A study on the chemical composition, properties and extraction kinetics of Holm oak (*Quercus ilex*) hemicelluloses using subcritical water in a tailor made cascade reactor

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Abstract

Holm oak (*Quercus ilex*) was submitted to a hydrothermal treatment using a cascade reactors and liquid fraction rich in hemicelluloses was obtained. The chemical composition as well as the molar mass were analysed during the experiments. The effect of temperature (130 – 170 °C) and reaction time on the conversion and molar mass of the obtained hemicelluloses was investigated. The results were compared to previously published data obtained using Norway spruce. The results show that the extraction rate depends strongly on the wood species used. The maximum yield (approximately 60%) was obtained at 170 ºC after 20 min. The molar mass of the hemicelluloses decreased during the extraction due to hydrolysis and the pH of the solution decreased as deacetylation occurred simultaneously. Temperature influenced significantly the hydrolysis rate of the macromolecules. Compared to Norway spruce (softwood), the average molar mass in Holm oak (hardwood) was lower under the same reaction conditions.

Keywords: Holm oak; Hemicelluloses; Molar mass; Extraction

1. Introduction

Dehesas, mainly used of silvopastoral purposes of pigs in south Europe have holm oak (Quercus ilex) as the main scattered tree. For instance, in south-western Spain, dehesa covers around 3 million ha, as indicated by Plieninger et al [1]. Regarding the hemicellulose composition holm oak and its possible use for hydrothermal treatments the information is almost null.

Lignocellulosic biomass is a versatile and plentiful raw material and hemicelluloses are one of its main compounds. The amount of hemicelluloses (dry weight) in sapwood is usually between 20 and 30% but the composition depends on the species. Besides the chemical composition, also the morphology of the wood matrix depends on the species and these factors influence significantly the extraction kinetics as well as the obtained carbohydrates and their potential for further valorization. Hemicelluloses are polysaccharides composed mainly of xylose, glucose, arabinose, galactose and mannose. Hardwood contains mainly xylans while softwood mostly consists of galactoglucomannans [2]. The dehydration of hexoses produces 5-hydroxymethylfurfural and pentoses, furfural.

During a hydrothermal process, acetyl groups are released from the hemicelluloses [3], which can catalyse the hydrolysis of hemicelluloses to shorter oligomers and, consequently, decrease the molar mass [4]. The knowledge of the chemical composition and molar mass of the extracted hemicellulose fractions is crucial for further applications. When targetting long-chained hemicelluloses, the pH is a key factor during the extraction. Krogell et al. reported in 2015 that when adjusting the pH to 4.8, the molar mass of hemicelluloses extracted from Norway spruce at 170 ºC was higher than the molar mass obtained without pH control [5]. Similarly, Tunc and Heiningen observed in 2011 that an increase in temperature decreased the average molar mass obtained in the extraction [6]. The structure of hemicelluloses is mainly amorphous and the molar mass is lower compared to cellulose, consequently it is easier to hydrolyse than cellulose. The hydrolysis of cellulose takes place at temperatures higher than 230 ºC, therefore, at lower temperatures mainly hemicelluloses are extracted [7,8]. Sattler et al. reported that the extraction of hemicelluloses from wood flakes begins at 120 ºC [5], and correspondingly Leppänen et al. observed that low amounts of hemicelluloses could be extracted already at 120 ºC-160 ºC [1], which indicates the practical lower temperature limit

for the extraction. However, Rissanen et al. showed that the extraction proceeds with the same mechanism and follows the same kinetic model as at higher temperatures even at 90 °C, albeit the extraction rate is naturally significantly slower [9].

Kilpeläinen et al. reported that 70% of xylan can be recovered from ground birch wood at 190 ºC in 30 min using a flow-through vessel, but the degree of polymerization of the xylans decreased significantly [10]. Both temperature and reaction time influence the process [11] and principally, a similar amount of the biopolymer can be extracted or hydrolysed at a higher temperature with less extraction time and vice versa. Hardwood species have more acetyl groups than softwood species, consequently more acetic acid is formed during the hydrothermal process increasing the reaction kinetics and promoting the formation of degradation product [4, 12].

