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Oligosaccharides and monomeric carbohydrates production from olive tree pruning biomass

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ABSTRACT

Using the severity factor, it has been possible to study cellulose and hemicellulose fractional conversion, sugar yields change and oligosaccharides variation through olive tree pruning biomass pretreatments with acid or liquid hot water under pressure. The temperatures tested were in the range 180–230 °C, operation time varying between 0 and 30 min and acid concentration used did not exceed 0.05 M. Complete hemicellulose solubilization in autohydrolysis was achieved using severity factors ($\log R_0$) close to 3.9 (most sugars are like oligomers), while if sulfuric acid 0.025 M is employed, this parameter could be smaller ($\geq$3.4). With these treatments, we have obtained cellulose conversions between 30 and 42% from liquid hot water experiments, 40–51% with sulfuric acid 0.025 M and 42–57% when the acid concentration was 0.05 M. The best results in terms of maximum yield in total sugars, D-glucose and D-xylose, with a low amount of acetic acid and hydroxymethylfurfural, was obtained at 200 °C, 0 min (what means that there is no time of temperature maintenance, only heating and cooling) and H_2SO_4 0.025 M.

1. Introduction

The olive tree is one of the most important crops in Spain. The pruning, operation that is usually applied to the branches and leaves after harvest, produces 5.0×10^6 to 5.5×10^6 t/year of lignocellulosic biomass (Moya, Bravo, Mateo, & Sánchez, 2008). The large volumes of waste generated, together with the great environmental damage caused by its uncontrolled burning has suggested the possibility of exploiting this biomass resource.

Hemicellulose is the second major polysaccharide in woody biomass, typically comprising 15–35% of the dry wood mass. It consists of heterogeneous polymers of pentoses (xylose and arabinose) and hexoses (glucose, mannose and galactose), which can be substituted with phenolic, uronic or acetyl groups (Lee, Venditti, Jameel, & Kenealy, 2011).

The acid hydrolysis of the hemicellulosic fraction of olive tree pruning can also lead to a valuable solid residue mainly formed of cellulose and lignin. Thus, this process can be conceived as the first stage of an integrated strategy for this residue utilization. The hemicellulosic sugars released from other lignocellulosic materials have been used to obtain ethanol, xylitol or single-cell protein.

We have tested some thermal processes for fractionating the olive tree biomass through treatments with liquid hot water (autohydrolysis) and in diluted acid media. While the hot compressed water treatment shows some favorable aspect associated with a lower environmental impact, limitation of equipment corrosion and a better selectivity for dissolving hemicellulose fraction without causing significant change in cellulose and acid-insoluble lignin, the sugars could be present as oligomers. In thermal acid treatment, the carbohydrate could be appear in free form to a greater extent, although resulting compound concentrations in the hydrolyzates (oligosaccharides, monosaccharides, acetic acid and sugars decomposition products) would depend on the operating conditions employed (Lee & Jeffries, 2011).

Both of these possibilities, individual sugars and oligosaccharides, could offer important applications. The first could be fermented to obtain products like ethanol with fundamental use as biofuel and other compounds of great industrial interest for their sweetening power, such as xylitol. The oligomers could be utilized as food additives, prebiotics or biopreservatives (Barreteau, Delattre, & Michaud, 2006), active principles for dermatological preparations, antioxidant activity products and for the prevention and treatment of infections.

Severe conditions of temperature and prolonged operation times, may facilitate hemicellulose solubilization although it could also generate significant amounts of inhibitors. In addition to hemicellulose sugars for fermentation to ethanol and xylitol, other products such as furfural can be made from these constituents by thermochemical reactions and reactive intermediates for catalytic conversion (Zhang, Kumar, & Wyman, 2013). So it might be necessary to find a set of operating values that would lead to a maximum of both, free and oligomeric forms.

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Table 1
Olive tree pruning biomass composition.

Composition	% of dry matter[a]
Moisture	7.5 ± 0.1
Cellulose	16.5 ± 0.4
Hemicellulose	20.8 ± 0.5
Acid-insoluble lignin	21.3 ± 0.5
Ash	4.7 ± 0.3
Extractives	21.4 ± 0.2
In water	18.8 ± 0.1
Xylose	2.7 ± 0.1
Glucose	5.8 ± 0.1
In ethanol	2.6 ± 0.1

[a] Average of at least three determinations.

Using the severity factor it would be possible to quantify the hardness of hydrolytic treatments considering the interaction between both temperature and operating time. However it is not possible to use an experiment design based on this parameter because of the inherent characteristics of this factor, although it has allowed also consider experimental aspects as might be the way that has been carried out the heating, maintenance and cooling stages involved in the different experimental tests performed.

The aim of this work was to find the optimal thermal pretreatment conditions to obtain hydrolyzates with the greatest concentration of sugars and lower of inhibitors as the first step in the recovery and use of olive tree pruning biomass and subsequent fermentation to ethanol and xylitol production.

