Fractionation of Hemicelluloses and Lignin from Rice Straw by Combining Autohydrolysis and Optimised Mild Organosolv Delignification

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An integrated strategy was followed to valorise rice straw, one of the most relevant biomass feedstocks available worldwide, to selectively recover solubilised hemicelluloses and lignin. The pathway encompassed the use of autohydrolysis to hydrolyse the hemicelluloses and an ethanol-based organosolv process to solubilise lignin. Several autohydrolysis conditions were assayed with the best results obtained at 210 ºC (log R_0 4.15), which enabled high removal of hemicelluloses, yielding an oligosaccharide-rich hydrolysate and a treated biomass with low content of hemicelluloses and enriched in cellulose and lignin. The effects of ethanol concentration (5 to 75%), and reaction time (0 to 24 h) on lignin removal under mild temperature (30 ºC) were studied. In optimal conditions (60.5% ethanol, 24h) the delignification yield reached 42%, whereas glucan solubilisation was below 17%. Lignin solubilisation yield was not influenced by the organosolv treatment duration while ethanol concentration favored the delignification up to 60.5% ethanol. The organosolv liquors contained economic interesting lignin-derived compounds such as vanillin, ferulic, and coumaric acids. The chemical composition and enzymatic digestibility of the treated biomass from autohydrolysis and organosolv delignification were compared, with the latter presenting an almost 10% higher enzymatic digestibility than the former.

Keywords: Agricultural residues; Autohydrolysis; Biomass fractionation; Biorefinery; Hemicelluloses; Lignin; Organosolv; Pretreatment; Rice straw

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INTRODUCTION

Rice straw is usually considered a waste material and is typically used only to a small extent for cattle beds, mulching, and combustion. Notwithstanding, its potential for valorisation within the biorefinery framework has been demonstrated as a major single feedstock for bioethanol production (Matsumura *et al.* 2005).

Environmental friendly processes, yielding separate streams that can be further used for different product lines are preferential, and their selectivity towards the target polymer is essential. One potential strategy is to carry out in a first step the hydrolysis of the hemicellulosic fraction by hydrothermal processing (autohydrolysis) before further processing. This is an effective and selective pre-treatment enabling a high production of soluble hemicellulosic oligosaccharides from the liquid phase and a high recovery of cellulose and lignin in the solid phase (Carvalheiro *et al.* 2009; Moniz *et al.* 2013).

For the fractionation of lignin, organosolv processes based on organic solvents such as low boiling-point alcohols (*e.g.*, methanol, ethanol, acetone, and/or organic acids), which can be easily recovered, have shown promising results (Binod *et al.* 2010; Girio *et al.* 2010; Bozell *et al.* 2011). The products that can be obtained include sulphur-free lignin fragments, which are useful for the production of lignin-based high value products due to their high purity, low molecular weight, and easily recoverable organic reagents (Garrote *et al.* 2008; Toledano *et al.* 2012). If applied directly to the lignocellulosic material, the organosolv treatment will yield a liquid stream containing both lignin and hemicellulose-derived products that will require major purification for lignin recovery (Harmsen *et al.* 2010). Furthermore, it will induce carbohydrate loss for the subsequent processes (Huijgen *et al.* 2010; Bozell *et al.* 2011; Toledano *et al.* 2013).

The use of sequential autohydrolysis and organosolv processes has the potential to be a strategic pathway, especially if organosolv delignification is carried out under mild conditions. A proposal for the selective fractionation of hemicelluloses and lignin aiming at the production of valuable compounds is shown in Fig. 1.

Fig. 1. Proposed pathway for the selective fractionation of the structural polymers from rice straw

In this work, the potential upgrading of rice straw by sequential autohydrolysis and organosolv delignification was investigated. The organosolv delignification was optimised in relation to reaction time and ethanol concentration by a Doehlert experimental design for maximum recovery of solubilised lignin. The lignin-derived products were characterised, and the upgrading potential of the remaining cellulose-rich solids (*i.e.*, for bioethanol production) was also evaluated.

EXPERIMENTAL

Materials

Raw material

Rice straw was kindly provided by Orivárzea (Salvaterra de Magos, Portugal). The raw material was dried, milled to particles smaller than 6 mm, homogenised, combined in a single lot, and stored in plastic containers at room temperature (Moniz *et al.* 2014).

