in less recalcitrant form, facilitating its degradation during the enzymatic hydrolysis [25]. Hence, the two-stage process allows to improve the separation of the polymers in the initial wood (cellulose, hemicellulose, and lignin) and thus their recovery with higher purity than in the one-stage process. Additionally, the operating conditions (i.e. pressure, temperature) of the two-stage process are less harsh than of the one-stage process consequently the decrement of the operating cost and material requirements.

The purpose of the current study is to evaluate the effects of organic or inorganic acids as catalysts on the beech wood during the prehydrolysis process and the subsequent effect on the chemical properties of extract lignin during Organosolv delignification.

2. Materials and Methods

2.1. Materials

In this study, the beech wood chips used were provided by JRS (Räuchergold®, type KL ¼, 2.5-3.5 mm). The chemical composition of the beech wood chips is 24.3 wt. % lignin, 43.0 wt. % glucan, 22.7 wt. % xylan, 5.6 wt. % acetic acid, 0.6 wt. % ash and 3.8 wt. % of others. Analytical grade sulfuric acid (SA), phosphoric acid (PA) and oxalic acid (OA) with a purity of 98 % (w/w), 85 % (w/w) and > 99 % (w/w), respectively, were purchased from VWR and used for the experiments. Ethanol with a purity > 93 % (w/w) was provided by the installations of Fraunhofer CBP (Leuna, Germany).

2.2. Method

Table 1 shows an overview of the conducted fractionation experiments, which consist of two stages: aqueous prehydrolysis and Organosolv delignification (Figure 1). The experiment No. 1 is considered as the reference for the verification of the improvement of the two-stage process versus only organosolv delignification. Both process stages were performed in 1 L autoclaves (Model 4600, Parr instruments, USA). Heating of the autoclaves was performed in a modified oven (Binder FP 115, Tuttlingen, Germany) while rotating the autoclaves at a rate of 100 rpm.

Table 1. Experimental conditions of prehydrolysis and organosolv delignification stages

Experiment No.	Stage	Conditions		
		Solvent	T (ºC)	t (min)
1	**1)** OS [a]	50 wt. % Ethanol + 100 mM H_2SO_4	155	160
2	**1)** PH [b]	Uncatalyzed water	175	60
	2) OS	50 wt. % Ethanol + 100 mM H_2SO_4	155	160
3	**1)** PH	Water + 20 mM H_2SO_4	175	60
	2) OS	50 wt. % Ethanol + 100 mM H_2SO_4	155	160
4	**1)** PH	Water + 20 mM H_3PO_4	175	60
	2) OS	50 wt. % Ethanol + 100 mM H_2SO_4	155	160
5	**1)** PH	Water + 200 mM $C_2H_2O_4$	175	60
	2) OS	50 wt. % Ethanol + 100 mM H_2SO_4	155	160

[a] OS = Organosolv delignification.
[b] PH = Prehydrolysis.

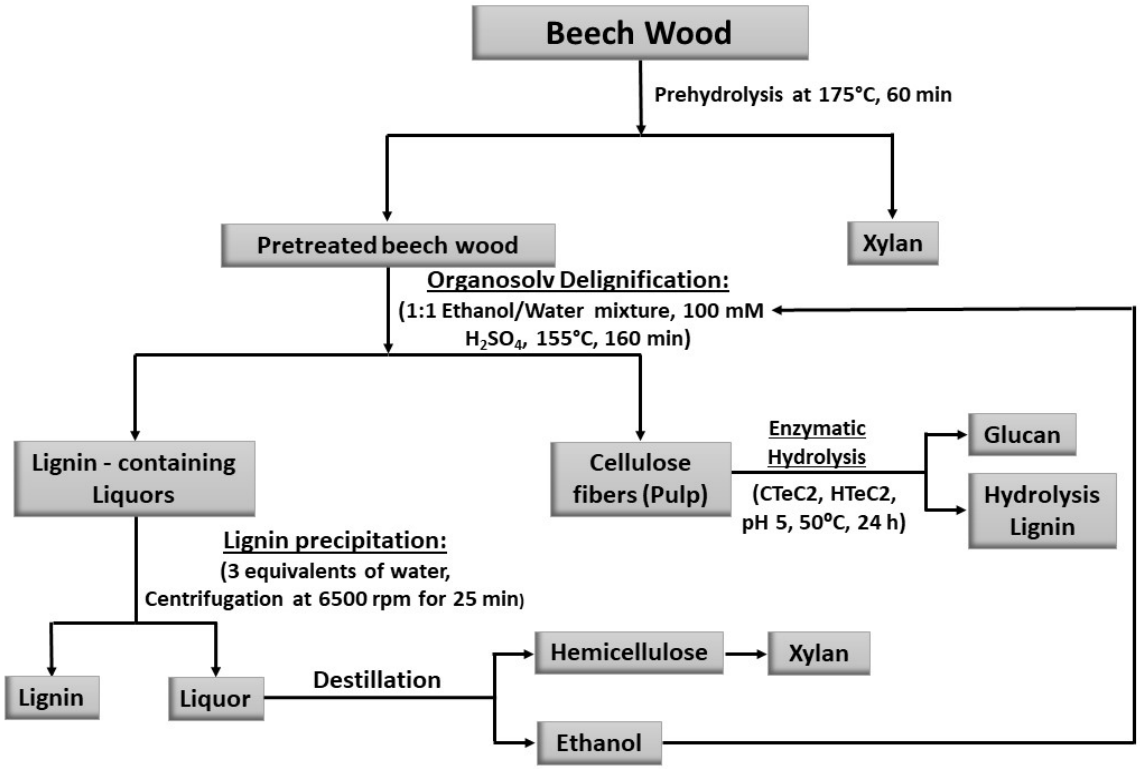

Figure 1. General scheme of the fractionation of beech wood by one or two-stage process.

2.2.1. Prehydrolysis stage

100 g of dry beech wood chips were suspended in acidified water 0-200 mM SA, PA or OA and heated in the autoclave reactor to a reaction temperature of 175 °C and kept for 60 min at the reaction temperature. The liquid to solid ratio (L/S) in all experiments was 4:1. After prehydrolysis, the autoclave was cooled down to a temperature below 40 °C. The prehydrolyzed beech wood chips were filtered quantitatively with a Whatman filter (Cellulose, 11µm) by using a vacuum pump. Then, the solid product was washed with 500 ml at 60 °C demineralized water and filtered as mention before. The two resulting liquors were combined (prehydrolyzate) and stored in a refrigerator at 4 °C for further analysis.

The prehydrolyzed wood chips were kept overnight in a refrigerator at 4 °C for the organosolv delignification stage.

2.2.2. Organosolv stage

The prehydrolyzed wood was mixed with 1:1 ethanol/water mixture and 100 mM of sulfuric acid to obtain a slurry with an L/S – ratio of 4:1 L/Kg dry wood. After heating the reactor to the desired reaction temperature (155 °C), the reactor was kept isothermal during the reaction time (160 min) and subsequently was cooled down to a temperature below 40 °C. After the organosolv delignification stage, the suspension was filtered as during the prehydrolysis stage. The liquid phase (black liquor) was collected and the solid (pulp) remaining after the process was washed with 1 L of 50 wt. % aqueous ethanol at 60 °C. In addition, the liquid phase (first wash) was collected and combined with the black liquor, then kept in a refrigerator. After that, the solid residues (pulp) were washed twice with water at 60 °C to get rid of ethanol. Half of the washed solid was dried at 105 °C for pulp yield determination (Equation 1), sugar analysis (glucan and xylan) and determination of residual lignin. The rest of the pulp was kept in the refrigerator at 4 °C for further enzymatic hydrolysis. Lignin was precipitated from the black liquor upon dilution with three equivalents of water and centrifugation at 6500 rpm for 25 min. The solid phase (organosolv lignin) was dried at 50 ºC under vacuum and the yield was calculated

according to equation 2, while the liquid phase was collected. Ethanol was recovered by distillation of the liquid phase and the remaining part is the hemicellulose fraction.

$$Pulp\ yield\ (wt.\%) = \frac{mass\ of\ dried\ pulp}{mass\ of\ total\ dried\ feedtock} * 100; \quad (1)$$

$$Lignin\ yield\ (wt.\%) = \frac{mass\ of\ dried\ lignin}{mass\ of\ lignin\ in\ the\ feed\ stock} * 100; \quad (2)$$

2.2.3. Enzymatic Hydrolysis

Enzymatic hydrolysis of pulp samples containing residual lignin was conducted according to a protocol developed at Fraunhofer CBP with reference to the application sheet provided by Novozyme ®. Briefly, a suspension of 10.5 g dry pulp in 70.5 ml 0.15 M citrate – phosphate buffer solution (pH 5.0) was stirred in an incubator (MaxQTM 8000, Thermo Scientific, Germany). Then, 1 g of antibiotic solution (10mg/ml kanamycin sulfate) was added to eliminate potential bacterial growth. Afterward, 6 g and 2.5 g of Cellic ® CTeC2 (cellulase) and HTeC2 (hemicellulase) enzyme solutions (Novozyme ®) were added to the suspension respectively. Then, the suspension was heated up to 50 °C with shaking at 120 rpm and the incubation continued for 24 h. The hydrolyzate was filtrated using vacuum filtration and the enzymes were deactivated by heating the solution to 100 °C for 10 min in a water bath. The hydrolyzate was stored at -18 ºC and the hydrolysis lignin was dried at 50 °C under vacuum for further analysis.

