



Case study

Towards a wheat straw biorefinery: Combination of Organosolv and Liquid Hot Water for the improved production of sugars from hemicellulose and lignin hydrolysis

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ABSTRACT

One major challenge of sustainable biorefineries relies on valorizing hemicellulose and lignin simultaneously. However, a single pretreatment will unlikely render high sugar and lignin yields. Liquid-Hot-Water (LHW) and Organosolv (OS) highlight as technologies to solubilize these components (in different proportions). A combined pretreatment may hydrolyze lignin and hemicellulose into different fractions, with higher overall yield and leaving cellulose available for further processing. Hence, it must be determined the stages' order to maximize hemicellulose/lignin hydrolysis. We performed both configurations (OS → LHW and LHW → OS) using wheat straw and determined sugar and lignin concentration and the dissolved lignin's molar-mass distributions. Both configurations reached similar sugar concentrations (~12 g/L), but LHW → OS reached a 1.6-times higher lignin extraction (7 to 11 g/L in the OS-stage). The extracted lignin molar mass in LHW → OS had lower polydispersity and molar-mass averages, favoring colloidal particle production. These results bring us closer to a sustainable biorefinery valorizing the different feedstock fractions.

1. Introduction

Developing sustainable biorefineries has become increasingly urgent to address the current fossil-based economy's environmental impacts and switch into a society based on sustainable development (Palmeros Parada et al., 2017). From their conception, biorefineries are proposed as a technological scheme to valorize the different fractions of the feedstock, and multiple advances have been achieved in terms of pretreatment, conversion technologies, and possible products from lignocellulosic biomass (Hendriks and Zeeman, 2009; Kamm and Kamm, 2004). Recalcitrance is the capacity of the lignocellulosic complex to resist chemical, enzymatic and physical attacks to deconstruct the polymeric structure. This property of lignocellulosic biomass still represents a challenge to fractionate the different components effectively and makes multiple proposed biorefineries to remain economically infeasible (Cardona Alzate et al., 2020; Hassan et al., 2019). One of the major challenges still relies on addressing the sugar production from the

hemicellulose fraction and lignin valorization simultaneously, as this would provide two different platforms to obtain value-added products, and cellulose could still be valorized either as a fiber or for enzymatic conversion (Galkin and Samec, 2016; Liu et al., 2019; Ragauskas et al., 2014).

Hemicellulose valorization by its conversion into sugars offers the possibility of a mixture of C5/C6 sugars that could be used as a substrate for fermentation (Michelin and Teixeira, 2016) or furan-based products (Aristizábal et al., 2015). Lignin valorization to value-added products has been considered recently as a key stage of sustainable biorefineries (Liu et al., 2019). Lignin is mostly underutilized in first-generation cellulosic projects as a residual stream for energy production –approximately 40% of “residual” lignin may cover the internal energy demand of a biorefinery (Boerjan et al., 2003). This leaves the potential for other applications where properties as biodegradability, biocompatibility, UV-resistance, and low toxicity are claimed. In this regard, lignin has been considered a potential feedstock for colloidal-particle

Abbreviations: AIL, Acid Insoluble Lignin; ASL, Acid Soluble Lignin; CLP, Colloidal Lignin Particles; LHW, Liquid Hot Water; OS, Organosolv; LHW → OS, Configuration 1: Liquid Hot Water followed by Organosolv; OS → LHW, Configuration 2: Organosolv followed by Liquid Hot Water; OS-1st, Organosolv performed as the initial stage; LHW-2nd, Liquid Hot Water performed subsequently after OS-1st; LHW-1st, Liquid Hot Water as the initial stage; OS-2nd, Organosolv performed subsequently after LHW-1st; M_w , M_n , Weight-average; M_n , number-average molar mass (M_n); Pd, Polydispersity index.

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production, namely, colloidal lignin particles (CLP). Some potential applications can be drug delivery systems, delivery of hydrophobic molecules, UV-barriers, antibacterials, and electrode materials, among others (Beisl et al., 2017). This shows both hemicellulose and lignin fractions' potential but brings out the key challenge of the pretreatment technologies for sugar fractionation and lignin valorization.

Multiple pretreatment clusters focused on the deconstruction of the lignocellulosic matrix have been proposed; each cluster focuses on hydrolyzing a specific fraction, namely, cellulose, hemicellulose, or lignin. For example, acid pretreatments focus on hydrolyzing hemicellulose, alkaline pretreatments focus on hydrolyzing lignin, and enzymatic pretreatments focus mainly on cellulose. Among these, Liquid Hot Water (LHW) highlights as a method for hemicellulose hydrolysis, as it only uses water as a reactant and is auto-catalyzed by the released acetic acid from the hemicellulose backbone, without further input of acids/bases (as it is the case of dilute acid hydrolysis). In addition, LHW produces less degradation products as furfural and hydroxymethylfurfural, and has lower capital costs (Agrawal et al., 2017; Satlewal et al., 2019). Regarding lignin hydrolysis, Organosolv (OS) pretreatment has been a promising approach, as it enables solubilizing part of the hemicellulose and removing most of the lignin (Huijgen et al., 2010). In many cases, the pretreatment stage's ultimate goal is to deconstruct the lignin–hemicellulose complex and increase cellulose accessibility to enzymatic attack –cellulose remains as the main target fraction to be valorized, either for the production of glucose via enzymatic hydrolysis or for fiber applications. However, a valorization of all of the different lignocellulosic complex fractions is a key factor in reaching a sustainable biorefinery (Serna-Loaiza et al., 2019).

In terms of a simultaneous valorization of the lignocellulosic complex's fractions, it is improbable that a single pretreatment will allow obtaining simultaneously high sugar yields and lignin concentrations (Liu et al., 2019). Therefore, a combination of pretreatments must be applied. In this regard, different combinations have been proposed. Steam explosion followed by alkaline pretreatment (Neves et al., 2016; Rocha et al., 2012) and Organosolv followed by LHW (Liu et al., 2019; Weinwurm et al., 2017; Win et al., 2016) are some examples. Specifically for the combination of OS and LHW, performing first the OS solubilizes part of the hemicellulose and most of the lignin, decreasing the feedstock's recalcitrance. This leaves a solid matrix with more-accessible hemicellulose, and therefore, sugar yields may increase when performing the LHW (Weinwurm et al., 2017). However, if this pretreatment combination is analyzed for lignin production, performing the LHW step initially would remove most of the hemicellulose and the lignin in a minor share, leaving then a solid matrix with lignin more accessible for the subsequent OS stage. Most of the pretreatments and studies carried out until now focus on removing either the hemicellulose or the lignin in order to valorize the other fraction, and consider the fraction to be removed as a residue to be removed rather than a component to be valorized. However, as both OS and LHW can solubilize hemicellulose and lignin (although each pretreatment in different proportion – OS hydrolyzing mainly lignin, whereas LHW hydrolyzes mainly hemicellulose), the question remains open: which should be the order of the stages to maximize sugar production and lignin extraction? Therefore, it is necessary to evaluate the sugar production, lignin extraction, and lignin molar mass for both configurations (LHW → OS and OS → LHW) to determine which pretreatment configuration has better results. Lignin molar mass is a key indicator to determine, as molar mass distribution partly governs lignins' reactivity and physico-chemical properties.