2. Materials and methods

The olive tree pruning biomass, collected during the pruning season, consisted of leaves, branches and pieces of trunks from olive trees and was obtained from an olive grove situated in Jaén, Spain. The material was air-dried, milled, screened to select the fraction of particles with a diameter between 0.60 and 0.425 mm and homogenized in a single lot.

2.1. Hydrolysis procedure

All thermal processes were conducted in a stainless steel discontinuous pressure reactor, stirred tank, Parr model 4842 (Moline, IL, USA). It has a maximum capacity of 2 dm^3 although the load should not exceed 1 dm^3. In order to perform the tests, 50 g of dry solid residue is loaded into the reactor with the necessary amount of ultrapure water or diluted sulfuric acid to work with an 1:5 S/L ratio; then, we close the equipment, set the agitation at 250 rpm, and the temperature is programmed (heating rate was between 5 and 6 °C/min). Cooling of the mixture is carried out by circulating cold water and then immersing the body of the reactor in an ice bath. Once the suspension is at 40–45 °C the reactor is opened. In order to calculate the treatment severity, the temperatures reached are recorded at different times of the process both in the heating phase and in the cooling. The residual solid is separated from the liquid hydrolyzate by filtration. The hydrolyzate, after the measurement of pH, is kept cool for further analysis while the water washed solid residue is dried at room temperature, weighed and characterized before use.

2.2. Analytical methods

2.2.1. Raw material and solid residues characterization

We analyzed the composition of both the original olive tree pruning biomass and the solid residues obtained after hydrolytic treatments. The experimental results of raw material in percentage of dry matter are shown in Table 1.

The solid fractions used in this work were characterized by the following parameters: moisture, by the TAPPI T 12 os-75; ash, according to TAPPI T 15 os-58; lignin by the procedure described by TAPPI T 222 os-74; neutral detergent fiber (NDF) and acid detergent fiber (ADF), by the method of van Soest and Robertson (1979). The percentages of hemicellulose and cellulose were calculated from the NDF and ADF values according to Eqs. (1) and (2).

$$\%\text{hemicellulose} = \%\text{NDF} - \%\text{ADF} \tag{1}$$

$$\%\text{cellulose} = \%\text{ADF} - \%\text{lignin} \tag{2}$$

Extractives were determined gravimetrically using a two-step secuencial extraction process by Soxhlet to remove water soluble material (inorganic compounds, non-structural sugars and nitrogenous substances, among others) and ethanol soluble material (chlorophyll, waxes or minor components) according to a procedure adapted from Sluiter, Ruiz, Scarlata, Sluiter, and Templeton (2008).

2.2.2. Hydrolyzates characterization

Concentrations of D-glucose, D-xylose, L-arabinose and hydroxymethylfurfural were determined by high performance ionic liquid chromatography (HPILC) using a Dionex instrument ICS 3000 (Dionex Corporation, Sunnyvale, CA, USA), equipped with a CarboPad PA20 analytical column (3× 150 mm) and guard column (3× 30 mm) using an isocratic pump with double valve position and an amperiometric detection system with AgCl as reference electrode. Elution was performed at 30 °C with NaOH 2 mM with a flow rate of 1.0 cm^3/min. The regeneration of the column was carried out eluting NaOH 200 mM for 15 min. Instrumental controls, data acquisition, and chromatographic integration were performed using Dionex Chromeleon software.The estimated content of oligosaccharides was performed by an indirect method carrying out a post-hydrolysis of the liquid fraction from the different hydrolytic treatments with 5 wt% H_2SO_4 at 115 °C for 60 min. Similar treatment conditions have been used by other authors, working with olive stones biomass (Cuevas et al., 2010) or maple wood (Zhang et al., 2013).

Acetic acid concentrations were measured using the method described by Bergmeyer and Mollering (1974), based on the enzymes acetyl coenzyme A synthetase and malate dehydrogenase. All the concentrations were determined by averaging at least two analytical data.

3. Results and discussion

Because of the inherent characteristics of the severity factor, we preferred to use a conventional methology to study the suitable conditions with a view to optimize the pretreatment. So, we performed 22 experiments varying temperature from 180 to 230 °C, operation time from 0 to 30 min, and sulfuric acid concentration between 0.00 and 0.05 M. The experiments conducted and the variables tested are shown in Table 2.

3.1. Parameters

From the concentrations of total sugars and several monomers generated in the hydrolytic process, D-glucose, D-xylose and L-arabinose, corresponding yields are determined for each experiment, defined by Eqs. (3) and (4).

$$Y_{\text{tot}} = \frac{\text{kg total sugars}}{\text{kg initial dry biomass}} \times 100 \tag{3}$$

$$Y_{\text{mon}} = \frac{\text{kg monomer}}{\text{kg initial dry biomass}} \times 100 \tag{4}$$

Table 2
Conditions, severity factors, hemicellulosic conversion, X_H, and cellulosic conversion, X_C, for the thermal treatments assayed.