2.2.4. Analysis

2.2.4.1. Composition of prehydrolyzate, lignin, hemicellulose and pulp

Liquid fractions (prehydrolyzate, hemicellulose, and hydrolyzate), as well as the solid fraction (lignin and pulp), were analyzed for the content of sugars (glucan and xylan) and degradation compounds (furfural, 5-HMF, and acetic acid). Additionally, residual lignin was determined in the pulp fraction and the content of acid-insoluble lignin (klason lignin)

and the acid-soluble lignin (ASL) were determined in lignin fraction. All the analyses were performed according to the NREL/TP-510-42618 protocol [26] in triplicate with an experimental error of less than 2 %.

For the determination, the solid sample was milled using a milling machine and hydrolyzed in two steps: (1) 12 M (72% w/w) sulfuric acid (30 °C, 1 h) and (2) 1.2 M sulfuric acid (121 °C, 100 min), while the liquid samples were hydrolyzed in the one-stage process by using 1.2 M sulfuric acid at 121 °C for 40 min. The solid residues (klason lignin) were determined gravimetrically, while the ASL was measured using UV-spectrophotometer (Specord ® 200 plus, Germany) at 280 nm. Sugars (glucan and xylan) and degradation compounds (5-HMF, furfural, and acetic acid) were analyzed with HPLC (Agilent 1260, Germany) equipped with refractive index (RID) and photodiode array (PDA) detectors and Aminex HPX-87H 300x7, 8mm (Bio-Rad) column at 65 ºC, 5 mM H_2SO_4 mobile phase and flow rate of 0.6 ml/min.

2.2.4.2. Molecular weight determination

An application note (Part No.: SADP2503008 + SADP3503008) provided by AppliChrom was used for the determination of the weight-average molecular weight ($\bar{M}_w$), the number-average molecular weight ($\bar{M}_n$) and the polydispersity ($\bar{M}_w/\bar{M}_n$) of the recovered lignin samples. The Analysis was performed using an HPLC (Agilent 1260, Germany) equipped with a refractive index detector (RID). The pre-column DMSO-Phil-250 (50 mm x 8 mm, 100-70000Da), DMSO Phil-P-350 (separation range 5-1500 Da) and DMSO-Phil-P-250 (100-70000Da) columns were used and the initial column oven temperature was 80 °C. All samples were eluted with 0.075 mol/L $NaNO_3$ (Sigma Aldrich, Germany) in DMSO at a flow rate of 0.5 ml/min. Dextran calibration standards with molecular weights of 180, 342,

504, 1080, 4400, 5000 and 9900 g/mol were used to prepare the calibration curve. Further, all samples were dissolved in eluent to get a final 4 mg/ ml.

2.2.2.3. Carboxyl and phenolic hydroxyl groups content

The content of carboxyl and phenolic hydroxyl groups of lignin was determined using potentiometric titration according to the protocol described by [27]. About 350 mg of dry lignin and 80 mg of *p*-hydroxybenzoic acid were dissolved in 30 ml of dimethylformamide under stirring until the lignin was dissolved. Then, the resulted solution was titrated with 0.05 M tetra-n-butylammonium hydroxide (TnBAH) in isopropanol. Two endpoints were determined during the titration, the first endpoint corresponds to the carboxyl content and the second endpoint corresponds to the total weak acid content, i.e. phenolic hydroxyl groups.

2.2.2.4. Fourier transform infrared (FTIR)

FTIR spectra of organosolv lignin samples measured by ATR spectrophotometer equipped with a single reflection diamond (Bruker Platinum-ATR Alpha series, Germany). The spectra of the recovered lignin were recorded in 400 to 4000 cm^{-1} regions with 2 cm^{-1} resolution and 30 scans.

2.2.2.5.Elemental analysis

For the determination of C, H, N and S content in lignin samples, an automatic elemental analyzer (EuroEA 3000 Series, EuroVector S. P.A., Milano, Italy) equipped with thermal conductivity detector (TCD) was used. Before the analysis, all samples were dried at 105 °C for 24 h. The oxygen content was calculated by difference according to equation 3. The ash content was calculated according to the protocol described in TAPPI T413 om-06 [28].

$$O\ (wt.\%) = 100\ \% - N\ (wt.\%) - C(wt.\%) - H(wt.\%) - S(wt.\%) - Ash\ (wt.\%); \quad (3)$$

3. Results and discussion

3.1. Composition of the prehydrolyzate

During the prehydrolysis stage, hemicellulose (main xylan) and amorphous cellulose are solubilized [29]. The hydrolysis of hemicellulose allows the separation of xylan from the initial beechwood before organosolv treatment, which is improved by using acid as a catalyst [30]. Besides, the use of different types of acids modifies the amount of xylan recovery in an independent stream and the xylan in the wood. Figure 2 shows that considering the use of water as uncatalyzed pretreatment (10.9 ± 0.4 wt. %), the application of SA as a catalyst in the prehydrolysis stage improved the recovery of xylan in an independent stream (11.3 ± 0.5 wt. %.). Similar results were found in previous studies [31, 32]. On the other hand, the use of PA and OA as a catalyst in the prehydrolysis stage decrease c.a. 0.5 times the recovery of xylan. This effect might be due to the fact that PA and OA prefer the cleavage of glycosidic bonds of xylo-oligosaccharides rather than dehydration and other side reactions [33].

Moreover, the use of acid during the prehydrolysis leads to the formation of degradants (5-HMF, furfural and acetic acid) that are not obtained by using water (Figure 2). This is a consequence of the reduction of the stability of xylan under acid conditions lead to the formation of 5-HMF and furfural by dehydration mechanism [34, 35, 36]. Furthermore,

furfural can polymerize with itself to produce another precursor, therefore, leads to enhance the recondensation reactions [37].

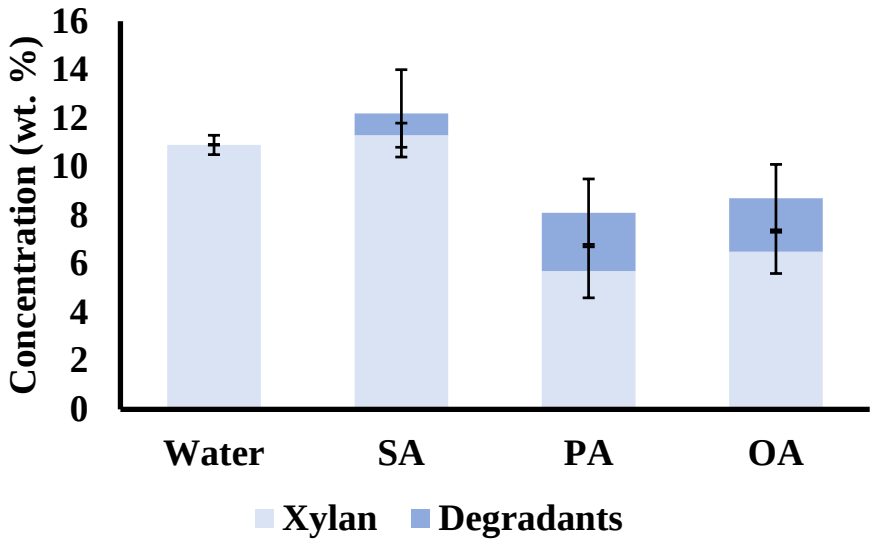

Figure 2. The composition of the prehydrolyzate after an uncatalyzed and acid catalyzed the prehydrolysis process. (Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

3.2.Yield and composition of the recovered lignin

Figure 3 shows a comparison of the recovery yield of the lignin from beech wood with a one-stage process and catalyzed and uncatalyzed a two-stage process. The use of a prehydrolysis stage with water results in a similar recovery yield, while the use of SA slightly increases the yield. This is in accordance with previous studies when SA was used as a catalyst [38, 39,34].