To address this matter, we performed both configurations (LHW → OS and OS → LHW) using wheat straw as feedstock and characterized the two liquid fractions in terms of the sugar concentration, lignin content, and the molar mass of the extracted lignin. This information can provide a better insight on which combination allows obtaining a sugar hydrolysate suitable for fermentation applications and a lignin solution that facilitates producing colloidal lignin particles. These analyses

should help us get one-step closer to a sustainable biorefinery that valorizes the feedstock's different fractions into value-added products.

2. Materials and methods

2.1. Raw material and reagents

The used wheat straw was harvested in 2019 in the region of Margarethen am Moos, state of Lower Austria, and stored under dry conditions until use. The particle size was reduced in a cutting mill, equipped with a 2 mm mesh, before pretreatment. The raw material was characterized in terms of the content of arabinan, galactan, glucan, xylan, mannan, lignin, extractives, ash, and moisture. The characterization was performed according to the National Renewable Energy Laboratory (NREL) NREL/TP-510-42618, NREL/TP-510-42622, and NREL/TP-510-42619 (Sluiter et al., 2012, 2008a, 2005).

Ethanol, abs. 100% a.r. (>99.8 vol% C₂H₅OH) was purchased from Chem-Lab NV (Zedelgem, Belgium). Ethanol/water mixture was prepared with ultra-pure water (18 MΩ/cm). Standards for carbohydrates (arabinose, galactose, glucose, xylose, and mannose), acetic acid (99.7%), 2-furaldehyde (furfural, 99%), hydroxymethylfurfural (HMF, 99%), and sulfuric acid (98%) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide (>99%) was purchased from Carl Roth (Karlsruhe, Germany). Polystyrene sulfonate sodium salts for HPSEC molar mass calibration were purchased from PSS Polymer Standards Services (Mainz, Germany) and Merck (Darmstadt, Germany), respectively.

2.2. Study design

The main objective of this work is to evaluate two pretreatment configurations. The first configuration consisted of performing LHW as the initial stage, followed by OS. This configuration is referred to as LHW → OS. The second configuration consisted of performing OS as the initial stage, followed by LHW. This configuration is referred to as OS → LHW. The objective is to determine the pretreatment configuration with better results in sugar production, lignin extraction, and lignin molar mass. Fig. 1 shows the general scheme of the process performed in this work. The general procedure was feeding the raw material to the reactor and carrying out the first process. Then, the mixture was separated, and the second process was carried out. In the end, there were two liquid fractions available (one coming from OS, lignin extract, and one from LHW, sugar extract) and a final solid residue.

For nomenclature and differentiation purposes, we decided to use the following acronyms: OS-1st refers to the Organosolv performed as the initial stage, and LHW-2nd refers to the LHW performed subsequently after OS-1st. Consequently, LHW-1st refers to the Liquid Hot Water as the initial stage and OS-2nd to the subsequent Organosolv. Finally, with "sugar extracts" and "lignin extracts," we refer to the liquid fractions obtained in the Liquid Hot Water (LHW-1st and LHW-2nd) and Organosolv (OS-1st and OS-2nd) processes, respectively. We used LHW → OS and OS → LHW (separated by an arrow) to refer to both of the configurations studied in this work.

2.3. Process conditions and description

2.3.1. Organosolv

The Organosolv (OS) conditions were based on a previous study (Beisl et al., 2018). The pretreatment was conducted using a 60%wt aqueous ethanol mixture as solvent at a temperature of 180 °C. The solid/liquid ratio was 1 g of dry solid per 11 g of solvent (solid loading of 8.3%wt). The moisture content of the solid was considered and subtracted from the prepared solvent. The total operation time was fixed at 60 min. The heating time was approximately 45 min, and the reactor was held at 180 °C for approximately 15 min. After these 60 mins of treatment, the reactor was cooled to room temperature.