The following technical lignins (Boeriu *et al.* 2014) were used: Soda wheat straw lignin (Soda WS) and soda lignin from mixed Sarkanda grass and wheat straw (P1000), were obtained from Greenvalue SA (Lausanne, Switzerland). Organosolv lignin from mixed hardwoods (Alcell) was obtained from Repap Technologies Inc. (Valley Forge, PA, USA).

Methods

Autohydrolysis of rice straw

Autohydrolysis treatments were carried out in a 2-L stainless steel reactor (Parr Instruments Co., USA). Temperature was controlled through a Parr PID controller (model 4842). The raw material was mixed with water in the reactor to a liquid-to-solid ratio of 10 (g water/g dry raw material). The reactor was heated to the final temperatures of 195, 200, 205, 210, 215, and 220 ºC, corresponding to a severity factor (log R_0) ranging from 3.66 to 4.36. After reaching the desired temperature, the reactor was rapidly cooled, the liquid and solid phases were separated, and the solid phase was washed and dried (Moniz *et al.* 2013). After selection of the optimised conditions at 210 ºC, several batches were performed to produce solids for used in organosolv studies, after thorough mixing into a homogenised lot.

Organosolv delignification

The solids obtained after autohydrolysis under optimised conditions (210 ºC) were subjected to organosolv delignification using different ethanol/water mixtures and duration periods, following the experimental design described in Table 1. A solid to liquid ratio of 1:10 (w/w) was used and all reactions were carried out using Schott flasks in an incubator set for 30 ºC and 150 rpm. Upon completion, the flask contents were filtered; the solid phase was washed twice with ethanol/water solution and dried at 45 ºC to be used for chemical characterisation and saccharification assays. The solid yield of the organosolv delignifications was determined as gram solid per 100 g of the autohydrolysed material (oven dry mass).

The yields of lignin (KL_R) (Eq. 1), glucan (Gn_R) (Eq. 2), and xylan (Xn_R) (Eq. 3) were calculated according to the following equations,

$$KL_R = SY \times \frac{KL}{KL_i} \tag{1}$$

$$Gn_R = \frac{Gn.SY}{Gn_i} \tag{2}$$

$$Xn_R = \frac{Xn.SY}{Xn_i} \tag{3}$$

$$Y_{KL} = 100 - KL_R \tag{4}$$

where *SY* is the solid yield (grams of solid recovered after treatments per 100 g feedstock), *KL*, *Gn*, and *Xn* refer to the polymer content in the solid samples after each treatment (autohydrolysis/organosolv delignification), Y_{KL} is the delignification yield, and Gn_i, Xn_i, and KL_i, refer to the corresponding polymer content in the sample prior to the treatment. All data are reported on a dry weight basis.

Experimental design

The experimental statistical design for optimisation of the organosolv delignification is based on a Doehlert distribution for two factors (Doehlert 1970; Ferreira *et al.* 2004). Twelve experiments (including four replicates at the center of the experimental domain) were carried out (Table 1). Two additional tests were carried out (experiment 11 and 12) to further explore extreme conditions of very low ethanol proportion. The factors studied were delignification time (X_1), which varied between 0 and 24 h, and ethanol concentration (X_2), which was varied between 0 and 100% (w/w).

Table 1. Number of Experiments, Coded Variables, and Real Variables Corresponding to Experimental Conditions of Time (X1) and Ethanol Concentration (X2) of the Organosolv Experimental Design

Experiment	Coded Variables		Real Variables	
	X_1	X_2	Time (h)	Ethanol (%, w/w)
1	0.0	0.050	12	52.50
2	0.0	0.050	12	52.50
3	0.0	0.050	12	52.50
4	0.0	0.050	12	52.50
5	1.0	0.050	24	52.50
6	-1.0	0.050	0	52.50
7	0.5	0.440	18	71.90
8	-0.5	-0.340	6	33.05
9	0.5	-0.340	18	33.05
10	-0.5	0.440	6	71.90
11	-0.5	-0.866	6	6.70
12	0.5	-0.866	18	6.70

The effects of variables were determined according to the following polynomial model, Eq. 5,

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \qquad (5)$$

where *Y* is the response variable, X_1 is time (h), and X_2 is ethanol concentration. β_0 is the intercept and represents the response in the center of the experimental domain, β_1 and β_2, the main factors, are the parameters directly associated with the time and the concentration of ethanol, respectively, representing the relative importance of each factor in the analysed response. The interaction parameter, β_{12}, indicates the combined effect of two factors. The quadratic terms ($\square_{11}$ and $\square_{22}$) are model tuning parameters that provide information about the geometric trend of the response surface.