Conversely, the effect was observed with the use of PA and OA during the prehydrolysis. There are two explanations for this observance. According to [34], hemicellulose and lignin are connected with ether bonds which are broken because of the acid used as a catalyst.

Then, the higher removal of hemicellulose during the prehydrolysis leads to the higher recovery of lignin during the organosolv stage. It might be that during the prehydrolysis lignin repolymerization may occur. This leads to the formation of carbonium ion intermediates, thereby enhancing the formation of new carbon – carbon bonds such as β-β, β-1 and β-5 [40,41]. Hence, the lignin extraction is hampered during the organosolv process.

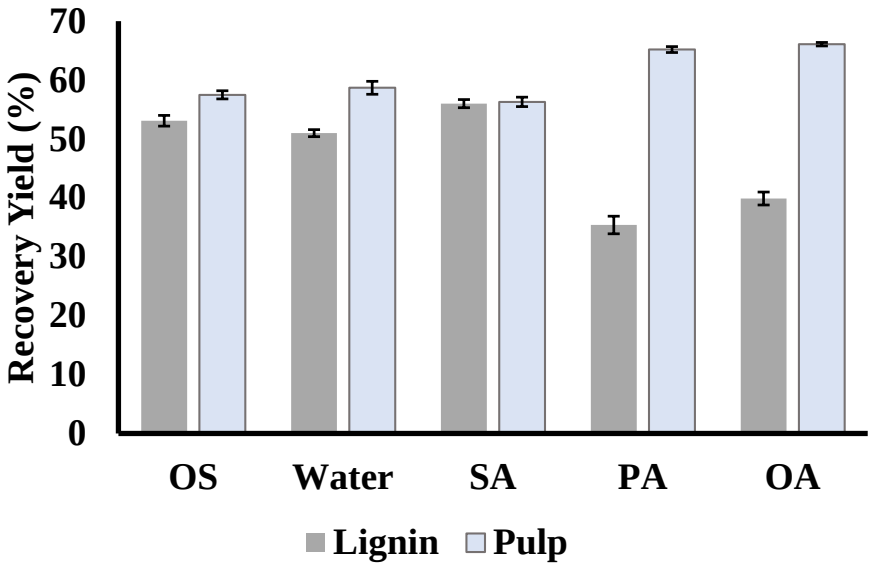

Figure 3. The yield of the recovered lignin and pulp after one-stage organosolv delignification and two-stage processes of catalyzed and uncatalyzed prehydrolysis followed by organosolv delignification. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

The use of a two-stage process also modifies the ratio of the components of the extracted lignin compared with a one-stage process. As can be seen in Figure 4, the main component extracted is Klason lignin (acid insoluble lignin), which slightly increases after the two-stage process. The highest percentage of Klason lignin was found by using SA (97.1 ± 0.4

wt. %). Considering the studies of Leschinsky [40,41], they stated that the amount of Klason lignin in the recovery lignin increment due to the condensation reactions of the dissolved lignin, sugar degradation compounds or the polyphenolic extractives [42, 43].

Figure 4 shows that the percentage of ASL was the highest (5.1 ± 1.6 wt. %) when one-stage was applied, or PA (4.3 ± 1.6 wt. %) was used as a catalyst during the prehydrolysis stage, because of the increase in the low $\bar{M}_w$ lignin when the pH value of the black liquor was dropped from 3.45 ± 0.02 to 2.51 ± 0.02. Additionally, the solubility of lignin was enhanced, and the dissolved compounds undergo different reactions like condensation with carbohydrates, intermolecular condensation and degradation reactions, which leads to the formation of ASL [42].

Although all the recovered lignin samples were washed with deionized water to remove the residual carbohydrate, sugar analysis results (Figure 4) showed the presence of carbohydrates (main xylan), which are similar to found in the literature [44]. The presence of covalent and ether bonds between the hemicellulose and lignin makes it difficult for the complete separation of the carbohydrates [45, 46, 47].

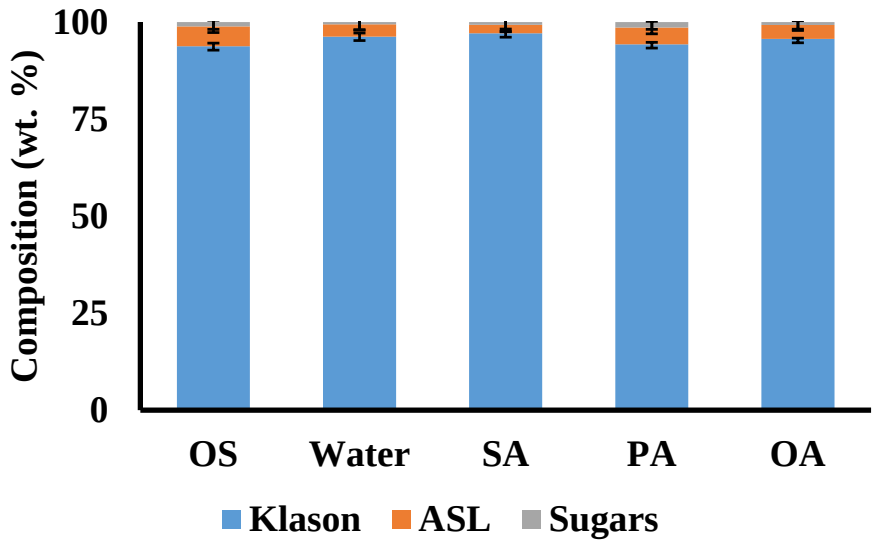

Figure 4. The composition of the centrifuged recovered lignin after one-stage and two-stage processes. (ASL: acid soluble lignin, Carbohydrates: total residual sugars in lignin, OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

3.3. Characteristic of the recovered lignin

The characteristics differ significantly from the type of the recovered lignin. Lignin obtained after the use of uncatalyzed water and PA during the prehydrolysis stage display a $\bar{M}_w$ which is 1.21 and 1.16 times respectively higher than lignin from the one-stage process (Figure 5). However, a low- $\bar{M}_w$ was obtained for the lignin recovered after using SA and OA as a catalyst during prehydrolysis. This high-$\bar{M}_w$ is associated with the incomplete removal of ester/ether linkage and the increment in aryl ether linkage [48]. However, the highest value of the ($\bar{M}_w/\bar{M}_n$) is obtained after the one-stage process, the values of $\bar{M}_w/\bar{M}_n$ obtained in this study with acid catalysed prehydrolysis is slightly lower than the results reported in previous studies [48, 49, 50]. This effect might be due to the

fact that prehydrolysis could facilitate the cleavage of the inter-unit bonds in lignin, therefore producing smaller fragments of lignin with low-$\bar{M}_w$. The dissolution of these low-$\bar{M}_w$ fragments and condensation reactions of lignin worked together to enhance the increase in the $\bar{M}_w$ and decrease the polydispersity.