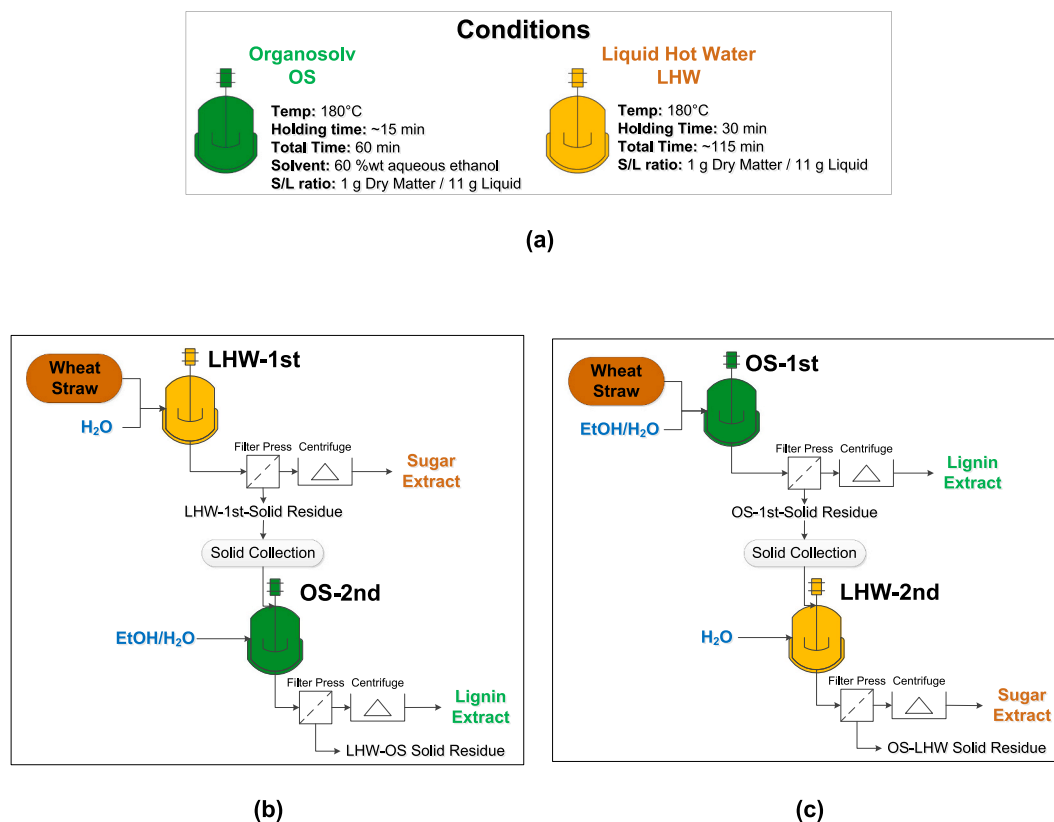


Fig. 1. General scheme of the OS ↔ LHW combinations and conditions. (a) Standard conditions for OS and LHW stages. (b) Scheme for the LHW → OS configuration. (c) Scheme for the OS → LHW configuration. OS: Organosolv. LHW: Liquid Hot Water. EtOH/H₂O: 60%wt aqueous ethanol. H₂O: Water.

2.3.2. Liquid Hot Water

The Liquid Hot Water (LHW) conditions were based on a previous study (Weinwurm et al., 2017). The conditions were 180 °C and a holding time of 30 min. The solid/liquid ratio was 1 g of dry solid per 11 g of solvent (solid loading of 8.3%wt). The moisture content of the solid was considered and subtracted from the prepared solvent. The holding time was fixed at 30 min. The heating time was approximately 45 min, and the reactor was held at 180 °C for 30 min. The total operation time was approximately 115 min, after which the reactor was cooled to room temperature. These conditions corresponded to a severity factor (R_0) of 11,100, which was calculated considering the time and temperature change corresponding to the heating (from 100 °C to 180 °C), temperature holding at 180 °C and cooling (from 180 °C to 100 °C), according to Eqs. (1) and (2). Δt_i is the time (min), T_i is the temperature (°C), and $Total R_0$ corresponds to the sum of each section's severity factor. The contribution of heating, holding, and cooling to the total severity factor were 21%, 76%, and 3%, respectively.

$$R_{0,i} = \Delta t_i * e^{\left(\frac{T_i - 100}{14.75}\right)} \quad (1)$$

$$Total R_0 = R_{0,Heating} + R_{0,Holding} + R_{0,Cooling} \quad (2)$$

2.3.3. Detailed description of the process

Both OS and LHW were carried out in a stainless steel high pressurize autoclave (Zirbus, HAD 9/16, Bad Grund, Germany) with a capacity of 1 L and maximum temperature and pressure of 250 °C and 60 bar, respectively. The autoclave had a controller that registered the reactor temperature every second; this information was used for the calculation of the severity factor. The stirrer was set to 200 rpm. The initial wet mass of wheat straw was approximately 32.31 g. The reactor was then heated to the operating temperature and cooled down after the set holding time.

The solid and liquid fractions were subsequently separated using a hydraulic press (Hapa, HPH 2.5) at 200 bar and a centrifuge (Sorvall, RC 6+) at 24,104 g for 20 min. The extract's density was determined using a density meter (DE45 DeltaRange, Mettler Toledo, Columbus, United States). The supernatant was stored at 5 °C until further analysis. The solid fraction was dried in a convection oven at 105 °C and stored.

For each configuration (OS → LHW and LHW → OS), the first process was performed in quadruplicate to collect enough solid sample to perform the second step in triplicate and maintain the same conditions (initial 30 g of dry solid sample, S/L ratio, and solvent volume). Each liquid fraction (sugar and lignin extract) was analyzed separately, and the solid fractions were mixed. Then, solid was taken to perform the second process, following the first extraction stage procedure.

2.3.4. Product characterization

Lignin and sugar extracts were characterized for sugars, degradation products, lignin, and lignin molar mass. Sugars and degradation products were characterized according to the NREL/TP-510-42623 (Sluiter et al., 2008b). Monomeric sugars were analyzed using HPAEC-PAD (ICS-5000, Thermo Scientific, USA) with deionized water as eluent. Oligomeric sugars were hydrolyzed (diluted sulfuric acid) at 120 °C and analyzed as monomers and this corresponded to the total sugars. A sugar recovery standard was used to account for losses. Oligomeric sugars were calculated as the difference between total and monomeric sugars. Furfural, HMF, and acetic acid were determined using HPLC (LC-20A HPLC system, Shimadzu, Japan) by UV and RI detection with a Shodex SH1011 analytic column at 40 °C with 0.01 N H₂SO₄ as mobile phase.

The lignin concentration was measured as acid-soluble lignin (ASL) and acid-insoluble lignin (AIL). The extract was dried, and the solid was submitted to the protocol established in the NREL/TP-510-42618 (Sluiter et al., 2012). AIL was determined by a gravimetric method and ASL by UV/VIS absorption at 205 nm using a Shimadzu UV-1800

spectrophotometer.

Lignin molar mass in both lignin and sugar extracts was analyzed in an aqueous phase (10 mM NaOH) using three TSK-GEL size exclusion chromatography (SEC) columns in series (PW5000, PW4000, PW3000, Tosoh Bioscience, Griesheim, Germany), kept at 40 °C in a column oven (Shimadzu, Japan) connected to an Agilent 1200 HPLC system with diode-array detection (Agilent, Santa Clara, USA). Calibration was done using a series of sodium polystyrene sodium salt reference standards with the following molar masses at peak maximum (M_p): 78,400 Da, 33,500 Da, 15,800 Da, 6430 Da, 1670 Da, 891 Da, and 208 Da.

3. Results and discussion

This work aims to identify the best order of the LHW and OS stages for maximized sugar production and lignin extraction. For this reason, we will present the results for sugars and degradation products, lignin yield, and molar mass, comparing in each section the obtained results for the configurations LHW → OS and OS → LHW. Table 1 shows the characterization obtained for the wheat straw. The raw material has a moisture content of 7.16%wt, and the percentage of error for each of the components is below 7.5%. The characterization accounts for 99.66% of the dry weight basis, which can be considered a complete raw material characterization.