Experiments	T (°C)	t (min)	$[H_2SO_4]$ (M)	$\log R_0$	X_H (%)	X_C (%)
1	190	0	0.000	3.21	60.40	29.84
2	200	0	0.000	3.43	86.12	38.57
3	210	0	0.000	3.71	90.01	44.43
4	220	0	0.000	4.14	100.00	51.66
5	230	0	0.000	4.38	100.00	58.41
6	210	15	0.000	4.65	100.00	63.40
7	190	30	0.000	4.17	100.00	51.03
8	230	30	0.000	5.35	100.00	77.40
9	180	0	0.025	2.89	68.90	31.91
10	190	0	0.025	3.17	98.12	40.02
11	200	0	0.025	3.44	100.00	44.42
12	230	0	0.025	4.51	100.00	64.74
13	180	15	0.025	3.61	99.63	45.82
14	190	15	0.025	3.91	98.78	53.42
15	200	15	0.025	4.20	100.00	60.05
16	210	15	0.025	4.49	100.00	65.22
17	190	30	0.025	4.17	100.00	59.22
18	230	30	0.025	5.35	100.00	n.d.
19	180	0	0.050	2.86	100.00	38.26
20	190	0	0.050	3.14	100.00	43.19
21	200	0	0.050	3.39	100.00	48.68
22	210	0	0.050	3.78	100.00	56.60

n.d., not determined.

Furthermore, characterization of the residues obtained after completion of each hydrolysis process allowed us to calculate the fractional conversion of hemicellulose, X_H, and cellulose, X_C, given by expressions (5) and (6) respectively.

$$X_H = \frac{\text{kg processed hemicellulose}}{\text{kg initial hemicellulose}} \times 100 \quad (5)$$

$$X_C = \frac{\text{kg processed cellulose}}{\text{kg initial cellulose}} \times 100 \quad (6)$$

In order to compare results in different hydrothermal treatment conditions and to determine a quantitative measure of intensity, and in accordance with various studies on the pretreatment of biomass with liquid hot water (LHW) hydrolysis, a severity factor, $\log R_0$, was employed. Although this does not provide an exact measure of the severity, it can be used to determine the aggressiveness of the treatment, (Galbe & Zachhi, 2007; Rogalinski, Ingram, & Brunner, 2008).

This severity factor, $\log R_0$, for hydrolytic pretreatments carried out with the olive tree pruning, has been evaluated according to the expression of Overend and Chornet (1987), who defined this factor as Eq. (7)

$$R_0 = \int_0^t e^{T-T_{ref}/w}\, dt \quad (7)$$

where T is the temperature of the reaction system, °C; t is the treatment time, min; and w is an empirical parameter related to the activation energy.

According to Montané, Overend, and Chornet (1998), the reference temperature has no appreciable influence on the analysis of data below 100 °C. It can be seen that there are no significant changes in the structure of certain polysaccharides (Garrote, Cruz, Domínguez, & Parajó, 2003), which has allowed us to establish this temperature as the reference value. Moreover, in relation to w there are many investigations that have considered a value of

Table 3
Total sugars and monomeric carbohydrates yields, %.

Experiments	$\log R_0$	Y_{tot}	Y_{glu}	Y_{xyl}	Y_{ara}
1	3.21	3.23 ± 0.02	n.d.	0.04 ± 0.01	0.64 ± 0.02
2	3.43	5.23 ± 1.00	2.67 ± 0.80	0.27 ± 0.01	1.04 ± 0.10
3	3.71	6.03 ± 1.06	3.17 ± 0.88	0.59 ± 0.02	1.16 ± 0.10
4	4.14	7.61 ± 0.94	n.d.	1.39 ± 0.02	0.52 ± 0.26
5	4.38	7.11 ± 1.65	2.81 ± 1.49	1.02 ± 0.20	0.06 ± 0.02
6	4.65	2.83 ± 1.14	1.87 ± 0.85	0.16 ± 0.06	n.d.
7	4.17	6.68 ± 1.65	2.82 ± 0.56	1.45 ± 0.54	0.44 ± 0.10
8	5.35	0.09 ± 0.02	0.05 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
9	2.89	8.26 ± 0.20	3.60 ± 0.24	0.37 ± 0.24	n.d.
10	3.17	10.88 ± 0.30	5.36 ± 0.30	1.29 ± 0.22	2.17 ± 0.40
11	3.44	12.72 ± 0.92	5.66 ± 0.14	2.99 ± 0.24	2.31 ± 0.48
12	4.51	5.29 ± 1.26	2.80 ± 0.18	0.73 ± 0.02	0.31 ± 0.04
13	3.61	16.00 ± 1.77	6.15 ± 0.78	5.37 ± 0.02	n.d.
14	3.91	15.75 ± 1.31	6.34 ± 1.11	5.85 ± 0.36	2.18 ± 0.34
15	4.20	11.29 ± 2.15	5.21 ± 0.02	3.78 ± 0.02	1.02 ± 0.42
16	4.49	3.68 ± 0.02	2.41 ± 0.02	0.43 ± 0.01	0.17 ± 0.06
17	4.17	10.25 ± 4.47	5.03 ± 2.11	4.09 ± 1.21	1.36 ± 0.48
18	5.35	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
19	2.86	17.50 ± 0.32	7.36 ± 0.04	5.66 ± 0.76	2.76 ± 0.08
20	3.14	24.79 ± 1.36	9.97 ± 2.13	9.35 ± 1.36	2.87 ± 0.10
21	3.39	26.64 ± 1.25	10.63 ± 0.89	10.98 ± 0.56	3.01 ± 0.36
22	3.78	20.56 ± 0.58	7.83 ± 1.23	8.99 ± 0.58	2.10 ± 0.42

n.d., not determined.