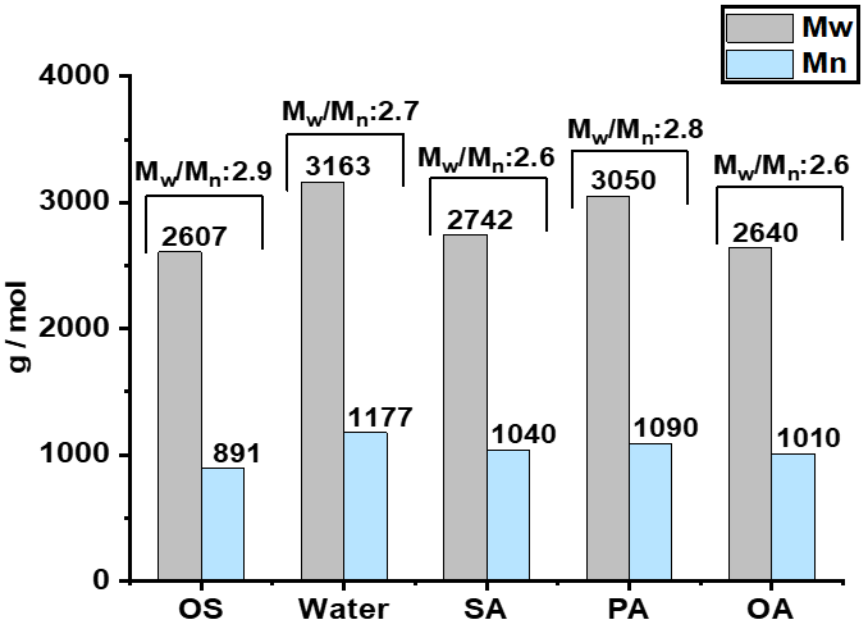

Figure 5. $\bar{M}_w$, $\bar{M}_n$ and $\bar{M}_w/\bar{M}_n$ of the recovered lignin after a one-stage and two-stage process. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid, $\bar{M}_w$: the weight-average molecular weight, $\bar{M}_n$: the number-average molecular weight, $\bar{M}_w/\bar{M}_n$: the polydispersity).

The organosolv lignin obtained by a one-stage process contains slightly lower carbon content than obtained by the two-stage process (Table 2). Lignins obtained after SA and PA catalyzed prehydrolysis with subsequent organosolv delignification show similar C (64 wt. %), H (6 wt. %), N (0.3 wt. %) and O (30 wt. %) contents (e.g. No. 3 and 4). Yet, the lignin recovered after uncatalyzed prehydrolysis followed by organosolv delignification

provided higher C and lower O contents (No. 2) compared with the other three lignin samples (No. 3 – 5). A possible explanation is newly generated hydroxyl groups in the lignin during the two-stage process of catalyzed and uncatalyzed prehydrolysis as well as during the organosolv delignification via hydrolysis of ether bonds and methoxy groups [51]. Additionally, the N content of lignin reflects contamination by protein residues from cell-wall. Consequently, N amounts are very low (about 0.3 wt. %), implying that the weak chemical bond between proteins and the recovered lignins [52].

Table 2. Elemental analysis results of the recovered lignin samples

No.	Lignin	Elemental composition (db wt. %)			
		C	H	N	O
1	OS	61.6 ± 0.2	6.2 ± 0.4	0.3 ± 0.2	31.9 ± 0.5
2	Water	65.2 ± 0.4	6.2 ± 0.4	0.3 ± 0.2	28.3 ± 0.6
3	SA	63.7 ± 0.3	6.0 ± 0.3	0.2 ± 0.1	30.1 ± 0.1
4	PA	63.9 ± 0.5	5.9 ± 0.1	0.3 ± 0.2	29.9 ± 0.5
5	OA	59.1 ± 0.2	5.6 ± 0.1	0.2± 0.1	35.1 ± 0.2

* db = dry basis

The content of carboxyl groups in the recovered lignin was determined to evaluate the degradation of the lignin structure. After the one-stage process and using PA and OA as catalysts, the highest amount of carboxyl groups was obtained (Figure 6). Additionally, only the use of OA produced lignin with the content of phenolic compounds similar to the one-stage process. This is due to the fact of using the organosolv delignification process with high proton levels enhances the loss of terminal groups in the lignin structure, therefore increasing the phenolic hydroxyl group content [49]. In addition, the content of

the phenolic hydroxyl groups is one of the factors as well as the purity (low carbohydrates and high Klason lignin) that determine the antioxidant activity of lignin [53].

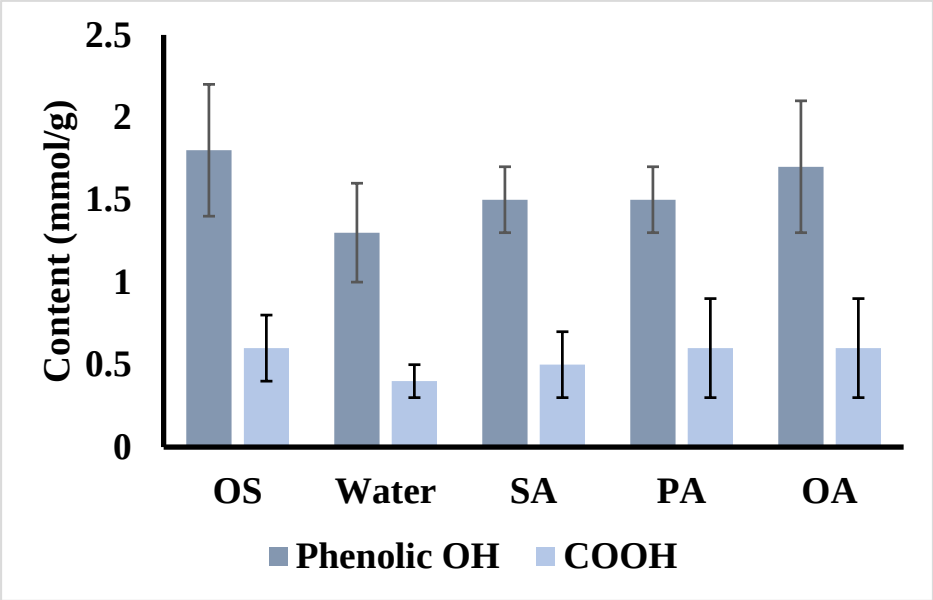

Figure 6. The content of carboxyl and phenolic hydroxyl groups of the recovered lignin after one-stage and two-stage processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

Figure 7 is shown the FT-IR spectra of the five lignin samples recovered after one and two-stage processes. The FT-IR spectra of the lignin without prehydrolysis was used as a reference for the comparison with catalyzed lignins. The literature data from [54, 55] used to assign the peaks for different functional groups of lignin structure. Assignment of the labelled peaks: C=O stretch in unconjugated ketones at 1735cm^{-1}, carbonyls and ester groups (mostly, from carbohydrate origin), aromatic skeletal plus C=O stretch at 1594 cm^{-1}, aromatic skeletal vibrations (G>S) at 1512 cm^{-1}, aromatic ring breathing (S and condensed G) at 1323 cm^{-1}, aromatic ring breathing (G) at 1270 cm^{-1}, C-H bending out of the plane in position 2 and 6 of S at 830 cm^{-1}. The peaks at 1594 cm^{-1} and 1323cm^{-1}

correspond to syringyl units (S) and at 1512cm^{-1} and 1270cm^{-1} correspond to guaiacyl units (G). Further, at 1120cm^{-1} and 1034cm^{-1} the aromatic in-plane C-H bending is observed for S and G units respectively, while the out-of-plane C-H bending of syringyl content is observed at 830 cm^{-1}.

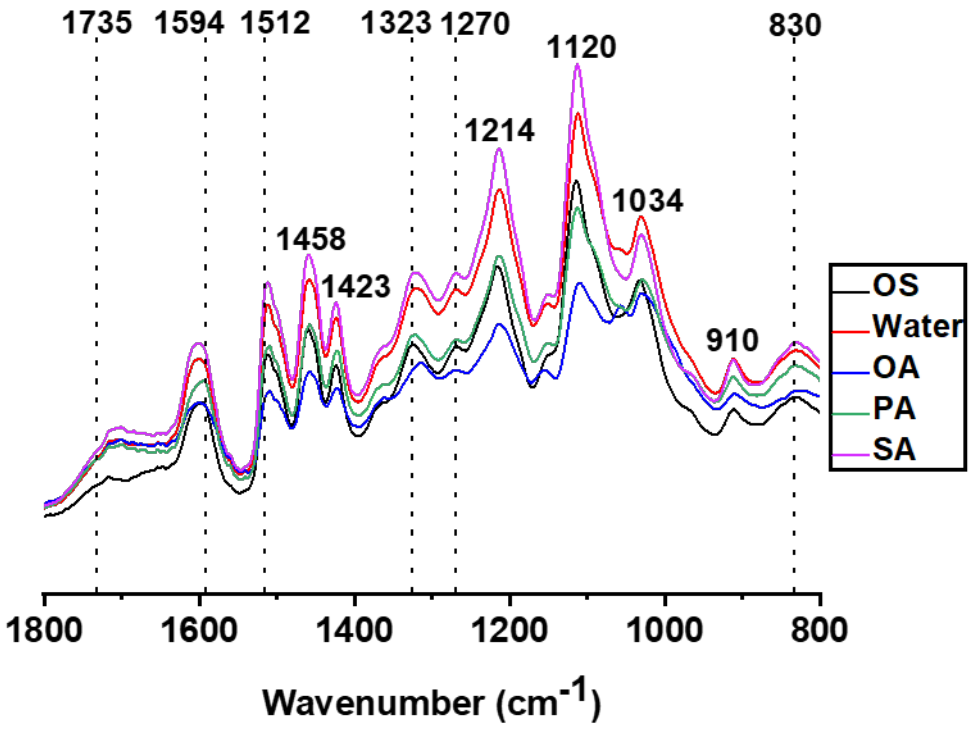

Figure 7. FT-IR spectra of recovered lignin after one-stage and two-stages processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