3.1. Sugars and degradation products

3.1.1. Sugars

This section compares the corresponding stages in terms of sugar production (monomeric and total sugars). Table 2 shows the concentrations for the determined sugars for the four analyzed stages. When comparing the respective counterpart stages, LHW-1st and LHW-2nd showed that each carbohydrate (except for xylose) is around the same magnitude order. For the case of xylose (main constituting carbohydrate of hemicellulose), performing OS-1st before increases slightly the concentration in the LHW-2nd extract (28.6 and 9.2% for monomeric and total sugars, respectively). For the OS-1st and OS-2nd stages, for both monomeric and total sugars, each carbohydrate (except for xylose) is higher in the OS-1st stage. Xylose concentration is the only carbohydrate showing a higher concentration in the OS-2nd stage (69 and 1099 mg/L, for monomeric and total sugars, respectively, compared to 8 and 142 mg/L for the OS-1st stage). This can be explained because performing the LHW-1st process initially leaves a more accessible solid for the subsequent OS-2nd stage, facilitating the remaining hemicellulose's further hydrolysis.

Based on this information, we summed up the C5 (arabinose and xylose) and C6 (galactose, glucose, and mannose) concentrations for both monomeric and total sugars and calculated the respective total concentration of sugars (C5 plus C6), as this allows comparing the overall yield of each configuration in terms of sugar production. Fig. 2 shows the summed concentration of monomeric and total C5 and C6 sugars. Fig. 2(a) and Fig. 2(b) correspond to Configuration 1 (LHW → OS); Fig. 2(c) and Fig. 2(d) correspond to Configuration 2 (OS → LHW).

When comparing the sugar production stages (LHW-1st and LHW-2nd), we can observe that performing OS-1st before LHW-2nd increases slightly the concentration of monomeric and total C5 sugars (32.3% and 10.3%, respectively), and therefore, the summed sugars have an 8% increase. This can be explained because the OS-1st solubilizes an initial fraction of the hemicellulose and removes part of the lignin; therefore, the sugar carbohydrates (remaining hemicellulose and

cellulose) are more accessible. However, for practical purposes, we can say that both combinations reached a similar concentration (between 1.2 and 1.6 g/L of monomeric C5 sugars, 0.17 g/L of monomeric C6 sugars, and 12–13 g/L of total sugars). This information is important because this may mean a similar behavior of the hydrolysate for different fermentation microorganisms. These results indicate that in terms of the sugars produced in the sugar stage (LHW-1st and LHW-2nd), the combined pretreatment showed no difference between configuration 1 and 2 (25%, 7.6 and 7.5% difference for monomeric C5 sugars, monomeric C6 sugars, and total sugars, respectively). However, it is necessary to analyze the degradation products and lignin results in the following sections to make an integral decision.

When comparing the lignin production stages (OS-1st and OS-2nd), we can observe that monomeric sugars remained under the same concentration level for both C5 and C6 sugars (0.1 g/L of monomeric C5 sugars, 0.01–0.06 g/L of monomeric C6 sugars, and 1–1.3 g/L of total sugars). However, performing LHW-1st before OS-2nd approximately triplicated the concentration of total C5 sugars (from 0.44 g/L in OS-1st to 1.21 g/L in OS-2nd). This is understandable, as LHW has a more intensive effect on the deconstruction of the lignocellulosic matrix; hence, the solid matrix is more accessible, and the OS-2nd stage hydrolyzes more hemicellulose compared to OS-1st. As the lignin extract would be subsequently used to produce CLP, sugars represent an impurity to be removed. According to Beisl et al. (2020), these carbohydrates might lead to undesired growth of microorganisms in final applications; however, there was no conclusive finding for the cited authors on the correlation between particle size, calculated surface, and the presence of carbohydrates (Beisl et al., 2020). Lignin precipitation is a physically driven phenomenon, meaning that carbohydrates in the respective solution do not implicate chemical reactions that would create lignin-carbohydrate complexes (LCC). LCC is present in the biomass and has to be broken up by pretreatment to solubilize both hemicellulose and lignin. The bond between hemicellulose and lignin is never cut perfectly; hence, some sugars are always attached to the lignin. However, there might be adsorption of the carbohydrates in the formation of the lignin particles. From an ideal perspective, a process with the lowest possible amount of impurities is desired. Performing LHW before OS does increase the impurities (meaning an increased concentration of sugars in the OS-stage) entering a subsequent CLP production stage; nonetheless, it is not conclusive to say that this will negatively affect the CLPs (Beisl et al., 2020; Zhao et al., 2020).

3.1.2. Degradation products

In this section, we will compare the corresponding stages in terms of degradation products. Fig. 3 shows the concentration of acetic acid, HMF, and furfural. Fig. 3(a) corresponds to Configuration 1 (LHW → OS), and Fig. 3(b) corresponds to Configuration 2 (OS → LHW). We observed that for the sugar production stages, furfural and HMF show similar concentration levels irrespective of the order (between 0.73 and 0.85 g/L for furfural and 0.03–0.04 g/L for HMF). For the lignin production stages, HMF showed a similar concentration level (below 0.01 g/L), but furfural was 4 times higher in OS-2nd (from 0.015 g/L in OS-1st to 0.061 g/L in OS-2nd). However, it is worth mentioning that the concentration levels of HMF and furfural in lignin production stages are considerably low. Acetic acid presents a different trend. For LHW-1st and OS-1st, both stages reach similar concentrations of acetic acid (1.81 and 1.41 g/L, respectively). The acetic acid coming from the acetyl groups present in hemicellulose acts as a catalyst for the hydrolysis of the hemicellulose into sugars (autohydrolysis effect) (Garrote et al.,

Table 1
Lignocellulosic characterization of the raw material.

Component	Arabinan	Galactan	Glucan	Xylan	Mannan	Lignin	Extractives	Ash
Weight percentage (%wt) ^a	2.13 ± 0.16	0.67 ± 0.01	35.31 ± 2.14	21.94 ± 0.60	0.72 ± 0.05	17.35 ± 0.99	20.45 ± 1.26	1.09 ± 0.07

^a Values expressed on a dry weight basis.

Table 2
Carbohydrate composition of the different streams obtained for the LHW and OS combinations.

