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Delignification Alternatives of Spent Solid from Autohydrolysis of Vine Shoots

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Vine shoots are residues obtained from an important agricultural activity as the viticulture. Although these residues have not received much attention yet, in the recent years the employment of these residues under a biorefinery approach has increased, being possible the obtaining of products of added value as polyphenols or lactic acid for instance. Dávila et al. (2016) carried out the hydrothermal treatment in a non-isothermal regimen in a range of temperatures between 180-215 ºC with a liquid/solid ratio of 8 Kg/Kg vine shoots, in order to obtain oligosaccharides which can be used as food ingredients due to their prebiotic effect.

In this previous study it was observed that the optimum temperature of the treatment to obtain the maximum oligosaccharides with the minimum formation of degradation products was 201 ºC. In this work, organosolv, acetosolv and alkali delignifications of the spent solid enriched in lignin and glucan were studied.

The alkali delignification, using 8 w/w % of NaOH at 124ºC for 105 min, was more promising than the other two treatments, being possible to remove 61% of the lignin present in the autohydrolized vine shoots. The lignin isolated in this process presented the highest content of Klason lignin (86%) and a high molecular weight (11401 g/mol) compared with the lignins isolated by applying the other delignification conditions.

1. Introduction

Lignin is the aromatic polymer most abundant in the nature and one of the mayor components of lignocellulosic biomass. It is part of the cell-walls of the biomass and it acts as a resin that holds the lignocellulosic matrix together as it fills the spaces between the cellulose and hemicelluloses (Erdocia et al., 2014). This macromolecular polymer is generated by enzymatic polymerization of the following monolignols; coniferyl alcohol, synapyl alcohol and p-coumaryl alcohol, by the formation of mainly interunit aryl-aryl type of bonds, although there are other 20 type of bonds (Sun et al., 2000). These polymerized monolignols lead to guayacyl, syringyl and p-hydroxyphenyl propane units respectively. Apart from the natural source, the extraction procedures also influence the physico-chemical characteristics of the lignin (Tejado et al., 2007).

Nowadays the main application given to the lignin is as low heating fuel for the generation of energy in the pulp and paper industry. However, different processes are being studied for the isolation of lignin from the lignocellulosic biomass in order to use it in industrial applications such as material/additives for wood panel products, biodispersants or polyurethane foams (Lora et al., 2002) or as a source of renewable products and commodity chemicals (Erdocia et al., 2014).

Furthermore, there is an ongoing trend to include the delignification process in a biorefinery scheme, in order to enable the valorization of all the fractions of the lignocellulosic biomass. Romaní et al. (2011) carried out an organosolv delignification of hydrothermally pretreated Eucalyptus globulus in order to improve the enzymatic susceptibility of the remained cellulose.

In the present work, the delignification of hydrothermally pretreated vine shoots under non-isothermal regimen at 201ºC was carried out. Under this condition, the solubilization of the hemicelluloses gave the maximum oligosaccharides concentration, obtaining a solid enriched in lignin and cellulose (Dávila et al., 2016).

Although the high quantities of vine shoots generated per year, 2-4 tons/hectare (Nabais et al., 2010) there are not many delignification studies using these residues. Bustos et al. (2005) carried out the delignification of prehydrolyzed vine shoots with NaOH, in order to use the delignified solid for saccharifications. Jiménez et al. (2006) tried different delignification treatments on the vine shoots in order to employ the obtained cellulose

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pulp. They observed that NaOH and Kraft pulping removed a 61.9 and 75.3 % respectively of the lignin contained in the original solid. They also observed that when softer delignification conditions were employed, using ethanol or ethylene glycol, the delignification was less efficient, removing only 26.1 and 32.4 % of the lignin in the original solid. However, neither of these researchers analyzed the characteristics of the lignins obtained in the different treatments.

In this work the delignification treatments carried out were sulfur-free as the lignin obtained resembles more closely to the structure of the native lignin (Lora et al. 2002) and the cellulose obtained presents higher purity (Karsltöm et al. 2014) which is beneficial working under a biorefinery approach. The delignification treatments employed in this study were alkali, organosolv and acetosolv using the hydrothermally pretreated vine shoots under non-isothermal regimen at 201ºC as raw material. The isolated lignins were chemically characterized and analyzed by HPSEC and FTIR. Apart from the characterization of the lignins, the efficiency of each delignification treatment was also analyzed.

2. Materials and methods

2.1 Raw material

Vine shoots from the grape variety Hondarribi Zuri supplied by the local winery Aldakoa S.L. (Basque County, Spain) were employed during the study. After the collection, they were air-dried, milled and sieved to obtain a fraction with a particle size smaller than 0.4mm. The milled vine shoots were stored in a dark and dry place until they were used.