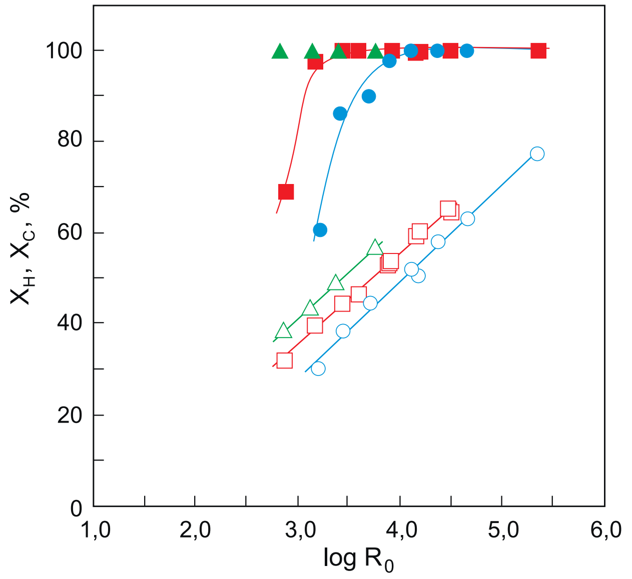

Fig. 1. Variation of X_H (solid symbols) and X_C (hollow symbols) with the severity factor in the thermal treatments made with H_2SO_4 0.00 M (circles), 0.025 M (squares) and 0.05 M (triangles).

this parameter equal to 14.75 for materials of similar characteristics to olive tree pruning biomass, (Cuevas, Sánchez, Bravo, Cruz, & García, 2009; Fernández-Bolaños et al., 2001; García, Cuevas, Bravo, & Sánchez, 2010).

The severity factor as described above can be used only if the temperature remains constant during the reaction (isothermal process). As this is not the case due to the progressive heating times, a modified severity factor is used, considering the temperature as a function of residence time in the reactor, so that now expression (7) remains as stated in Eq. (8), where $T(t)$ represents the function relating the temperature of the reaction system to the treatment time.

$$R_0 = \int_0^t e^{T(t)-100/14.75}\,dt \qquad (8)$$

Calculating R_0 from Eq. (8) will therefore allow us to measure the combined effect of both variables (temperature and time) in a given treatment, as well as compare pretreatments carried out under different conditions.

In hydrolysis experiments, in general, three distinct phases can be distinguished: heating, holding and cooling. Thus, in order to calculate severity factor, the functions $T(t)$ have been used, which obey the following settings: linear ($T = a_1 + b_1 t$), constant and third-order