Sugar type	Sugar	Configuration 1				Configuration 2			
		LHW-1st		OS-2nd		OS-1st		LHW-2nd	
		Conc. (mg/L)							
Monomeric Sugars	Arabinose	478	± 24	31	± 3	94	± 41	659	± 23
	Galactose	100	± 5	7	± 0.7	7	± 1	123	± 6
	Glucose	62	± 5	3	± 1	42	± 19	31	± 2
	Xylose	736	± 151	69	± 4	8	± 2	947	± 128
	Mannose	23	± 4	0.2	± 0	11	± 6	18	± 5
Total Sugars	Arabinose	895	± 68	111	± 5	303	± 112	1098	± 55
	Galactose	576	± 15	54	± 3	145	± 42	630	± 21
	Glucose	1274	± 34	87	± 5	324	± 79	1254	± 107
	Xylose	8939	± 207	1099	± 29	142	± 25	9754	± 736
	Mannose	303	± 9	33	± 2	37	± 12	216	± 37

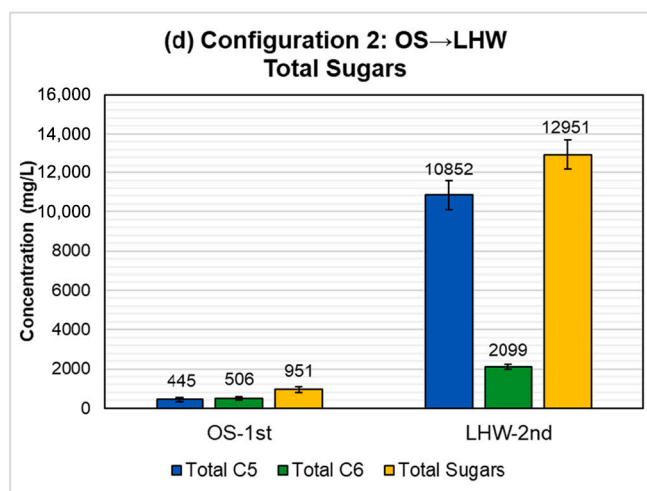
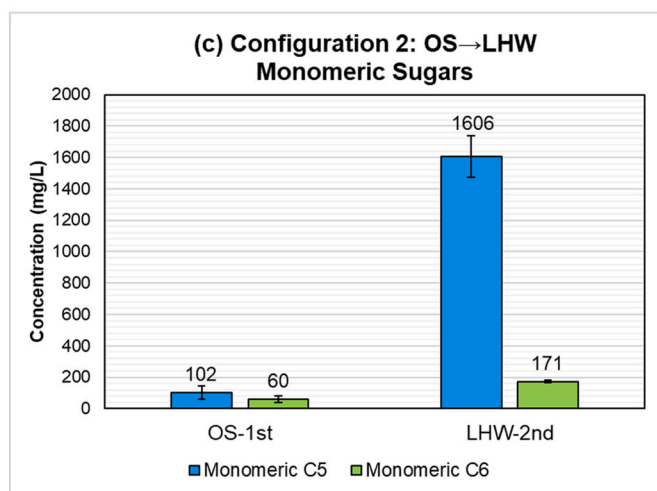
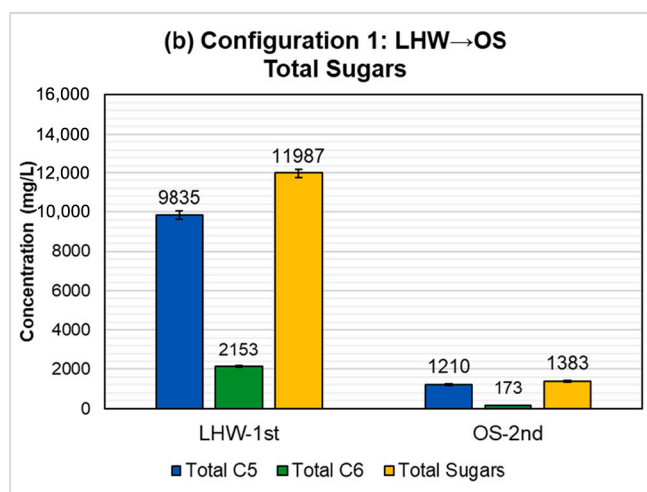
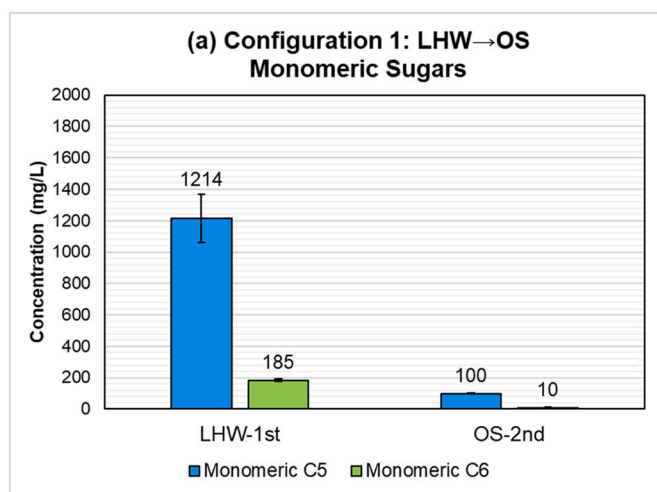


Fig. 2. Comparison of the summed C5 and C6 monomeric and total sugars of the OS → LHW combinations. Number above bars indicate the average, and error bars indicate the standard deviation.

1999). When present in a sugar production stage, it can be associated with promoting the production of sugars; for lignin production stages, the presence of acetic acid and consequent hemicellulose hydrolysis increases the accessibility of the solid matrix for lignin solubilization. This means that LHW-1st decreases the concentration of acetic acid in the subsequent OS-2nd extract (as observed in Fig. 3(a)). Therefore, we

can say that the Configuration 1 (LHW → OS) provides better overall results in terms of degradation products: The highest concentration of acetic acid is released, catalyzing the hydrolysis of the hemicellulose into sugars. The remaining solid then has a more accessible matrix to extract the lignin, without the necessity of adding an acid that would enhance the removal of hemicellulose.