The obtained lignin samples had rather similar FT-IR spectra indicating that the lignin after one stage organosolv delignification and two-stage of catalyzed and uncatalyzed prehydrolysis followed by organosolv delignification processes had not undergone major structural modification. However, the obtained lignin samples showed different intensities in some bands. [56]. The wavelength (1594, 1323 and 1512, 1270) in the FT-IR spectrums

corresponded to the S and G rings respectively of the hardwood lignin [50]. The peak at 1703 cm^{-1} corresponds to the formation of ester due to the esterification reaction of the aromatic unit and alcohol of the propane chain during the combined fractionation process [55].

3.4. Composition of hemicellulose

A co-product after the separation of the lignin and liquor by centrifugation is aqueous phase rich in carbohydrates. This liquor is distilled in a Snyder column at 81 ºC to recover the ethanol, and the remaining liquid phase is mainly water with soluble carbohydrates. This liquor contains residual hemicellulose (quantify as xylan) that was not possible to separate along the process. As was expected, the liquor after two-stage contains less amount of xylan than with a one-stage process, because the prehydrolysis stage removes most of the initial hemicelluloses as shown in figure 8.

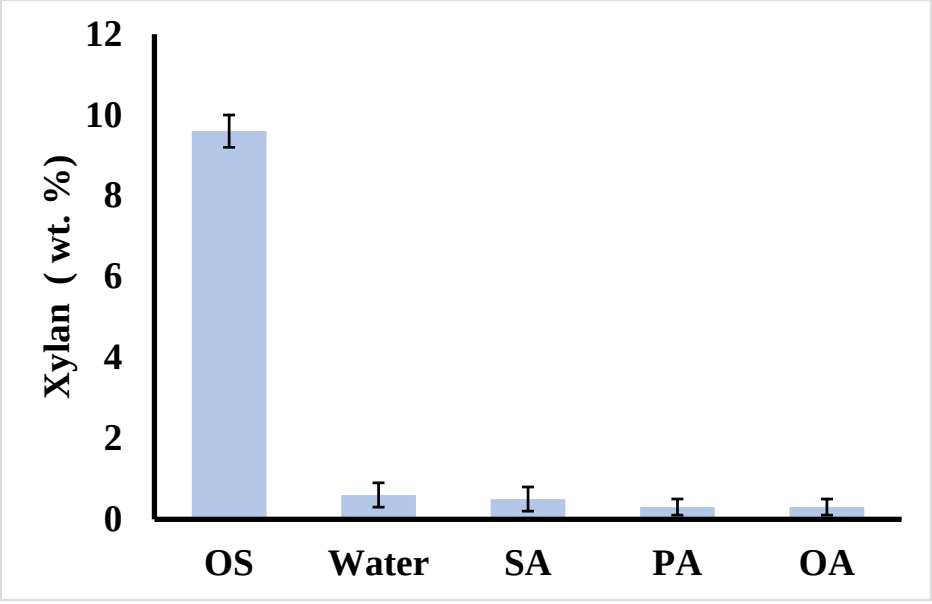

Figure 8. The composition of hemicellulose after one-stage and two-stage processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

3.5.Composition of pulp and enzymatic hydrolysis

The solid fraction (pulp), obtained after the one-stage process, and uncatalyzed and catalyzed two-stage processes of prehydrolysis followed by organosolv delignification was characterized before enzymatic hydrolysis. The pulp is mainly composed of cellulose (glucan) and residual xylan amounts (Figure 9). It is necessary to notice that, during the prehydrolysis, the crystalline structure of the cellulose was not possible to hydrolyze (glucan units was not solubilized in the liquid phase). Owing to the process conditions were not harsh enough to break down the hydrogen bonds between the adjacent glucose units and β glycosidic bonds that linked glucose units in cellulose [57]. The highest amount of glucan was found in the fiber after the one-stage process (73.0 ± 0.2 wt. %), uncatalyzed prehydrolysis (74.1 ± 0.1 wt. %) and SA catalyzed prehydrolysis (72.2 ± 0.3 wt. %). The remaining hemicellulose in the fiber that cannot be possible to hydrolyse due to the covalent bonds was quantified as xylan. The lowest amount of xylan was found in the pulp treated with SA as a catalyst during the prehydrolysis stage (Figure 10).

Therefore, it can be assumed that the treatment with SA is the most efficient to remove the hemicellulose before organosolv treatment, which is in agreement with previous studies [58, 34]. In addition, the pulp obtained after using SA as catalyst contains the lowest content of residual lignin quantified (8.7 ± 0.9 wt. %). On the other hand, the pulp obtained after the use of PA and OA contains the highest amount of xylan and residual lignin, it

might be to the fact that both of them produced the lowest recovery yield to lignin (Figure 1).

shows that the pulp obtained after PA catalyzed prehydrolysis and organosolv delignification has the highest residual lignin content (15.1 ± 0.5 wt. %) while the pulp obtained after SA catalyzed prehydrolysis and organosolv has the lowest content (8.7 ± 0.9 wt. %).

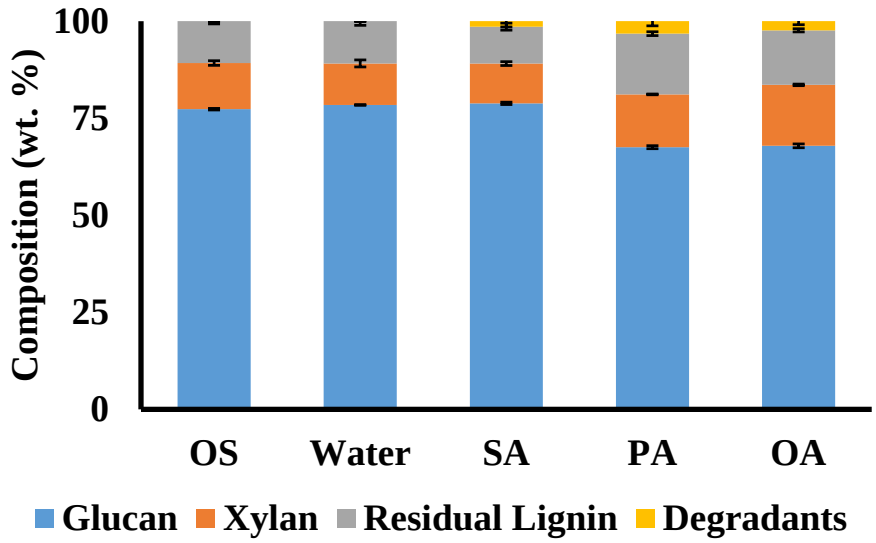

Figure 9. The composition of pulp after one-stage and two-stage processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

To evaluate the efficiency of a two-stage process, enzymatic hydrolysis was conducted using the cellulosic fiber obtained after each process. The results of the enzymatic hydrolysis with CTeC2 and HTeC2 reflected as glucan are shown in Figure 10. The cellulose obtained during the one-stage process has a higher tendency to get hydrolyzed to (86.3 ± 0.3 g/L), while the lowest (34.6 ± 1.1 g/ L) was obtained after using PA catalyzed

prehydrolysis. As shown in Figure 9, the use of PA (15.1 ± 0.5 wt. %) of residual lignin, which has a significant effect on the enzymes used for the enzymatic hydrolysis, inhibiting the depolymerization of cellulose and the production of monomeric sugars [59].The lower amount of lignin also implies a higher number of active sites and the 1-beta glucoside bonds of the cellulose for the enzymes [60]. These results approaching those found in the literature [61, 62, 63].