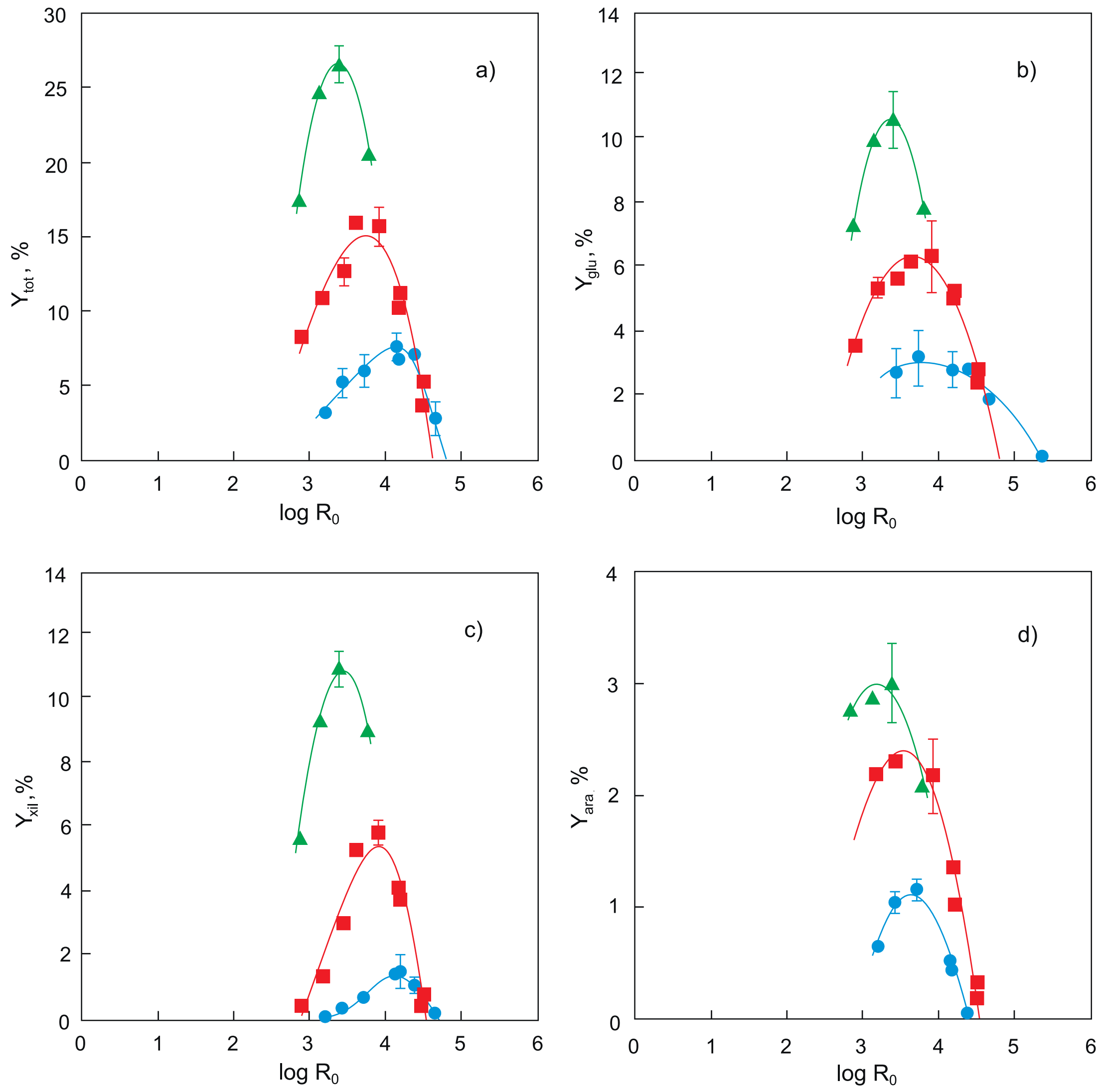

Fig. 2. Total sugars (a), D-glucose (b), D-xylose (c) and L-arabinose yields (d) vs. severity factor for the thermal treatments made with H_2SO_4 0.00 M (circles), 0.025 M (squares) and 0.05 M (triangles).

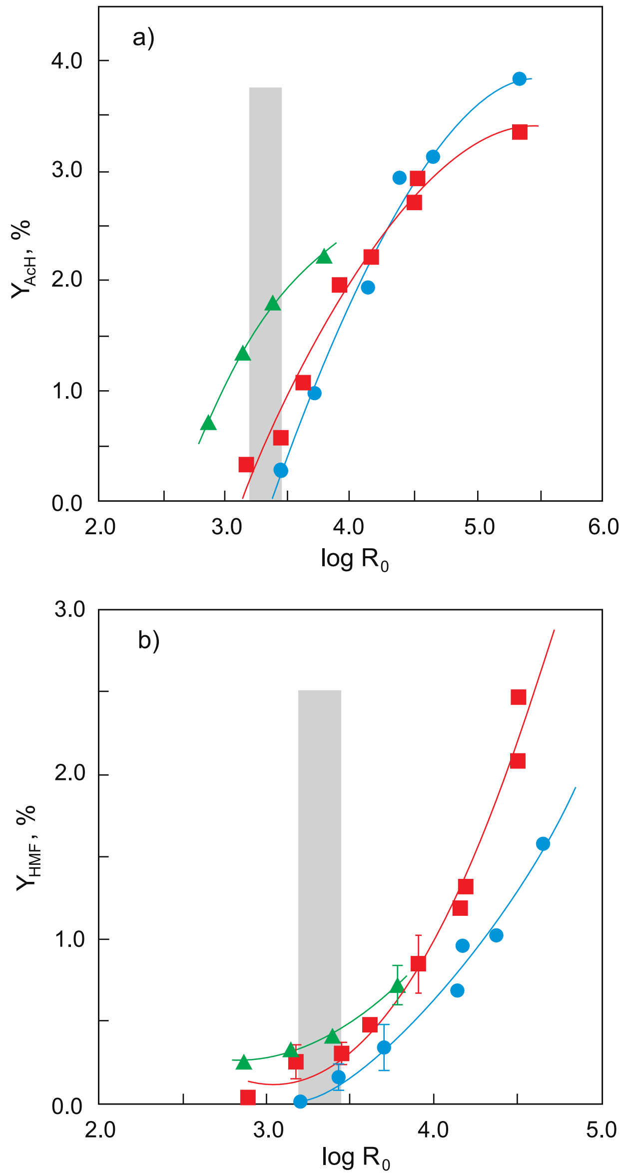

Fig. 3. Acetic acid yield (a) and HMF yield (b) vs. severity factor for the thermal treatments made with H_2SO_4 0.00 M (circles), 0.025 M (squares) and 0.05 M (triangles).

polynomial for heating, maintenance and cooling, respectively. The overall severity factor is determined as the sum of those corresponding to the three phases.