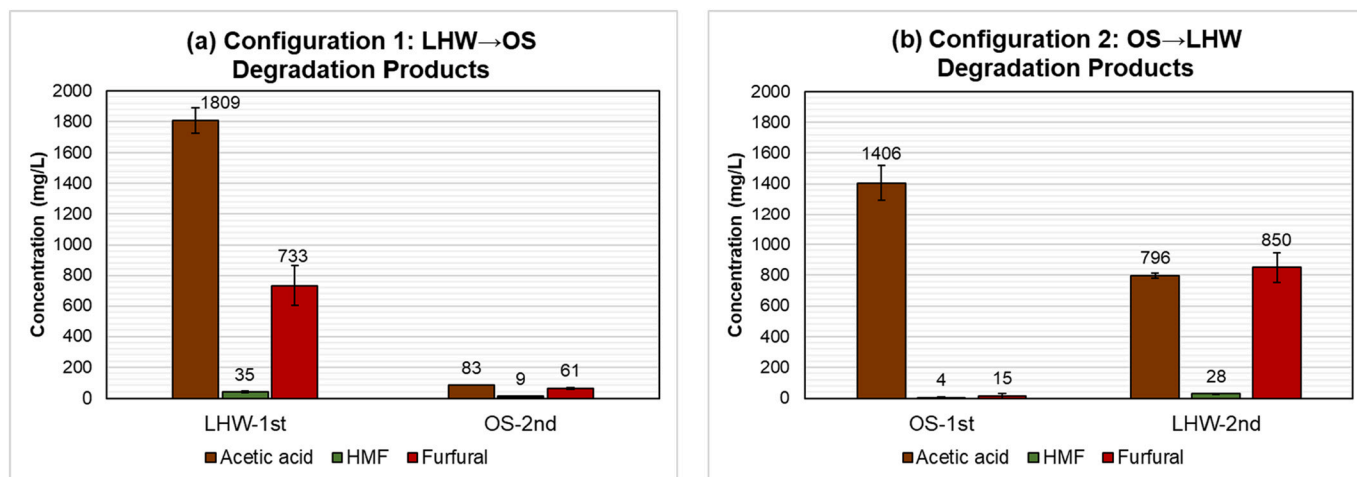


Fig. 3. Comparison of the degradation products of the OS ↔ LHW combinations. Number above bars indicate the average, and error bars indicate the standard deviation.

3.2. Lignin

This section compares the corresponding configurations in terms of the lignin extraction, as shown in Fig. 4. Fig. 4(a) to Configuration 1 (LHW → OS), and Fig. 4(b) corresponds to Configuration 2 (OS → LHW). Configuration 1 shows a 1.6 times increase in the acid insoluble and total lignin concentration in OS-2nd when compared to OS-1st (from 5.9 to 10.1 g/L and 6.81 to 10.86 g/L for AIL and total lignin, respectively). This is an excellent result associated with the lignocellulosic matrix's depolymerization achieved in the LHW-1st stage, making the lignin more accessible. Besides, the ratio between AIL/ASL increased from 6.51 (OS-1st) to 12.89 (OS-2nd). As the ASL in both stages is around the same level (between 0.78 and 0.91 g/L), more AIL is being extracted when LHW-1st is performed; this result is essential for the production of CLP, as AIL is the bigger fraction of the extracted lignin which is precipitated.

On the other hand, even though LHW is not a process focused on lignin solubilization, some lignin is still solubilized, and this lignin is lost for the OS stage and subsequent CLP production. However, the results showed that even when 4.6 g/L of total lignin is extracted in the LHW-1st (2.4 g/L correspond to AIL), the overall increase in the OS-2nd stage yield compensates this loss. In addition, Configuration 1 (LHW → OS) suggests a higher delignification of the solid, which means the resulting solid fraction is also better suited for further applications (e.g., fiber

applications, paper applications, and/or enzymatic hydrolysis). Section 3.4 below will discuss in more detail this topic.

3.3. Molar mass of the lignin

In this section, we will compare the corresponding stages in terms of the molar mass distribution of the dissolved lignins (Fig. 5). When comparing the area-normalized HP-SEC chromatograms of the single-step treatments OS-1st and LHW-1st, huge differences are evident in the observed molar mass distributions: while in OS, high-molar-mass contributions are strong, LHW is dominated by low-molar-mass contributions, especially by the very intense peak at around 26 mL elution volume. Further, the peak at 24.5 mL was derived from p-hydroxycinnamic acids like p-coumaric acid and ferulic acid (Zikeli et al., 2016), and it is significantly stronger in LHW compared to OS (Fig. 5).

The determined molar mass averages for the respective sugar and lignin extracts after one-step LHW and one-step OS treatment show rather strong differences (Table 3): Weight-average (M_w) of the lignin extracts (OS-1st and OS-2nd) was almost double compared to the lignin in the sugar extracts (LHW-1st and LHW-2nd), and the polydispersity index (P_d) was higher too (Table 3). OS-2nd showed significantly lower M_w , number-average molar mass (M_n), and P_d than the lignin extract after OS-1st. Further, higher total lignin yield of OS-2nd compared to

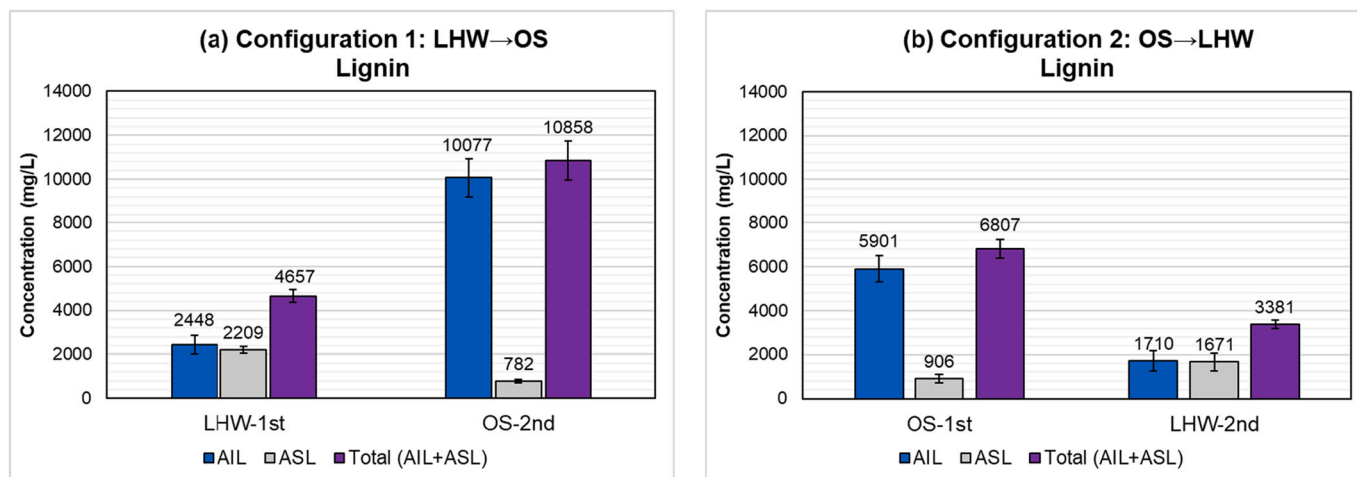


Fig. 4. Comparison of the lignin concentration of the OS ↔ LHW combinations. AIL: Acid Insoluble Lignin; ASL: Acid Soluble Lignin. Total Lignin corresponds to the sum of AIL and ASL. Number above bars indicate the average, and error bars indicate the standard deviation.