Data fitting and statistical analyses were carried out with Microsoft Excel® 2010 (USA), and the best delignification conditions were determined by using the Solver Tool based on the best-fit equation obtained, using a constrained model. Coded representation of the variables was used for all calculation purposes.

Chemical characterisation of raw material and processed solids

The materials were ground in a knife mill (IKA, Germany) to a particle size smaller than 0.5 mm, and the moisture content was determined by oven-drying at 100 ºC to a constant weight. The ash content was determined using the NREL/TP-510-42622 protocol (Sluiter *et al.* 2005).

The samples were hydrolysed to determine the amounts of glucan, xylan, arabinan, and acetyl groups using acid hydrolysis. The acid-insoluble residue was considered as Klason lignin after correction for ash, and the quantification of macromolecular compounds was carried out as previously described (Moniz *et al.* 2013).

Chemical characterisation of the autohydrolysis liquors

The autohydrolysis liquors were analysed for monomeric sugars, acetic acid, and furan derivatives by high performance liquid chromatography (HPLC) (Agilent 1100 Series, Waldbronn, Germany), using an Aminex HPX-87H column (Bio-Rad, USA) in combination with a cation H^+-guard column (Bio-Rad) as described before (Moniz *et al.* 2013).

Elution took place at 50 ºC with 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. The HPLC was equipped with a diode array detector (DAD) and a refractive index detector (RI). For oligosaccharides quantification, another sample was hydrolysed with 4% (w/w) H_2SO_4 (Moniz *et al.* 2013) and analysed by HPLC as described above.

Total phenolic compounds

Total phenolic compounds in the organosolv liquor were determined by the Folin-Ciocalteu colorimetric method according to Singleton *et al.* (1999). Briefly, 100 µL of the organosolv sample was mixed with 5 mL of the 1/10 (v/v) diluted Folin-Ciocalteu reagent and 4 mL of 7.5% Na_2CO_3. Absorbance was measured at 765 nm after 15 min incubation at 45 ºC. Total phenolic compounds are expressed as mg GAE mL^{-1} (gallic acid equivalents).

Capillary zone electrophoresis

The organosolv liquors were analysed by capillary zone electrophoresis (CZE) to obtain their phenolic profile using an Agilent System device (Waldbronn, Germany), with diode-array detector (DAD). ChemStation data software and a fused-silica uncoated (I.D. 50 µm and 62/56 cm effective length) extended light path capillary from Agilent were used. A 30 kV voltage was applied and injection was done at 50 mbar for 6 s. A 15 mM borate in 10% MeOH was used as an electrolyte adjusted to pH 9.1 and temperature at 25 ºC. The capillary was preconditioned between runs by flushing with 0.1 M NaOH (3 min) followed by buffer (3 min). Detection was at 200 and 280 nm and compounds were identified by electrophoretic comparison (migration times and UV spectra) with authentic standards.

Molecular weight characterisation

The molar mass distribution of the lignin fragments solubilised in the organosolv liquors were analysed by alkaline size exclusion chromatography (SEC) using a TSK gel Toyopearl HW-55F column, 0.5 M NaOH as eluent, UV detection at 280 nm, and calibration with sodium-polystyrene sulfonates (Gosselink *et al.* 2010). Calculations included M_p (peak molecular weight), M_n (number average molecular weight), and M_w (weight-average molecular weight and polydispersity (PD, M_w/M_n).

Enzymatic hydrolysis

The enzymatic digestibility of the untreated (raw material), autohydrolysis pretreated, and organosolv delignified solids of rice straw were evaluated based on NREL/TP-510-42629 protocol (Selig *et al.* 2008). The results are expressed as percentage of glucose released after 72 h enzymatic hydrolysis in relation to initial glucose. All assays were carried out at least in duplicate and the results are corrected for enzyme and biomass blank tests.

RESULTS AND DISCUSSION

Selective Fractionation of Rice Straw Hemicelluloses

To selectively fractionate hemicelluloses, six temperatures for the autohydrolysis pretreatment were studied ranging from 195 to 220 ºC (corresponding to severity factors of log R_0 3.66 to 4.35). Previous studies at a smaller scale demonstrated that optimal conditions for hemicellulose hydrolysis of rice straw ranged within this temperature interval (Moniz *et al.* 2014). Figure 2 and Table 2 show the effect of autohydrolysis on the macromolecular components of rice straw. The variation of xylan, glucan, lignin, and total pentoses (oligomeric and monomeric) as a function of the severity factor shows that autohydrolysis was selective for the hydrolysis of the hemicellulosic fraction (Fig. 2). The hydrolysis of xylan increased with temperature and attained large values above 200 ºC, for which more than 70% of the initial xylan was solubilised. A similar trend was obtained in other autohydrolysis studies using rice straw, corn straw, and wheat straw (Carvalheiro *et al.* 2009; Moniz *et al.* 2013, 2014).