In this way, the expressions obtained for the severity factor in the heating, $(R_0)_{heat}$, and maintenance phases, $(R_0)_{man}$, would be those corresponding to Eqs. (9) and (10), where t_c is the time in which the maximum treatment temperature is reached, t_r is the time the temperature remains constant and T_{man} is the maintenance temperature.

$$(R_0)_{heat} = \frac{14.75}{b_1}\left[e^{a_1-100+b_1 t_c/14.75} - e^{a_1-100/14.75}\right] \qquad (9)$$

$$(R_0)_{man} = (t_r - t_c)e^{T_{man}-100/14.75} \qquad (10)$$

In the cooling zone, we have used a numerical method of integral resolution (trapezoidal method) as the analytical method for solving integral equation (8) would be a too laborious process, while the calculation procedure used does not involve any significant error risk. Table 2 summarizes the values of the severity factors calculated for each of the experiments performed.

3.2. Cellulose and hemicellulose conversions

For each of the solid residues obtained in the assayed pretreatment conditions, we have analyzed the composition of moisture, lignin, cellulose, hemicellulose and ash. It has been possible to calculate the fractional conversions of hemicellulose (X_H) and cellulose (X_C), obtaining the data as shown in Table 2.

In all experiments the percentage of lignin recovered was close to 100%, slightly higher in some cases, mainly in the experiments of greater severity. The increase of this fraction was also observed by Carvalheiro, Esteves, Parajó, Pereira, and Gírio (2004) and may be explained by the condensation of lignin with sugars or degradation products such as furfural, (Montané, Salvadó, Farriol, Jollez, & Chornet, 1994). This hypothesis of non-degradation of the lignin fraction allows us to refer the cellulose and hemicellulose to the raw material percentages.

From the discussion of these values we can infer that in absence of acid (hydrothermal process), the total conversion of hemicellulose achieved for severity factors close to 3.9, while with acid 0.025 M is achieved for log R_0 values over 3.4, Fig. 1. These data are similar to García et al. (2010), who for the complete solubilization of the hemicellulose from olive pruning debris by autohydrolysis established the limit at 3.5. On the other hand, Cuevas et al. (2009) ranged this value for olive stones biomass at above 4.0.

Moreover, as shown by the data gathered in Fig. 1, the cellulose conversion rises with an increment in severity factor, and that the hemicellulose conversion is superior, for a value of log R_0, the higher the concentration of H_2SO_4 employed.

Although some authors indicate that the reactions of depolymerization of cellulose typically start at temperatures above 260 °C (Bonn, Concin, & Bobleter, 1983), others place the temperature limit at 210 °C (Martínez et al., 1997). In any case, the high conversion rate of cellulose obtained in some of the experiments may reveal the existence of cellulose in relatively disordered (amorphous) zones that are more accessible and susceptible to any change in their structure.

With the pretreatment performed fractional conversions of cellulose were achieved for severities in the range of 3.2–3.7, which vary between 30 and 42% for treatment with LHW, 40–51% when using acid at 0.025 M, and 42–57% when the acid concentration is 0.05 M. For corncobs autohydrolysis at 179 °C and 23 min, the content of cellulose in the hydrolysis residue, as percentage of original cellulose in raw material, was appreciable, just a small part (20%) was depolymerized to oligomers and glucose (Nabarlatz, Ebringerová, & Montané, 2007). Cellulose conversions of 30% were obtained using olive tree pruning as raw material, heating the system to 200 ± 1 °C and immediately afterwards cooled to room temperature (García et al., 2010).

Table 4
Maximum total sugars and main monosaccharides yields, %, for the thermal treatments assayed.

$[H_2SO_4]$ (M)	log R_0	Y_{tot}^{max}	log R_0	Y_{glu}^{max}	log R_0	Y_{xyl}^{max}	log R_0	Y_{ara}^{max}
0.000	4.08	7.46	3.82	3.01	4.19	1.35	3.65	1.10
0.025	3.73	14.97	3.65	6.35	3.91	5.33	3.47	2.36
0.050	3.37	26.62	3.34	10.60	3.44	10.88	3.19	3.00