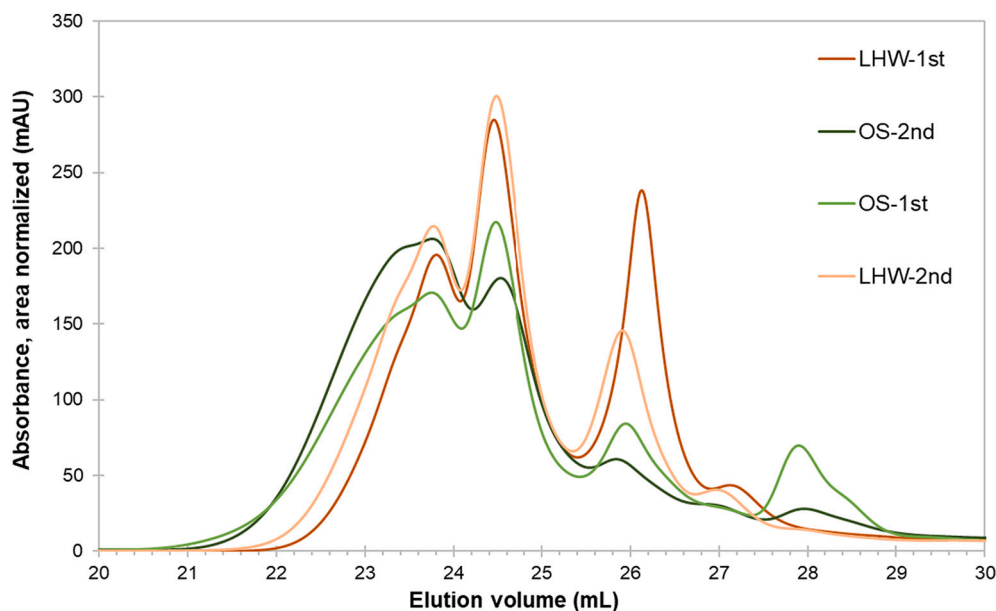


Fig. 5. HP-SEC elution curves of the sugar and lignin extracts for the LHW → OS and OS → LHW configurations.

Table 3

Weight-average (M_w), number-average molar mass (M_n), and polydispersity index (P_d) of the lignin and sugar extracts determined by HP-SEC.

Configuration		M_w (Da)	M_n (Da)	P_d
1	LHW-1st	558	388	1.44
	OS-2nd	780	485	1.61
2	OS-1st	1039	509	2.04
	LHW-2nd	547	399	1.37

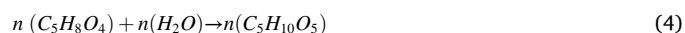
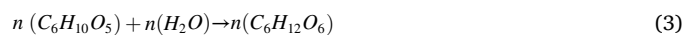
single-step OS treatment was determined (Fig. 4), indicating a strong positive influence of the preliminary LHW-1st treatment by opening-up fiber structure resulting in higher delignification of wheat straw as well as less polydisperse lignin being extracted in the subsequent OS treatment. The lower polydispersity of the solubilized lignin is also illustrated by the HPSEC elution curves of the respective extracts of OS-1st and OS-2nd. The absorbance of medium mass lignin is much higher after OS-2nd, while high-molar-mass contributions observed for the OS extract are absent in the OS-2nd extract (Fig. 5), resulting in a lower M_w . In terms of molar mass averages, a shift to higher M_w and M_n is registered for the sugar extract LHW-2nd compared to LHW-1st. The respective HP-SEC elution curve confirms this shift to higher molar mass in Fig. 5.

3.4. Composition of the solids after each stage

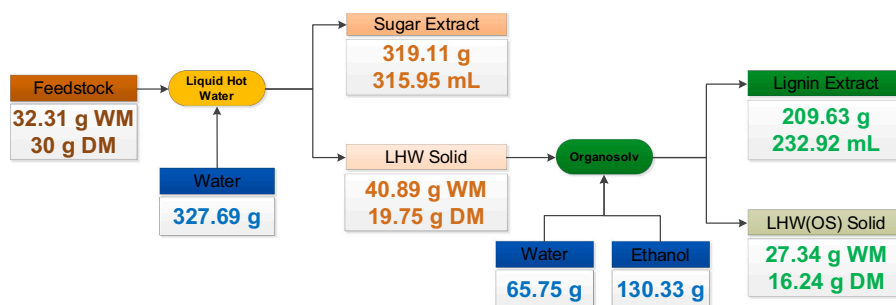
After identifying that Configuration 1 (LHW → OS) showed the overall best results for both lignin and sugar production, we decided to propose a theoretical mass balance for the solids in this sequential treatment. This way, in addition to characterizing the liquid fractions, we are identifying how the components of the feedstock are distributing among the different streams. Fig. 6 shows the mass balances for the LHW → OS configuration. Fig. 6(a) shows the theoretical mass balance of the sequential LHW → OS treatment of wheat straw. We took as base the collected sugar extract, the moisture content of the solid leaving the LHW-1st after the pressing, and based on this amount, we calculated the solvent required for the OS-2nd stage. Then, we used the same proportions obtained in the OS-2nd experiments to calculate the amount of lignin extract and the respective solid. We proceeded to calculate the mass balance using the density of the extracts (1.01 g/mL and 0.9 g/mL for sugar and lignin extracts, respectively). We provide all the

information related to each extraction's mass balance in the Appendix (Appendix A and Appendix B). This same approach was performed for the OS → LHW configuration and is also presented in the Appendix C. During the experimental tests, the collection process, pressing, and centrifugation implicated certain losses of material, which accounted for approximately 12%wt on the LHW stage and 7%wt on the OS stage, compared to the initial total loaded mass. The main assumption we made in this theoretical mass balance is that there were no losses, and these losses were added to the respective extracts.