2.2 Hydrothermal processing of the vine shoots

The hydrothermal treatment of the vine shoots and the analysis of the obtained solid were carried out following the procedure carried out by Dávila et al. (2016). The milled vine shoots were mixed with water in a 1.5 L stainless steel Parr reactor in a liquid to solid ratio of 8 Kg/Kg (oven dried basis). The hydrothermal process was carried out at 201 ºC under non-isothermal regimen, controlling the temperature by a Parr PID control. The solid and liquid phases were separated by filtration, being the solid phase washed and stored at 4 ºC without being dried.

In order to characterize the spent solid obtained in the hydrothermal treatment an aliquot of the solid stored at 4 ºC was air dried, milled and sieved in order to obtain a particle size smaller than 0.25 mm. The milled spent solid was subjected to a quantitative acid hydrolysis with H_2SO_4 72 % (TAPPI T-249-em-85) and the liquid and solid phases were separated by filtration. The liquid phase was employed for the determination of monosaccharides (glucose, xylose and arabinose), galacturonic and acetic acid and degradation products (5-hydroxymethylfurfural and furfural). The analysis was carried out by High Performance Liquid Chromatography (HPLC) using a Jasco LC Net II / ADC chromatograph equipped with a refractive index detector and using a 300 x 7.8 mm Aminex HPX-87H column (Bio-Rad Laboratories, USA). 20 µL of the sample were eluted with 0.005 M H_2SO_4 at a flow rate of 0.6 mL/min at 50 ºC. The oven-dried weight of the solid phase obtained was considered as Klason lignin. The analyses were carried out by triplicate.

2.3 Delignification procedures of the hydrothermal spent solid

The spent solid stored obtained after the hydrothermal treatment at 201 ºC under non-isothermal regimen and stored at 4 ºC was subjected to organosolv, acetosolv and alkali delignification employing different conditions.

In order to carry out the organosolv delignification, the autohydrolized solid was mixed with an aqueous solution of 50 % (w/w) of ethanol in a 1.5 L stainless steel Parr reactor using a liquid to solid ratio of 8 Kg/Kg (oven dried basis). The mixture was kept at 200 ºC for 90 minutes and after being cold, the solid and liquid phases were separated by filtration. The precipitation of the lignin contained in the black liquors was carried out by the addition of 4 volumes of acidified water and it was isolated by filtration and neutralized with water.

The acetosolv delignification was carried out by modifying the procedure proposed by Erdocia et al. (2014). The hydrothermally obtained spent solid was mixed with an aqueous solution of 65 % (w/w) of acetic acid with 0.073 % (w/w) HCl in a liquid to solid ratio of 12 Kg/Kg (oven dried basis) using a 1.5 L stainless steel Parr reactor. The process was carried out at 130 ºC for 90 minutes and the solid and liquid phases were separated by filtration. The black liquors were treated with 4 volumes of water and the precipitated lignin was isolated by filtration.

An alkali delignification treatment was carried out using the hydrothermally treated spent solid using the following conditions. The spent solid was mixed with an aqueous solution of 8 % (w/w) of NaOH in a solid to liquid ratio of 10 Kg/Kg (oven dried basis) and autoclaved at 124 ºC for 105 minutes. The precipitation of the lignin contained in the alkali black liquor was carried out by the addition of H_2SO_4 96 % until pH 2 was reached. The isolated lignin was separated by filtration and neutralized with water.

The solid phases obtained after each delignification treatment were washed with the solution employed in the delignification treatment and then with water. The washed solid phases were air dried, subjected to gravimetric and moisture determinations in order to analyzed the solid yield and then subjects to the same analytical processes as the spent solid obtained after the hydrothermal process.

2.4 Lignin characterization

The composition in monosaccharides, acid soluble lignin and Klason lignin of the isolated lignins from the different delignification processes was determined by a modification of the acid hydrolysis carried out by Erdocia et al. (2014). The acid hydrolysis was carried out with 72 % H_2SO_4 at 30 ºC for 1h and then the samples were diluted to 12 % (w/w) H_2SO_4 and kept in the autoclave for 1h at 121 ºC. The solid and liquid phases obtained after the hydrolysis were separated by filtration and the oven-dried solid was considered as Klason lignin. The monosaccharides were determined in the liquid phase by HPLC using the procedure carried out in the characterization of the hydrothermally obtained spent solid.

The determination of the acid soluble lignins (ASL) in the liquid phases was carried out using an UVmini-1240 spectrophotometer (Shimadzu Corporation). The samples were diluted with H_2SO_4 1 M until the absorption measured at 205 nm was between 0.1 and 0.8 (TAPPI UM250 um-83). The ash content of the lignin was analyzed by a thermogravimetric analysis: between 3 and 5 mg of the isolated lignins were tested in a TGA/SDTA RSI analyzer 851 Mettler Toledo under an oxygen atmosphere at a heating rate of 25 ºC/min from 25 to 850 ºC.