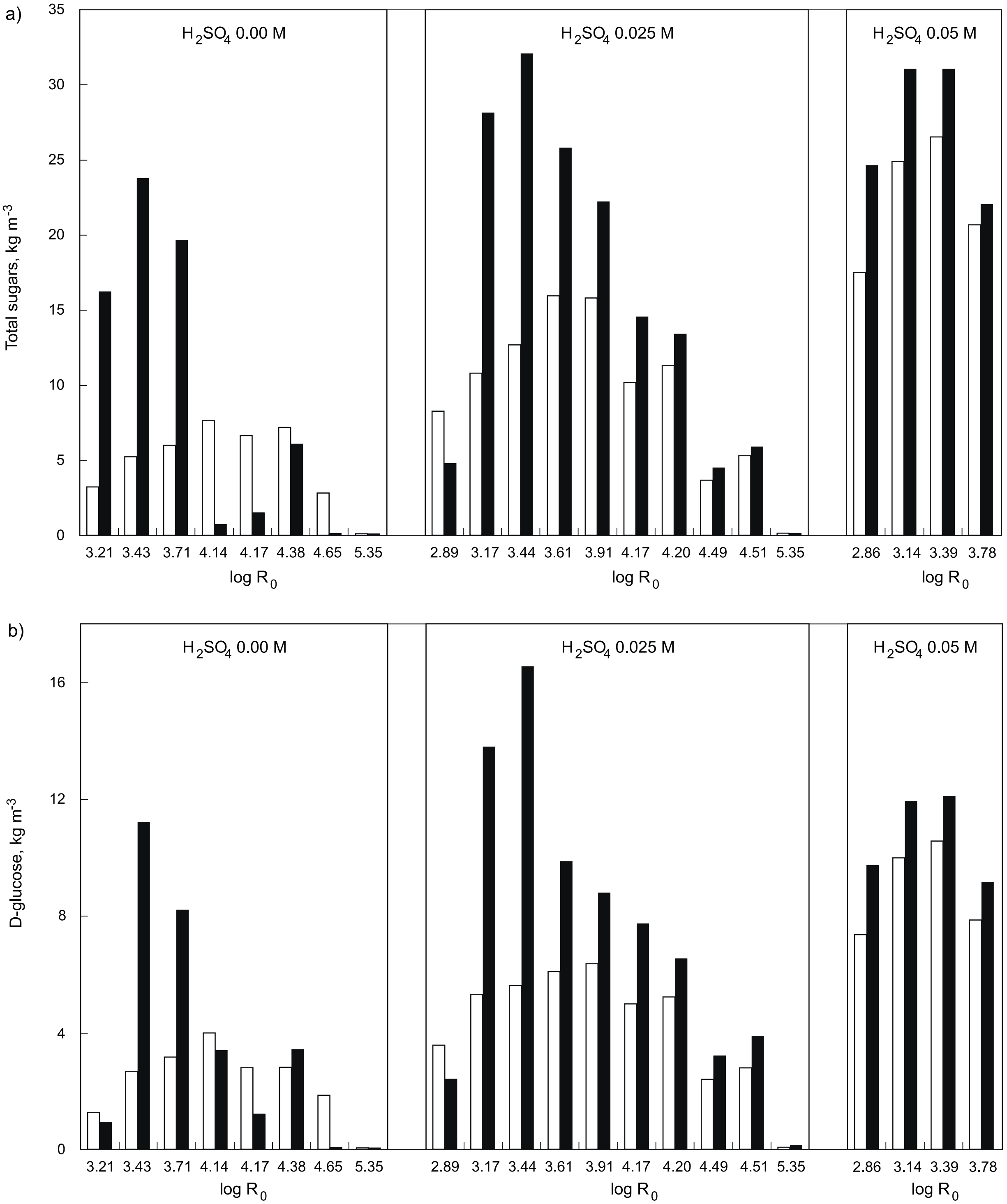

Fig. 4. (a) Total sugars and (b) D-glucose before (hollow columns) and after (filled columns) the oligosaccharides determination vs. severity factor.

The experimental values obtained for the fractional conversion of cellulose have been adjusted through multiple regression to an equation based on the severity factor and the concentration of H_2SO_4 employed in the pretreatment, obtaining Eq. (11), $r^2 = 0.991$.

$$X_C = -34.22 + 20.85(\log R_0) + 246.82\,[H_2SO_4] \qquad (11)$$

Eq. (11) allows to calculate the value of X_C for any couple of $\log R_0$ and concentration of H_2SO_4 values. Thus, theoretically we would get a total conversion of the cellulose with a severity factor of 6.44 if no sulfuric acid is used, 6.14 if the acid concentration is 0.025 M or 5.85 if H_2SO_4 0.05 M is employed.

3.3. Sugars yields

Yields values of total sugars, D-glucose, D-xylose and L-arabinose were obtained from the monomer concentrations. These values are shown in Table 3 together with the corresponding standard deviation values. After hydrothermal treatment, the arabinose loss found in the hydrolyzates increased with the severity factor (experiments 1–6) (Kabel et al., 2002).

When pretreatments are carried out using LHW, sugar yields are lower than using acid, which coincides with the findings of Wyman, Dale, Elander, Ladish, and Lee (2005). Thus the highest sugar yields are obtained in experiments with a higher concentration of acid, reaching the maximum at 200 °C and 0 min ($\log R_0 = 3.39$).

Moreover, the fact that for severity factors close to 3.4 in acid treatments, where it has been shown that hemicellulose is totally degraded (experiments 11 and 21), yields of D-glucose and D-xylose were markedly increased with acid concentration, which may suggest the presence of oligosaccharides in the liquid, susceptible to depolymerization due to the action of the acid employed.