The amount of solubilised pentoses in the liquid increased in parallel with xylan hydrolysis to reach a 41% maximum of the initial amount at 210 ºC, which corresponds to a concentration of 11.6 g/L. The maximum production of pentoses obtained in this work was similar to that obtained in other studies with rice husk (Nabarlatz *et al.* 2007), but lower than those obtained with corn straw (Moniz *et al.* 2013). Contrasting with xylan solubilisation, glucan remains essentially in the solid phase: maximal solubilisation corresponded to only 20% of the initial glucan in the raw-material. These values are in agreement with the known behavior of autohydrolysis treatments that are characterised by a low solubilisation of the cellulose fraction.

Lignin content in the solid followed a similar trend, which is typical of autohydrolysis pretreatment. Up to a temperature of 210 ºC, the remaining lignin in the solid was always close to 100% of the initial amount, showing that the treatment did not substantially affect this component. From 210 ºC onwards, lignin in the solid decreased, *e.g.*, about 30% at 215 ºC, and it increased again for the most severe condition studied. This lignin increase is also typical of these processes and can be associated with condensation reactions of lignin with sugars and degradation products, such as furfural, leading to the formation of insoluble compounds (Ramos 2003) that are quantified as Klason lignin.

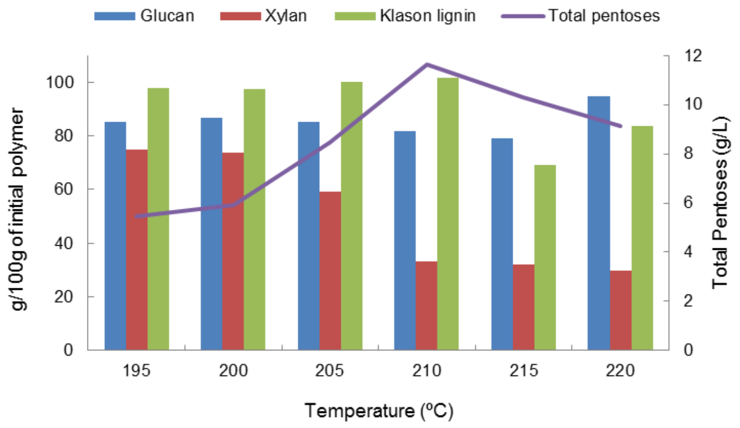

Fig. 2. Effect of autohydrolysis temperature on glucan, xylan, and Klason lignin yield in the treated solids (bars) and total soluble pentoses concentration in the liquid phase (line)

The composition of the raw material and the solid phase resulting from the autohydrolysis of rice straw for different severity conditions is presented in Table 2. For the milder conditions (195 and 200 ºC), the solubilisation of the solid phase was about 20%, but increased with increasing severity of the treatment *e.g.*, 35% at 210 ºC.

Glucan was practically not affected by the treatments, and the processed solids obtained in the most severe condition contained 53.2% glucan; this corresponds to glucan enrichment of the solid as compared to the raw material by 30%. Xylan content was highly affected by the pretreatment and decreased with increasing severity to reach 8% in the autohydrolysed solids at 220 ºC. Lignin was almost not solubilised (Fig. 1), and therefore lignin content generally increased with pretreatment severity. The highest value was obtained for 210 ºC.

The autohydrolysis temperature leading to small losses of glucan and lignin in the solid and to considerable xylan solubilisation with the highest amount of total pentoses in solution was 210 ºC. This temperature was selected as the optimum autohydrolysis condition that enables both the production of pentose rich liquors (mostly oligosaccharides (Moniz *et al.* 2014) and solids enriched in cellulose and lignin for further processing in the organosolv studies.