The chemical structure of the different lignins was analyzed by Fourier transform infrared spectroscopy (FTIR) using a PerkimElmer Spectrum Two FT-IR spectrometer. The spectrum was recorded from a range between 4000 and 600 cm^{-1} accumulating a total of 8 scans in transmission modes with a resolution of 4 cm^{-1}.

The lignins were also subjected to High Performance Size Exclusion Chromatography (HPSEC) to determine their average molecular weight (Mw), number average (Mn) and their polydispersity. The analyses were carried out using a Jasco LC Net II/ADC chromatograph equipped with a refractive index detector using two consecutive Varian Polymer Laboratories PolarGel-M columns (300 mm x 7.5 mm). The samples were dissolved and eluted (20 µL) with a solution of DMF with 0.1 % of lithium bromide using a flow rate of 0.7 mL/min and working at 40 ºC. The calibration of the HPSEC was carried out using polystyrene standards with molecular weights between 62,500 and 266 g/mol) provided by Sigma Aldrich.

3. Results and discussion

3.1 Yield of the delignification treatments

In this study organosolv, acetosolv and alkali treatments were assayed for the delignification of the spent solid obtained after the hydrothermal treatment of the vine shoots at 201 ºC in a non-isothermal regimen. The composition of the spent solid which is shown in Table 1 differs completely from the composition of the vine shoots of the variety Hondarribi Zuri, as this latest contained 33.0 % glucan, 27.0 % hemicelluloses, 26.7 % lignin, 2.6 % ashes and 3.1% extractives, everything on oven dry basis (Dávila et al. 2016). During the hydrothermal treatment, the effective solubilization of the hemicelluloses took place, giving a solid enriched in lignin and glucan, with low hemicellulose content.

In the Table 1 the yields of the different delignification treatments and the composition of the delignified solids are shown. As it can be seen, the organosolv and acetosolv delignifications were the less effective treatments between the ones studied, which is in agreement with what it was reported by Jiménez et al. (2006). The organosolv and acetosolv delignifications leaded a 12.6 and 25% of delignification yield, while in the alkali delignification a 61 % of the lignin of the original solid was removed.

Table 1: Composition of the solids obtained by the different delignification strategies.

	Hydrothermal	Organosolv	Acetosolv	Alkali
Yield (%)		82.2	70.3	53.4
Lignin (%)	46.8	49.7	49.8	33.8
Glucan (%)	39.9	44.6	49.9	55.4
Hemicelullose (%)	5.7	0	0	0
Delignification yield (%)		12.6	25.0	61.4

Table 2: Composition of the isolated lignins by the different treatments

	Organosolv	Acetosolv	Alkali
Klason lignin (%)	81.00	83.40	86.00
ASL (%)	1.12	1.24	1.24
Glucose (%)	0.36	0.57	0.44
Xylose (%)	0.30	0.43	2.81
Ash (%)	8.68	4.63	2.13

It was also observed that the delignification conditions influenced both the efficiency of the lignin removal and the composition of the isolated lignins, as it can be observed in the Table 2. All the isolated lignins contained around 80 % of Klason lignin, obtaining the purest lignin after the alkali delignification. It can also be observed that the alkali lignin presented the highest saccharide content. According to Huijgen et al. (2014), the presence of these carbohydrates could be attributed to the fact that they could remain covalently link to the lignin or they could be trapped in the lignin precipitation stage. Although the higher saccharide content in the alkali lignin, it contained higher Klason lignin than what it has been reported before.

Toledano et al. (2013) and Sequeiros et al. (2014) reported respectively that the alkali lignin extracted from olive tree pruning and from almond shell only contained 4.55% and 12.87% of Klason lignin, due to the high saccharide and ash content. However, the high purity of the alkali lignin obtained in this study could be due to the pretreatment of the vine shoots, as in the pretreatment great part of the hemicellulosic saccharides were removed.

3.2 Lignin structure and molecular weight

The molecular weight distribution of the lignins isolated by the different delignification treatments were analyzed by HPSEC, being the molecular weight (Mw), the number-average (Mn) and the polydispersity (Mw/Mn) shown in the Table 3. The results presented in this Table could be used only for comparative analysis as the calibration curve was done with polystyrene standards.

Table 3: Molecular weight and polydispersity of the lignins isolated by different delignification conditions.