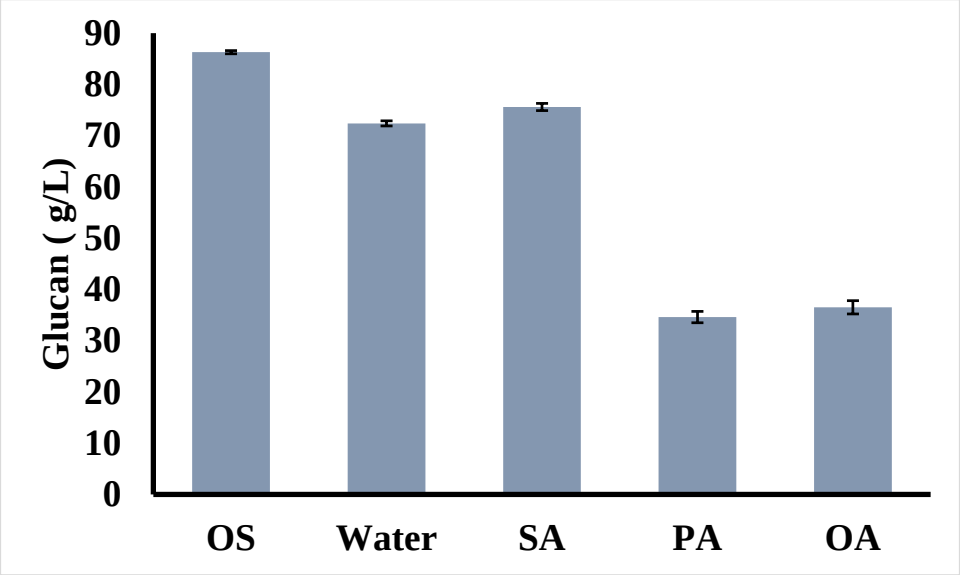

Figure 10. Cellulose digestibility of pulp after one-stage and two-stage processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

4. Conclusion

In this study, the yield of lignin and its characteristics, as well as the yields of carbohydrates, their degradation products through a one-stage organosolv process and two-stage process, were compared. In detail, the effect of uncatalyzed (water) and catalyzed with sulphuric, phosphoric and oxalic acids was evaluated during the two-stage process. The results showed that the SA catalyzed prehydrolysis enhanced the removal of xylan (11.3 wt. %) while using PA and OA diminished it to 5.7 wt. % and 6.5 wt. %, respectively. Moreover, SA catalyzed prehydrolysis has a positive effect on the organosolv delignification process by improving the yield of the recovered lignin to 56.0 wt. % and improved the enzymatic hydrolysis of the cellulosic pulp (glucan content increased from 72.4 g/L to 75.6 g/L). The use of PA and OA as a catalyst in the prehydrolysis results in lignin with a low amount of xylan and carboxyl groups. In comparison, the lignin recovered from the one-stage process has low $\bar{M}_w$, low sugar content and high content of phenolic hydroxyl groups. Furthermore, the best enzymatic digestibility (86.3 g/L) in this work was for the pulp obtained after one-stage organosolv delignification.

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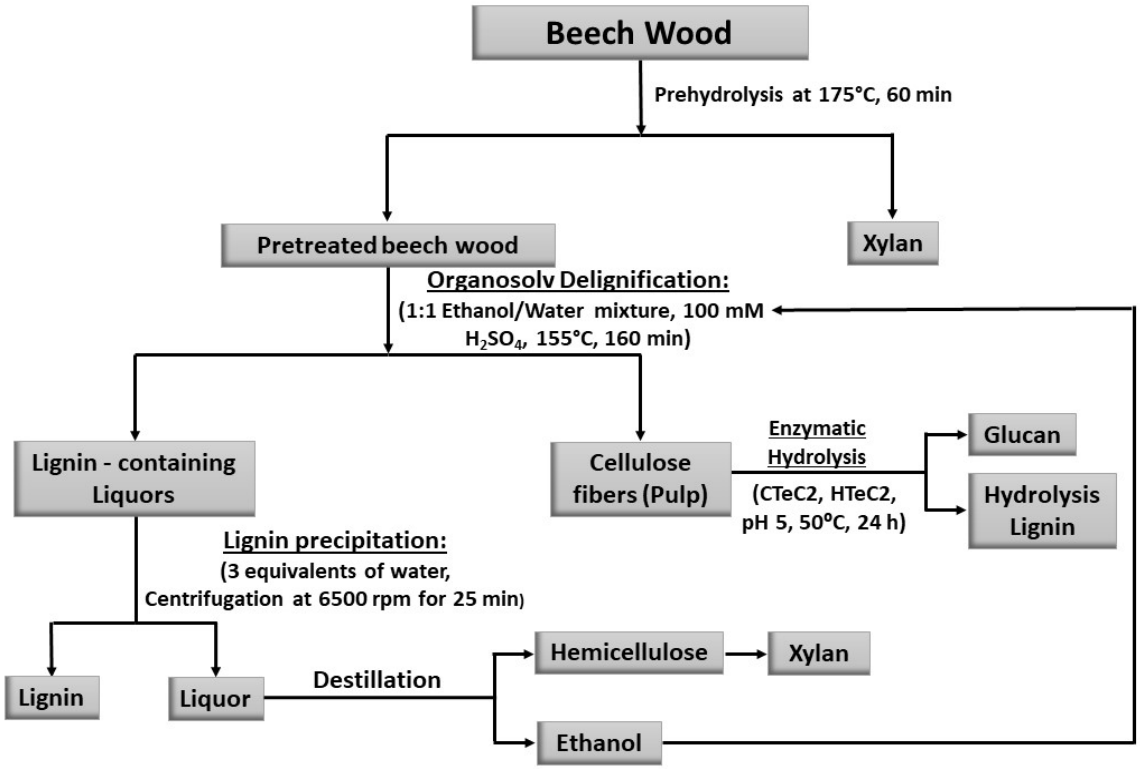

Figure 1. General scheme of the fractionation of beech wood by one or two-stage process.

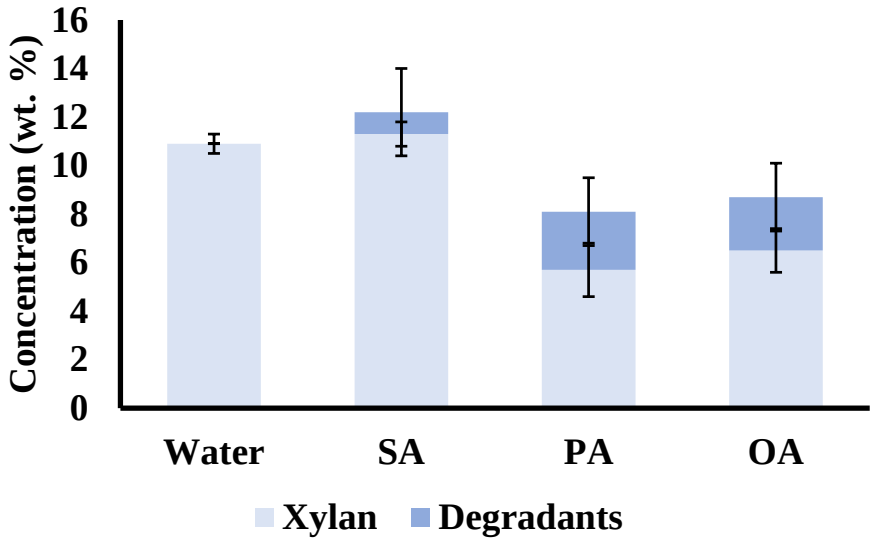

Figure 2. The composition of the prehydrolyzate after an uncatalyzed and acid catalyzed the prehydrolysis process. (Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