Table 2. Raw Material Composition, Solid Yield, and Solids Composition after Autohydrolysis at Different Temperatures

		Temperature, ºC (log R_0)					
	Raw material	195 (3.66)	200 (3.80)	205 (3.95)	210 (4.15)	215 (4.25)	220 (4.36)
Solid yield[a]	-	80.21	79.66	74.86	65.14	67.63	67.21
Xylan[b]	20.46	19.10	19.17	16.03	10.29	9.96	8.39
Glucan[b]	40.90	43.39	45.06	46.28	50.89	49.04	53.18
Klason lignin[b]	14.43	17.60	17.87	19.19	22.32	15.16	16.54

[a] (g/100 g raw material); [b] (g/100 g autohydrolysed solid)

Organosolv Process Optimisation

Table 3 presents the experimental conditions of the delignification experiments and the corresponding responses regarding delignification yield (liquid phase) and xylan and glucan yields (solid phase). The delignification yields, *i.e.*, percent of lignin solubilised, ranged from 30.6 to 41.7% for experiments 1 to 10, whereas lower values were obtained for experiments 11 and 12, 19.7% and 10.8%, respectively. Reaction time favored delignification to only a small extent, and substantial delignification was already obtained for very short reaction times. The highest lignin solubilisation corresponded to a delignification yield of 41.7% and was obtained in experiment 5 (52.5% ethanol and 24 h). The ethanol content influenced lignin removal as seen by comparing experiments 7 and 10 (71.9% ethanol) and 8 and 9 (33.05% ethanol), for which the delignification yields were approximately 40 and 30%, respectively,

Experiment 6 (52.5% ethanol, and 0 h) had substantial delignification yield (38.3%) which shows that there is a very rapid reaction of lignin with ethanol, probably of the more reactive moieties, and with immediate solubilisation of the lignin fragments. Rice straw lignin is considerably reactive to ethanol, and over one third of the autohydrolysed rice straw lignin becomes solubilized at near ambient temperature (30 ºC). This does not happen for untreated rice straw (data not shown). Neither does it happen for wood lignin, for which high temperatures (*e.g.*, 165 ºC) are needed to attain similar delignification (Pereira *et al.* 1986). An important factor for the enhancement of lignin solubilisation is the solid matrix opening caused by the autohydrolysis that the material underwent before the organosolv process. By dissolving the hemicelluloses during the thermal hydrolysis, there was a disruption of the lignin-carbohydrate linkages, and the macromolecular cell wall arrangement that should facilitate access and reactivity to subsequent processes. In fact, the lignins obtained had a low mean molecular mass when compared to technical lignins (Fig 4). The delignification time was not an important factor if only partial lignin extraction was the objective, but a minimum ethanol concentration was important as shown by experiments 11 and 12 where the delignification yields were lower (19.7 and 10.8%).

Table 3. Experimental Conditions of Organosolv Delignification and the Corresponding Responses

Experiments	Real Variables		Responses		
	Time (h)	Ethanol (%)	Delignification yield (%)	Glucan yield (%)	Xylan yield (%)
1	12	52.50	32.95	83.52	99.82
2	12	52.50	37.84	87.99	102.25
3	12	52.50	39.74	83.82	100.61
4	12	52.50	38.98	84.61	101.41
5	24	52.50	41.69	85.16	104.56
6	0	52.50	38.28	85.44	107.41
7	18	71.90	39.69	84.14	105.77
8	6	33.05	30.60	86.37	101.97
9	18	33.05	33.03	89.02	101.08
10	6	71.90	38.28	82.72	99.31
11	6	6.70	19.69	96.28	95.34
12	18	6.70	10.75	101.84	107.38

The delignification yield obtained for the different experiments was lower than other published values for rice straw organosolv delignification (Sun and Sun 2002;

Romani *et al.* 2011; Sindhu *et al.* 2012). This result can be ascribed to the very mild temperature used in this study (30 ºC) in comparison with the more severe conditions used in other organosolv studies, *e.g.* temperatures usually higher than 70 ºC together with the use of catalysts (*e.g.*, H_2SO_4 or HCl).

The amount of glucan and xylan remaining in the solid was analyzed. In contrast to lignin, the polysaccharides were almost not affected by the mild organosolv treatment, demonstrating the selectivity of this process towards lignin. After delignification, both glucan and xylan yields were high, with complete xylan recovery in almost all experiments. Higher xylan recovery was found in this work when compared to previous studies (El Hage *et al.* 2010). In the case of glucan, there was no solubilisation in experiments that used low ethanol concentrations for which glucan yield was close to 100%; in the other cases the glucan yields were about 85%. Glucan yields are in agreement with those obtained by direct delignification of raw wheat straw (Huijgen *et al.* 2010). In other studies (Caparros *et al.* 2007; Romani *et al.* 2011) with a similar hydrothermal pretreatment before organosolv delignification, glucan yields were lower (ranging 70 to 87%).