Fig. 2 shows the total sugar yield and the main monomers vs. the severity factor values for the experiments performed with different sulfuric acid concentrations. As can be seen, for a fixed severity factor value the yields are greater the higher the concentration of acid used. The adjustments made for each concentration of H_2SO_4 correspond to second or third grade polynomials. These equations have made possible to determine the maximum yield as well as the corresponding severity value at this conditions, Table 4.

In general, as shown in Table 4, the corresponding maximum values of the several yields increase when using a higher concentration of sulfuric acid in the hydrolytic process, also entailing a decrease in the severity of the treatment.

From this point of view, and in order to obtain higher yields, we would have to work with the highest concentration of acid and severity factors in the range from 3.19 to 3.44. However, these conditions could result in further degradation of the monomers obtained and a higher concentration of compounds which would be toxic-inhibitory to the microorganisms used in the subsequent fermentation process (Mussatto & Roberto, 2004; Söderström, Pilcher, Galbe, & Zacchi, 2003).

In order to test this, we determined the concentrations of acetic acid and hydroxymethylfurfural (HMF) at the end of each of the experiments, which allowed us to calculate the corresponding yields of these products, Y_{AcH} and Y_{HMF}, defined using Eqs. (12) and (13).

$$Y_{\mathrm{AcH}} = \frac{\text{kg acetic acid}}{\text{kg initial dry biomass}} \times 100 \qquad (12)$$

$$Y_{\mathrm{HMF}} = \frac{\text{kg HMF}}{\text{kg initial dry biomass}} \times 100 \qquad (13)$$

The representations of Y_{AcH} and Y_{HMF} are shown in Fig. 3, in which the range of severity factors corresponding to the maximum yields of monomers are shaded.

It can be seen that the yields, both in acetic acid and HMF, increase significantly when using a higher concentration of sulfuric acid. In an experiment performed with a $\log R_0 = 3.4$, Y_{AcH} values rise from 0.11 to 0.73 and 1.80% when H_2SO_4 is used at concentrations of 0.00, 0.025 and 0.050 M, respectively, which is a great rise (7 and 16 times higher) when sulfuric acid is used.

These yields, for this severity factor ($\log R_0 = 3.4$), are equivalent to acetic acid concentrations lower than those reported by Palmqvist, Grage, Meinander, and Hahn-Hägerdal (1999), indicating that not only are fermentation inhibitors, but that for concentrations below 9 g/dm^3, acetic acid could stimulate ethanol production if there is no presence of furfural.

As for the performance of HMF, for an experiment conducted with $\log R_0 = 3.40$, Y_{HMF} values go from 0.12 to 0.24 and 0.43% when using H_2SO_4 at concentrations of 0.00, 0.025 and 0.050 M, respectively, which again means important increases (from 2 to 4 times higher) when using sulfuric acid.

3.4. Oligosaccharides production

As it can be seen in Table 3, for similar severity factors (close to 3.4 for which the hemicellulose is fully degraded), Y_{glu} and Y_{xyl} values appear to increase remarkably with acid concentration. It suggests the existence of oligomers, which are susceptible to be depolymerized.

Thus, the analytical determination of oligosaccharides was performed for each of the hydrolyzates obtained, allowing to quantify the total concentrations of sugars and D-glucose before and after testing, Fig. 4. The presence of oligosaccharides to a greater or lesser extent can be obtained by comparison between these values. From the data collected in these figures, in the hydrothermal treatments with liquid hot water, most of the sugars are found as oligomers.

In general it can be seen that for experiments in which the hemicellulose is already fully hydrolyzed ($\log R_0 = 3.9$ with hot water and 3.4 with acid), the amount of oligosaccharides is minimal for the higher concentration of acid and maximum for the experiments with 0.025 M sulfuric acid.

In the assays performed at 200 °C and 0 min with liquid hot water (experiment 2) and 0.025 M acid concentration (experiment 11), it might be interesting to carry out a subsequent acid hydrolysis to convert these oligomers into simple sugars and a joint processing of the liquid fraction with the residual solid. This would avoid a preliminary separation of the two fractions and to have the possibility of obtaining more fermentable sugars from pretreated solid.

4. Conclusions

From the results, it is evident that, in the range of variables studied, the hemicellulose fraction is completely hydrolyzed to severity factors near 3.9 or 3.4 when using only water or 0.025 M sulfuric acid, respectively. Moreover, the conversion of cellulose increases with the severity factor, noting that the values of X_C are greater, for a given $\log R_0$, at higher concentrations of H_2SO_4.

With regard to the possibility of using the pretreatment liquid fraction obtained as fermentation liquor, for ethanol and xylitol production, the maximum concentration of monosaccharides with an acceptable severity factor (3.8), low yields in acetic acid (2.2 kg kg^{-1}) and HMF (0.7 kg kg^{-1}) corresponds to treatments performed at 190–200 °C, 0 min and H_2SO_4 concentration 0.050 M. However, noting the maximum concentration of oligosaccharides as well as monosaccharides generated with the lowest possible severity, the most suitable pretreatments for subsequent acid hydrolysis correspond to experiments carried out at 200 °C and 0 min, either with H_2SO_4 at 0.025 M or with ultrapure water.

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