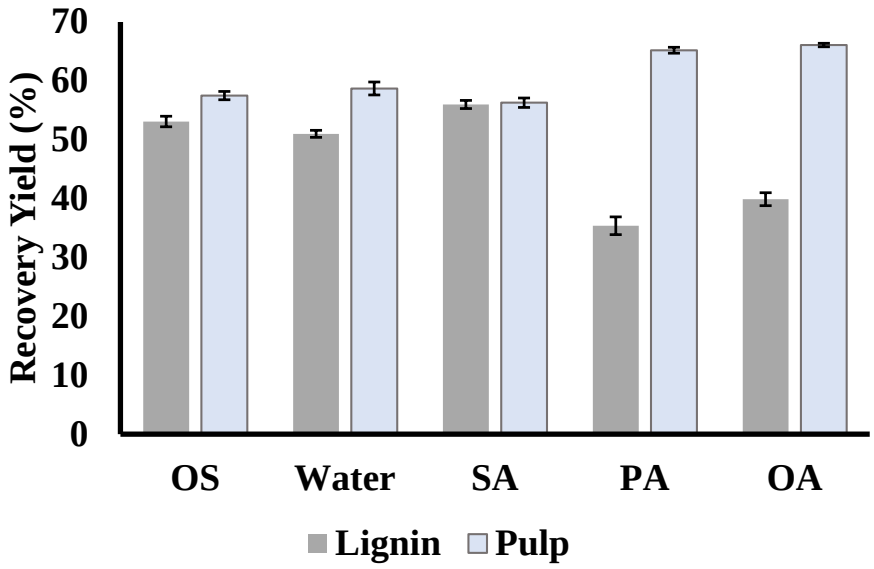

Figure 3. The yield of the recovered lignin and pulp after one-stage organosolv delignification and two-stage processes of catalyzed and uncatalyzed prehydrolysis followed by organosolv delignification. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

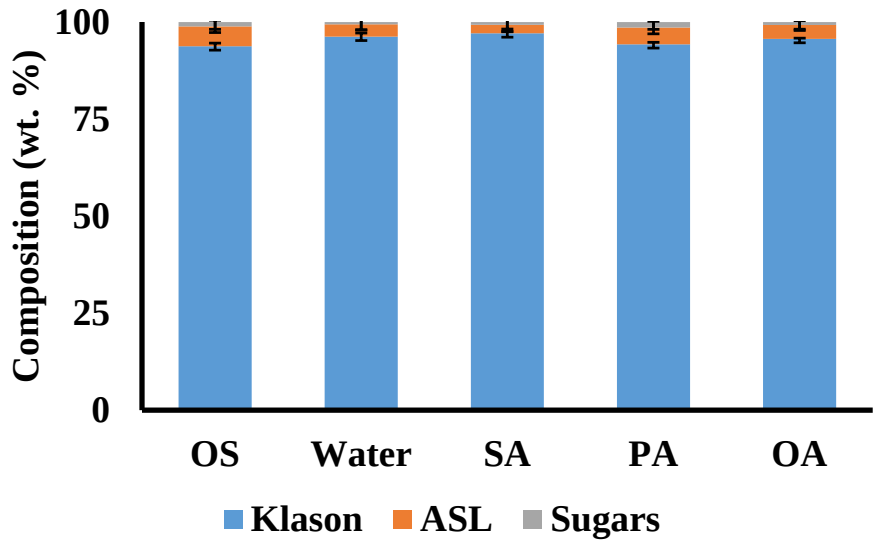

Figure 4. The composition of the centrifuged recovered lignin after one-stage and two-stage processes. (ASL: acid soluble lignin, Carbohydrates: total residual sugars in lignin, OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

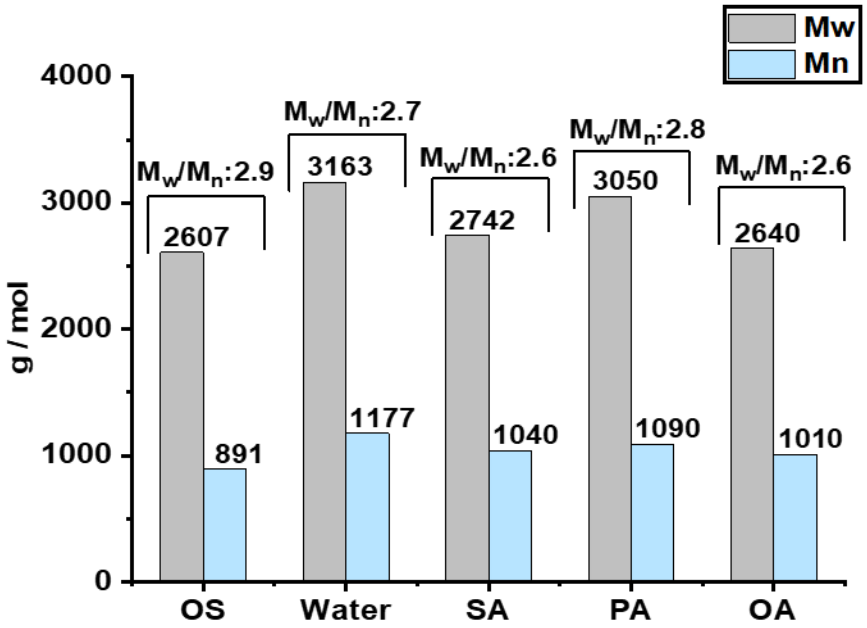

Figure 5. $\bar{M}_w$, $\bar{M}_n$ and $\bar{M}_w/\bar{M}_n$ of the recovered lignin after a one-stage and two-stage process. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid, $\bar{M}_w$: the weight-average molecular weight, $\bar{M}_n$: the number-average molecular weight, $\bar{M}_w/\bar{M}_n$: the polydispersity).

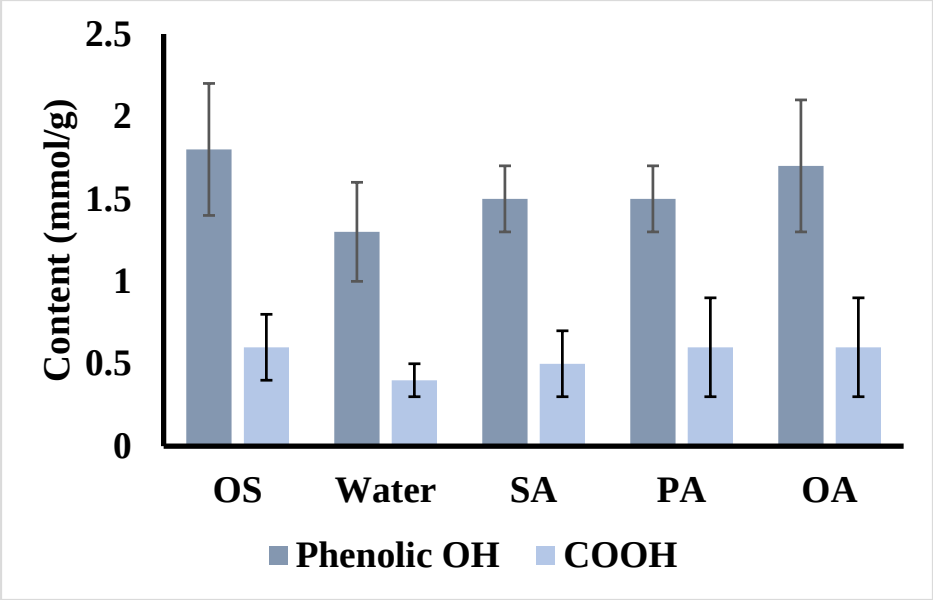

Figure 6. The content of carboxyl and phenolic hydroxyl groups of the recovered lignin after one-stage and two-stage processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

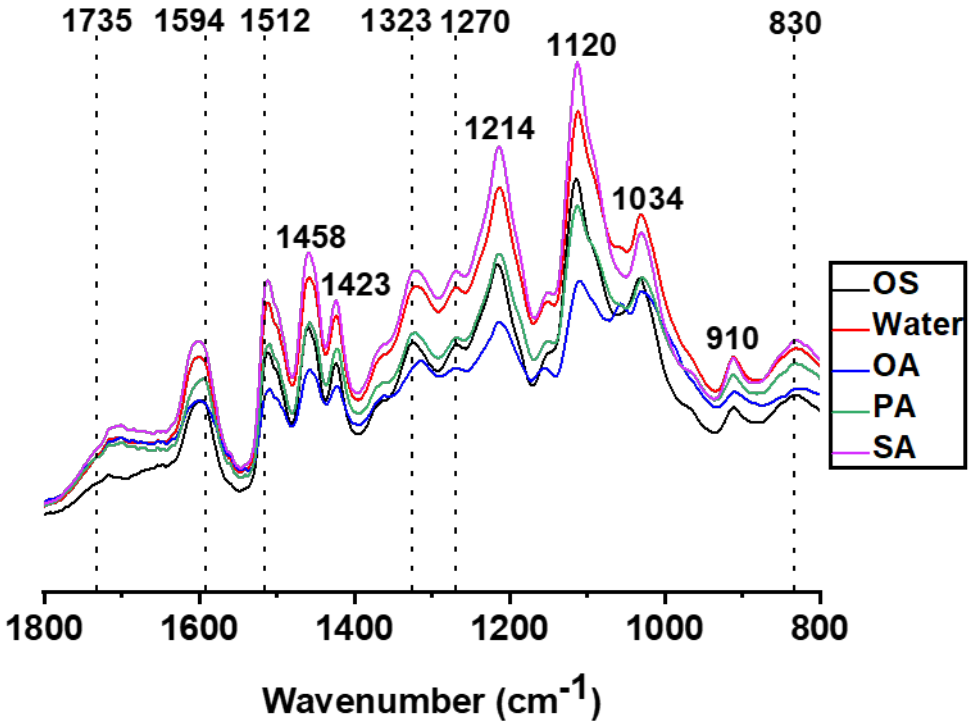

Figure 7. FT-IR spectra of recovered lignin after one-stage and two-stages processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