The influences of time and ethanol concentration in solubilised lignin yield and on glucan and xylan yields were modelled, and β parameters were calculated. The responses that can be correlated with the studied variables by the proposed equation Eq. 1 are shown in Table 4.

Table 4. Regression Coefficients and Statistical Parameters Measuring the Correlation and Significance of the Experimental Design

Parameters	Responses		
	Delignification yield (%)	Glucan yield (%)	Xylan yield (%)
β_0	38.86 ± 0.35 (0)*	84.31 ± 0.50 (0)*	100.68 ± 1.21 (0)*
β_1	1.83 ± 0.40 (0.01)*	0.42 ± 0.54 (0.47)	0.65 ± 1.45 (0.66)
β_2	11.81 ± 0.77 (0)*	-6.20 ± 1.07 (0)*	0.37 ± 2.88 (0.90)
β_{12}	-1.15 ± 1.69 (0.53)	0.70 ± 1.39 (0.63)	-1.47 ± 3.72 (0.71)
β_{11}	1.10 ± 0.62 (0.15)	1.30 ± 0.89 (0.21)	5.07 ± 2.30 (0.06)
β_{22}	-25.39 ± 1.55 (0)*	7.76 ± 1.69 (0*)	3.73 ± 5.43 (0.43)
R^2	0.998	0.988	0.714

All values are presented in the form "coefficient +/- standard error (*p*-value)
* Denotes coefficients significant at the 99% confidence level.

For delignification yield and glucan yield, the R^2 values were above 0.9, *i.e.*, these compounds effectively correlated to the studied process variables by the proposed equation, with statistically significant regressions. For xylan yield, R^2 was approximately 0.7.

According to the regression coefficients obtained, ethanol concentration (β_2) was the factor that had the greatest impact and positive delignification yield. The parameter β_1, referring to delignification time, also affected delignification yield, although to a much smaller extent. The quadratic term ($\square_{22}$) was negative and statistically significant, suggesting that although there was a trend of increasing delignification with increasing ethanol concentration, this occurred only to a certain point, after which the delignification yield was not favored by the ethanol concentration increase (Fig. 3). The coefficients $\square_1$, $\square_2$, and $\square_{22}$ were statistically significant at a confidence level of $p < 0.05$.

Glucan yield was negatively affected by the ethanol concentration, and low concentrations led to an enhanced glucan recovery, as expected. The results obtained for $\square_{22}$, also statistically significant, were therefore contrary to those obtained for the delignification yield. For xylan yield, none of the factors were statistically significant, and it can be concluded that xylan was not degraded by this treatment ($\square_0$ = 100.68%).

The response surface of delignification yield *versus* time (X_1), and ethanol concentration (X_2) (Fig. 3) made it possible to select the optimal delignification conditions: 1 h time and 60.5% ethanol concentration, for which a delignification yield of 42.4% can be predicted. In a previous study with rice straw, a delignification yield of 48% was obtained using more severe conditions (60% ethanol, 1% H_2SO_4, and 70 ºC) (Sun and Sun 2002). Higher delignification yields were obtained using organosolv at high temperatures, for sugar cane bagasse at 195 ºC and 30% ethanol (Mesa *et al.* 2011), and wheat straw at 180 ºC, 40% ethanol, and 0.1% NaOH (Mesa *et al.* 2011; Ruiz *et al.* 2011).

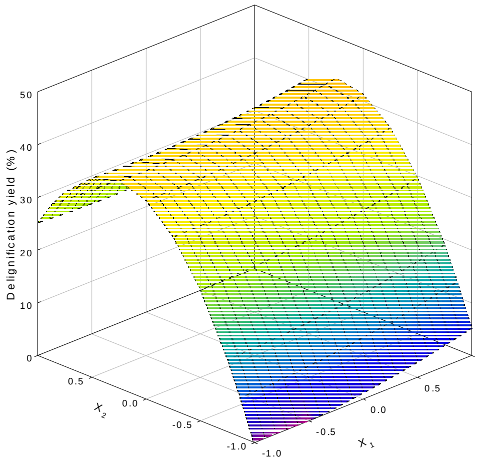

Fig. 3. Response surface for delignification yield in relation to time (X_1) and ethanol (X_2) concentration

Lignin Molecular Weight

The soluble lignin products obtained in the organosolv liquors of experiments 4, 5, and 6 (12, 24, and 0 h, respectively) were analysed for molecular weight and compared with technical lignins: Alcell (organosolv hardwood lignin), P1000 (soda lignin from wheat straw and Sarkand grass), and SodaWS (soda wheat straw lignin). The rice straw lignins obtained in this work were very similar and had low mean molecular weight, showing that delignification time did not affect the molecular weight of the solubilised lignins. These lignins showed substantially lower molecular weight and lower heterogeneity than all the technical lignins tested (Fig. 4).