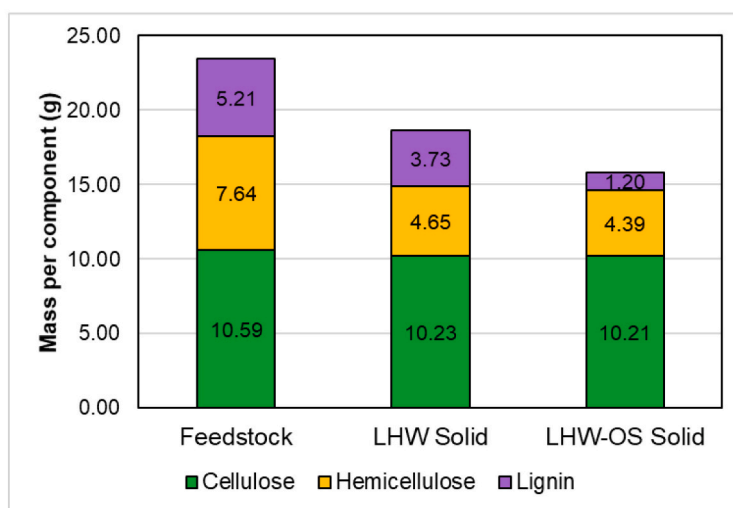
Table 4 shows the composition of the solids on the sequential stages. These values were calculated based on the concentrations obtained in the respective liquid fraction. We calculated the respective oligomer's theoretical amount that must have been consumed to produce the monomer's amount (e.g., arabinan to arabinose, etc.) based on the stoichiometry of the hydrolysis reaction. Eqs. (3) and (4) show the hydrolysis reaction for both C5 and C6 oligomers into the respective monomer.



Based on this information, we analyzed the change in the distribution of monomers and lignin among the three solids obtained in the process, namely, the feedstock, the solid after the LHW-1st (LHW Solid), and the solid after the subsequent OS-2nd (LHW-OS Solid). This information is shown in Table 5 and Fig. 6(b). Given that each of the monomeric sugars has a very different proportion, we decided to group them considering that glucan corresponds to cellulose and the other carbohydrates (arabinan, galactan, xylan, and mannan) as hemicellulose. We can observe that on the LHW stage, hemicellulose had a conversion of approximately 40%, 28% of the lignin is removed, and cellulose remains mostly intact (only 3% is removed). Then, on the subsequent OS-2nd stage, lignin solubilization reached 68%, while hemicellulose and cellulose remained in the solids, with conversions below 6% in both cases. These results are very interesting as we can observe that the final solid contains more than 60% cellulose, and it remains almost intact throughout the treatment (only 4% of the initial cellulose is converted into sugars). This means that after the subsequent LHW → OS process, the remaining solid can be used for material applications, for example, paper production. Of course, the paper's quality that can be produced from this solid must be evaluated, but these results



(a)



(b)

Fig. 6. Mass balances for the LHW → OS configuration. (a) Theoretical mass balance of the sequential LHW → OS treatment of wheat straw. (b) Distribution of cellulose, hemicellulose, and lignin between the three solid fractions of the LHW → OS sequential process.

Table 4

Composition of the solids after LHW-1st and OS-2nd stages.

	Feedstock (g)	LHW solid	OS-2nd solid
Arabinan	0.64	0.39	0.37
Galactan	0.20	0.04	0.03
Glucan	10.59	10.23	10.21
Xylan	6.58	4.10	3.87
Mannan	0.22	0.13	0.12
Lignin	5.21	3.73	1.20
Ash	0.33	0.11	0.09

Table 5

Calculated composition of cellulose, hemicellulose, lignin and ash of the solids after LHW-1st and OS-2nd stages.

Component	Feedstock		LHW solid		OS-2nd solid	
	Mass (g)	Mass fraction (%wt)	Mass (g)	Mass fraction (%wt)	Mass (g)	Mass fraction (%wt)
Cellulose	10.59	44.58	10.23	54.62	10.21	64.23
Hemicellulose	7.64	32.14	4.65	24.84	4.39	27.60
Lignin	5.21	21.90	3.73	19.93	1.20	7.58
Ash	0.33	1.38	0.11	0.61	0.09	0.59

provide an outlook directed to material applications of the final solid instead of an energy-driven approach.

Another aspect of interest discussed is the relatively low conversion of hemicellulose and how this can be approached. In this work, we selected certain intermediate conditions for the LHW (180 °C and 30 min) compared to other LHW studies that operate in temperature and time ranges within 160–220 °C and 30–120 min (Kim et al., 2009; Li et al., 2017; Michelin and Teixeira, 2016; Pérez et al., 2008; Weinwurm et al., 2017; Zhuang et al., 2016). However, we consider that the approach, in this case, might not be increasing the time and/or temperature, as this also implicates an increase in the concentration of degradation products. At least from a theoretical perspective, an alternative that could be considered is a second stage of LHW (LHW → LHW → OS) under the same conditions. However, this is an outlook for further research that requires further evaluation addressing other aspects like energy consumption, costs, and environmental performance.

3.5. Further outlook of the research

This study covered additional steps into developing a biorefinery from wheat straw, aiming to the valorization of the entire feedstock. Further questions are opened based on the results obtained in this work. Regarding the lignin, the next step should be to precipitate and characterize the extracted lignin and the respective thereof prepared colloidal particles. Regarding the solids, the next steps should focus on

using the final solid for a material application (e.g., papermaking). This could provide key information about the suitability of the treatment and the quality of the product. This way, a stage focused on a material application with added-value could be considered. Finally, other elements that should be further studied for the development of the process are the influence of an intermediate washing of the solids (to remove solubilized components as sugars and degradation products) and, as previously discussed, even considering other stages to solubilize further the hemicellulose. In addition, even though LHW is a process to hydrolyze hemicellulose into sugars, it also solubilizes part of the lignin. Therefore, another topic of interest for further research is studying different conditions (temperature and time) for the LHW and determining the lignin solubilization in addition to sugar concentration. This way, it may be possible to identify other conditions that render the same sugar concentrations but remove less lignin, which could be then extracted in the subsequent OS-2nd stage.

4. Conclusions

This work tested the influence of the configuration of Organosolv and Liquid Hot Water stages for the hydrolysis of hemicellulose and lignin fractions in wheat straw. We show that Configuration 1 (LHW followed by OS) showed a better overall performance in terms of sugar production (~12 g/L of total sugars) and lignin extraction yield (11 g/L of total lignin). The lignin extract showed better indicators in terms of the molar mass (lower polydispersity and molar mass average), which allows saying that the production of CLP can be facilitated. The remaining solid after the treatment consists mainly of cellulose, which may be used for material applications as pulp and paper.

CRedit authorship contribution statement

Sebastián Serna-Loaiza: Conceptualization, Investigation, Writing – original draft. **Florian Zikeli:** Investigation, Writing – review & editing. **Johannes Adamczyk:** Investigation, Writing – review & editing. **Anton Friedl:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biteb.2021.100667>.

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