	Organosolv	Acetosolv	Alkali
Mw (g/mol)	1902	6579	11401
Mn (g/mol)	915	1639	1348
Mw/Mn	2.08	4.01	8.46

It can be observed that the molecular weight varied strongly with the delignification conditions. The delignification efficiency seems to be related with the molecular weight of the obtained lignins; Coral Medina et al. (2015) observed that the high delignification yield was related with the removal of larger molecules of lignin. The lignin obtained in the alkali treatment presented the highest molecular weight. Toledano et al. (2013) and Coral Medina et al. (2015) also obtained alkali lignin isolated from olive tree pruning and oil palm empty fruit with 10875 g/mol and 12580 g/mol, respectively.

The chemical structure of the lignins isolated from the hydrothermally pretreated vine shoots with different delignification conditions was analyzed by FTIR as it can be observed in the Figure 1. Although the intensity of the bands seem to vary significantly depending on the delignification treatment, all the isolated lignins present the characteristic bands observed in lignins. The assignation of the bands was carried out taking into account what it has been previously reported in literature (Erdocia et al., 2014; Coral Medina et al., 2015).

A wide band corresponding to the O-H stretching vibration in aromatic and aliphatic hydroxyl group is observed at 3350 cm^{-1}. The bands observed at 2930 cm^{-1}, 2850 cm^{-1} and 1460 cm^{-1} which are common for all the samples corresponded to C-H bonds vibrations in CH_3 and CH_2 groups. The band observed at 1700 cm^{-1}, which intensity varies between the obtained lignins, corresponded to the stretching of non-conjugated carbonyl groups with the aromatic skeletal of the lignin. The intensity of this band in the acetosolv lignin is higher than in the other lignins, which could be due to the esterification of some of the hydroxyl groups of the lignin by the organic acid used (Villaverde et al., 2009).

The characteristic bands of the monolignols present in the structure of the lignin were also observed in the FTIR spectra. The presence of the guaiacyl units was reflected in the bands observed at 1513, 1425, 913 and 1030 cm^{-1} corresponding the last three bands to the C-H deformation, C-H out of plane and C-H in plane bending in the guaiacyl ring. The presence of syringyl units was corroborated by the bands observed at 1330 and 833 cm^{-1} , corresponding the last one to the out of plane C-H bending (Villaverde et al., 2009). The bands observed at 1596, 1215 and 1115 cm^{-1} also are related to the lignin structure as they correspond to a peak of aromaticity, to the C-O vibrations in the syringyl and guaiacyl units and to the C-H in plane deformation for aromatics in these monomers.

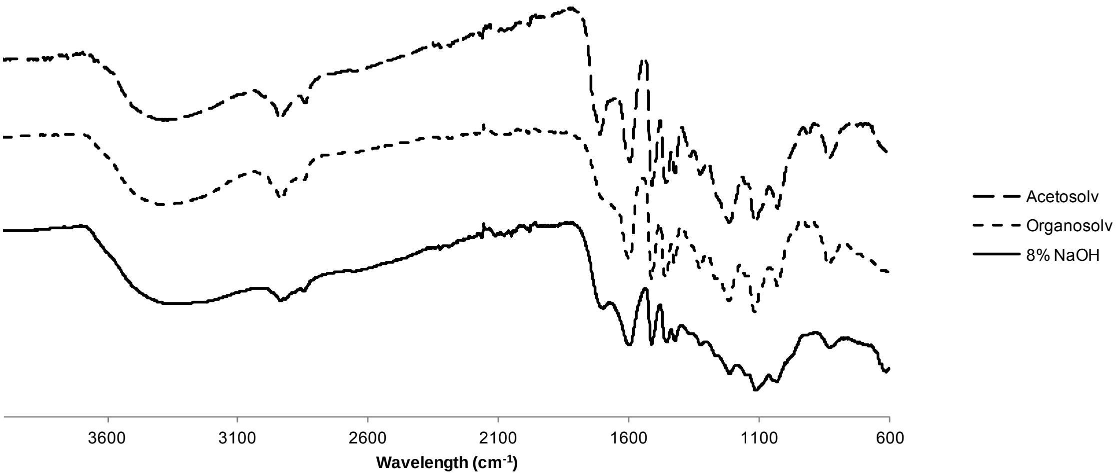

Figure 1: FTIR spectra of the lignins isolated using different delignification conditions.

4. Conclusions

Different delignification treatments were carried out on the spent solid obtained from the hydrothermally treated vine shoots at 201 °C in non-isothermal regimen, concluding that the alkali treatment were more promising than the organosolv and acetosolv delignifications.

The employment of 8 % (w/w) of NaOH at 124 °C for 105 minutes produced a delignification of 61 % of the lignin in the original solid, obtaining a lignin which contained a 86 % of Klason lignin and a molecular weight of 11401 g / mol. In basis of the results obtained in this work, it would be feasible to include this delignification step in a biorefinery scheme in order to obtain a solid enriched in cellulose which could be subjected to enzymatic hydrolysis yielding dissolutions rich in glucose and a lignin fraction that could be used for biomaterials.

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