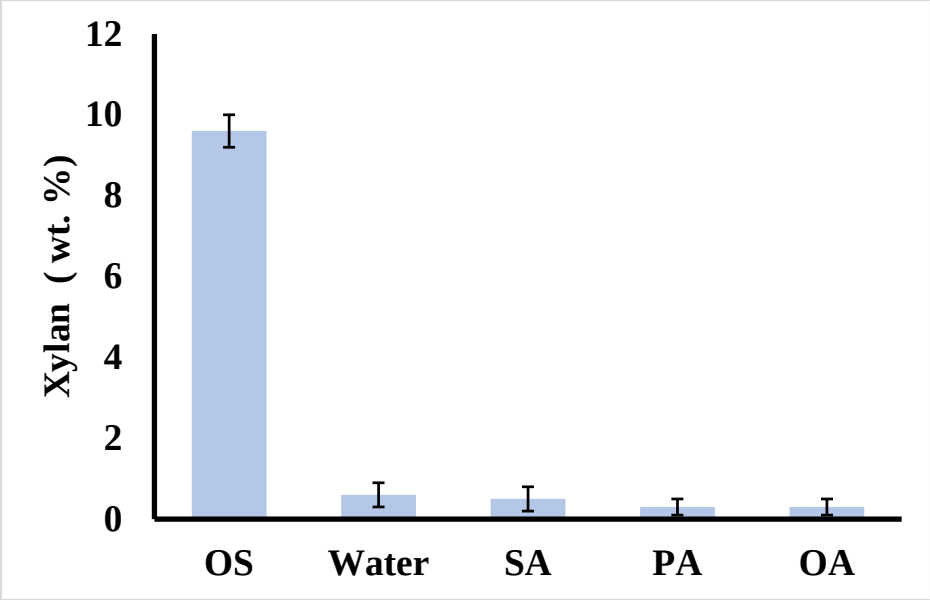

Figure 8. The composition of hemicellulose after one-stage and two-stage processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

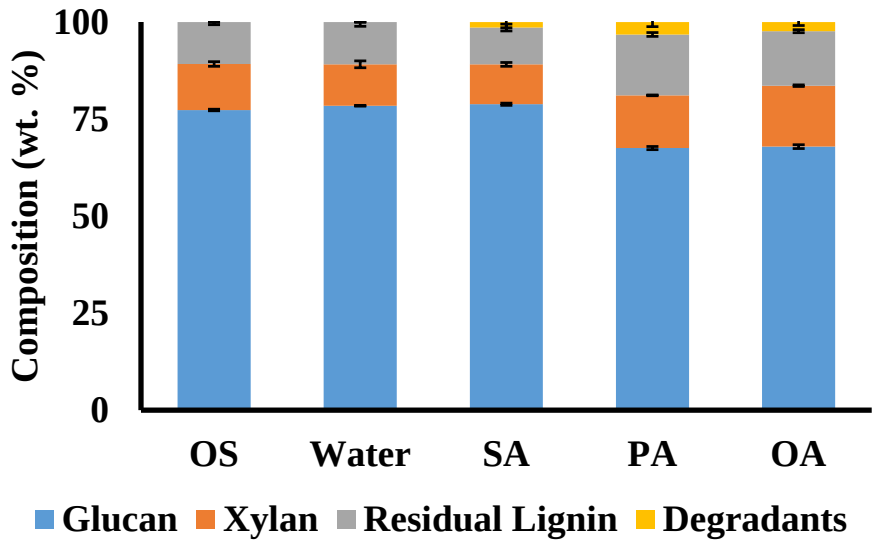

Figure 9. The composition of pulp after one-stage and two-stage processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

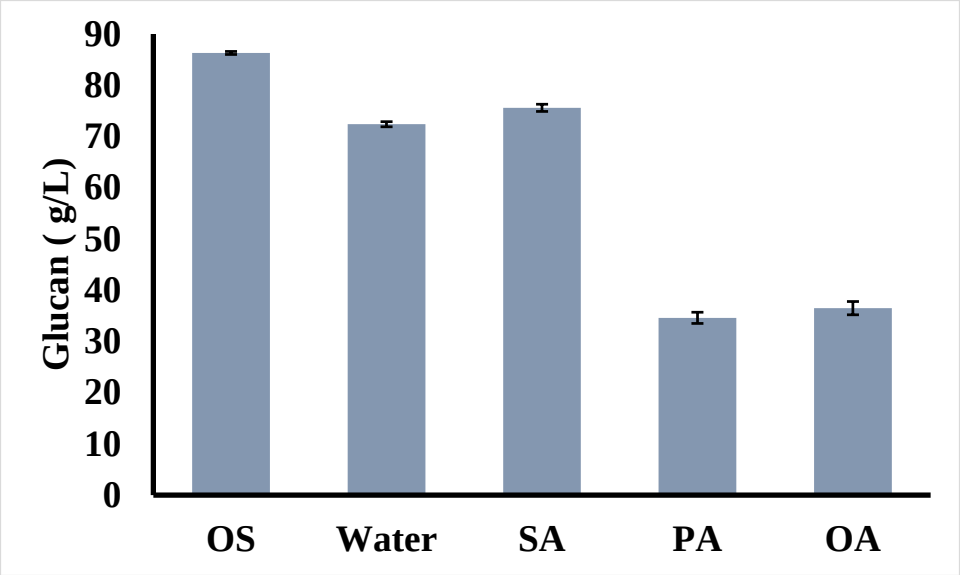

Figure 10. Cellulose digestibility of pulp after one-stage and two-stage processes. (OS: one-stage organosolv process, Water: uncatalyzed prehydrolysis, SA: Sulfuric acid, PA: Phosphoric acid, OA: Oxalic acid).

Table 1. Experimental conditions of prehydrolysis and organosolv delignification stages

Experiment No.	Stage	Conditions		
		Solvent	T (ºC)	t (min)
1	**1)** OS [a]	50 wt. % Ethanol + 100 mM H_2SO_4	155	160
2	**1)** PH [b]	Uncatalyzed water	175	60
	2) OS	50 wt. % Ethanol + 100 mM H_2SO_4	155	160
3	**1)** PH	Water + 20 mM H_2SO_4	175	60
	2) OS	50 wt. % Ethanol + 100 mM H_2SO_4	155	160
4	**1)** PH	Water + 20 mM H_3PO_4	175	60
	2) OS	50 wt. % Ethanol + 100 mM H_2SO_4	155	160
5	**1)** PH	Water + 200 mM $C_2H_2O_4$	175	60
	2) OS	50 wt. % Ethanol + 100 mM H_2SO_4	155	160

[a] OS = Organosolv delignification.
[b] PH = Prehydrolysis.

Table 2. Elemental analysis results of the recovered lignin samples

No.	Lignin	Elemental composition (db wt. %)			
		C	H	N	O
1	OS	61.6 ± 0.2	6.2 ± 0.4	0.3 ± 0.2	31.9 ± 0.5
2	Water	65.2 ± 0.4	6.2 ± 0.4	0.3 ± 0.2	28.3 ± 0.6
3	SA	63.7 ± 0.3	6.0 ± 0.3	0.2 ± 0.1	30.1 ± 0.1
4	PA	63.9 ± 0.5	5.9 ± 0.1	0.3 ± 0.2	29.9 ± 0.5
5	OA	59.1 ± 0.2	5.6 ± 0.1	0.2± 0.1	35.1 ± 0.2

* db = dry basis