Organosolv treatments produce more homogeneous lignin fractions with defined molecular mass distribution, chemical group functionalities (Gouveia *et al.* 2012), and low molecular weight (Lora *et al*. 2008). The obtained results were in agreement with these studies.

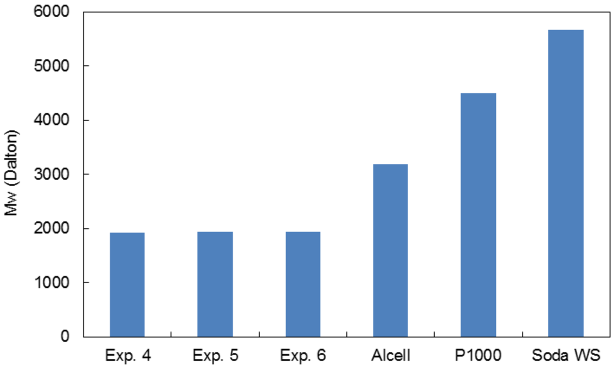

Fig. 4. Molecular weight of lignins after autohydrolysis and organosolv delignification (Exp. 4, 5, and 6) and technical lignins

Lignins of low molecular weight have been reported to be adequate as extenders or as components of phenolformaldehyde resins because of their high reactivity, in comparison with lignins with high percentages of high molecular weight molecules (El Mansouri and Salvado 2007; Tejado *et al.* 2007).

Phenolic Compounds

The total phenolic compounds in solution were analysed for all experiments. Total phenolic concentration was relatively low ranging from 1.1 to 2 g/L (data not shown) and was not in relation with organosolv conditions.

The phenolic compounds were separated using capillary electrophoresis, and identification was done by comparison of spectra and migration times with those of authentic standards (Fig. 5). Some phenolic compounds could be identified with a good matching: vanillin, ferulic, and coumaric acids. However a major compound (10.014 min migration time) could not be identified, but its spectrum (denoted by X) indicated that it should be a flavonoid. The presence of flavonoids in rice straw has been reported (Karimi *et al.* 2014).

Ferulic acids, coumaric acids, and vanillin have been identified in rice straw by other authors (Sun *et al.* 2002; Garrote *et al.* 2007; Buranov and Mazza 2008), and also in corn, wheat, and flax straws in different proportions being rice straw lignin the one that presents higher ferulic acid content (Buranov and Mazza 2008). These phenolic compounds are well known for their bioactivities, particularly as antioxidants (Binod *et al.* 2010).

Enzymatic Digestibility

The effects of the autohydrolysis pretreatment and organosolv delignification on the cellulose digestibility were evaluated by enzymatic hydrolysis of the remaining solid fractions. Table 5 shows the enzymatic digestibility of untreated rice straw, and on the solid fraction obtained after autohydrolysis and organosolv delignification.

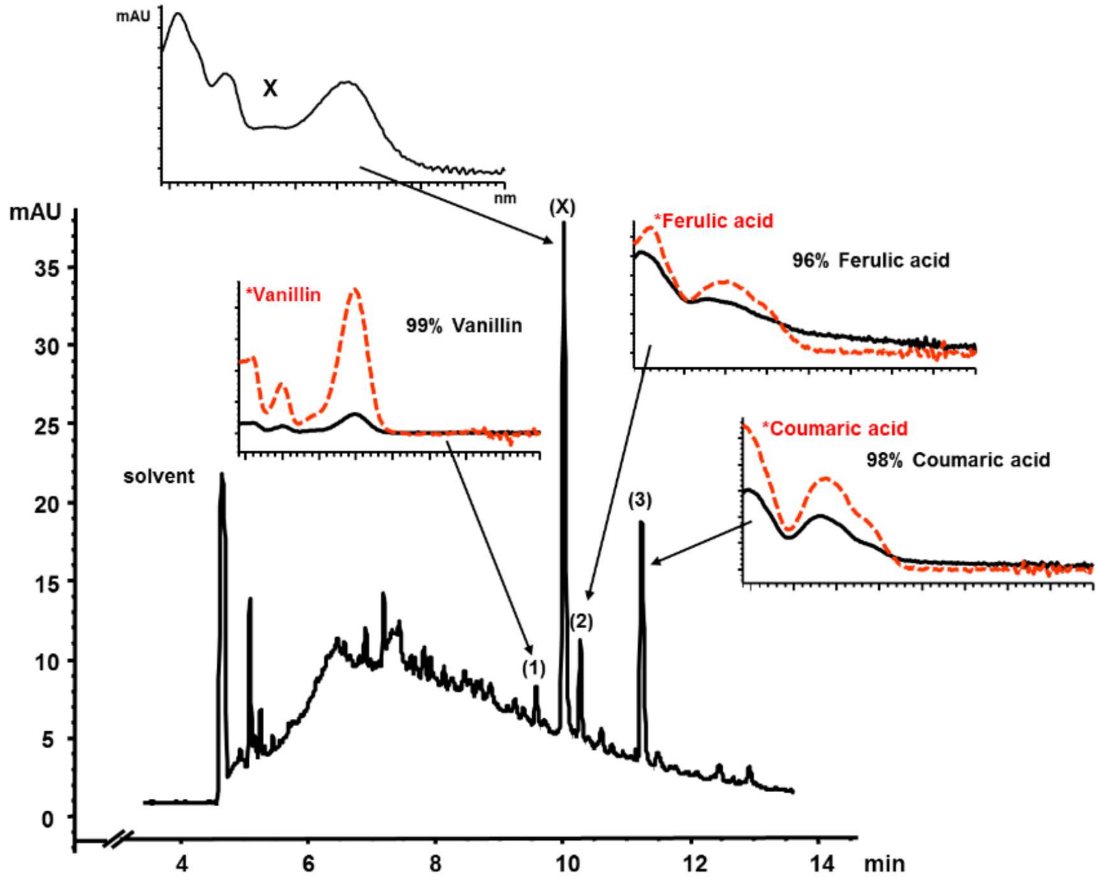

Fig. 5. Electropherogram (200 nm) showing the phenolic profile for the sample obtained after organosolv delignification (experiment 10) with matching % compared to authentic standards

The autohydrolysis induced a high increase in digestibility and higher severity led to increased digestibility. This did not occur with the organosolv conditions tested, and the enzymatic digestibility of the remaining solids was not affected significantly (68.5% *vs*. 62.8%, Table 5). The maximum glucose yield of 87.9% was obtained after autohydrolysis at 220 ºC. These results are in range of those obtained after hot water treatment of rice straw at 200 ºC for which 82% enzymatic digestibility was reported (Zhong *et al.* 2009; Imman *et al.* 2013), but lower than those obtained for rice straw after dilute acid hydrolysis (Kim *et al.* 2012) or AFEX (Zhong *et al.* 2009), and for wheat straw after autohydrolysis (Rossberg *et al.* 2014).

Table 5. Enzymatic Digestibility of the Untreated Raw Material, of the Solid Obtained before Autohydrolysis at 210 ºC (AH 210 ºC) and 220 ºC (AH 220 ºC), and after Autohydrolysis at 210 ºC and Organosolv Delignification

	% Enzymatic digestibility
Raw material	32.78 ± 3.23
AH 210 ºC	62.81 ± 0.05
AH 210 ºC + Organosolv	68.54[a] ± 2.06
AH 220 ºC	87.85 ± 3.83
[a] average of the 12 organosolv experiments	

All the organosolv experiments were tested for enzymatic digestibility. Glucose yields ranged between 71.9 and 67.2% (data not shown), and neither time nor ethanol concentration had a significant influence on the enzymatic digestibility of the remaining solid. The maximum increase of glucose yield by enzymatic hydrolysis in the organosolv solids when compared to the 210 ºC pretreated solids was 14.5% (experiment 7).

CONCLUSIONS

1. Autohydrolysis followed by mild organosolv treatment is an effective strategy for the valorisation of rice straw.
2. The hydrolysate obtained from autohydrolysis was rich in pentoses (mainly the oligomeric form). The solid fraction was enriched in lignin and glucan, and it showed a high enzymatic cellulose hydrolysis.
3. The mild organosolv treatment enabled a significant delignification at room temperature, which is significantly lower than the temperatures usually required for organosolv processes. The lignin extracts contained biological active compounds and the glucan-enriched solid had a high enzymatic digestibility.
4. To the best of our knowledge, this is the first report of a significant delignification at such mild conditions, and it is only possible because it is used a pre-treated material, further supporting the proposed integrated valorization strategy.

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