In this study, the extraction of hemicelluloses from Holm oak (*Quercus ilex*) was investigated using subcritical water. Holm oak has not been widely studied previously and the composition of Holm oak hemicelluloses has not been previously reported to the knowledge of the authors. The focus was on the effect of temperature and reaction time on the yield of carbohydrates and the molar mass of the product. The degradation of the sugars monomers was not desired. The effect of the raw material on the extraction was evaluated by comparing the results with data obtained with softwood. The results can be used to optimize the reaction conditions to obtain a high yield of hemicelluloses with low degradation or to target a specific value of molar mass.

2. Materials and Methods

2.1 Materials

The Holm oak (*Quercus ilex*) sapwood was milled and sieved to a particle size between 1.25 and 2 mm. This size fraction was selected to minimize the influence of internal mass transfer on the kinetics during the extraction. The chemical composition of the hemicelluloses in the raw material was determined and the following values were obtained: 0.186 mg/mg xylose, 0.007 mg/mg rhamnose, 0.011 mg/mg mannose, 0.002 mg/mg glucuronic acid, 0.028 mg/mg glucose, 0.018

mg/mg galacturonic acid, 0.019 mg/mg galactose, 0.014 mg/mg arabinose and 0.018 mg/mg 4-O-methylglucuronic acid (Figure 1).

2.2 Experimental

The experiments were carried out in a cascade reactor comprising five reactors connected in series [13]. The volume of each reactor was 200 ml. A metallic filter was used at the top of the reactor to prevent the loss of the solid raw material with the flow. The recirculation flowrate was set to 150 $L \cdot h^{-1}$ and the pressure was 2.9 bar higher than the boiling point of water at the reaction temperature. The reactors were equipped with heating jackets as well as with PID controllers. The temperature was measured continuously inside and outside of the reactor for control purposes. The pressure of the system was measured before the first reactor and after the last reactor. The experimental temperatures were between 130 and 170 ºC. Rissanen et al. observed that the yield was only 10% at 120 ºC using the same pilot plant, consequently the temperatures selected were higher than 120 ºC [14]. Each reactor was charged with 5 g of drywood (25 gr in total) and filled with distilled water and kept overnight to pre-wetted the raw material. The rest of the system was filled (by-pass mode) and the amount of water inside in the system was measured. The liquid/solid ratio was approximately 160 in the reactor to avoid thermodynamic limitations during the extraction. The liquid inside in the by-pass part of the system was rapidly heated to the desired temperature. After that, the by-pass section was opened allowing the circulation of the hot water through the reactors. At that precise moment time was set to zero (i.e. reaction started). From a macroscopic point of view the reaction system behaved like a perfectly agitated batch reactor, as the flowrate was high and it was operated in recirculation mode. Figure 2 shows a simplified scheme of the experimental device.

When a predetermined sampling time was reached (see Table 1), one of the reactors was again by passed and cooled down rapidly. The cooling was performed by quenching the reactor with cold water. Five liquid and solid samples were obtained from a every single experiment.

2.3 Analysis methods

2.3.1 Analysis of pH

The pH was measured with a Phenomenal pH meter using a refillable glass electrode model 221 with a built-in PT 1000 temperature sensor.

2.3.2 Hemicelluloses content

The total solid content of carbohydrates was first determined by weighting the mass of a sample before and after oven drying. After that, a certain amount of liquid or solid sample, which contained about 0.1 mg of carbohydrates was freeze-dried under vacuum. The calibration samples were prepared using a carbohydrate calibration solution. 2 mL of 2M HCl/MeOH anhydrous was added to the samples and the samples were heated to 100 ºC for 3 h. The excess acid was neutralized with 170 µL of pyridine. After that, 1 mL of the internal standards sorbitol (0.1 mg/mL in MeOH) and resorcinol (0.1 mg/mL in MeOH) was added. Then, the solution was evaporated under nitrogen gas at 50 ºC and silylated using 150 µL of pyridine and HMDS and 70µL of TMCS. The derivatised samples were analysed by a gas chromatographic method with flame ionization detection.

About 1 µL of the silylated sample was injected through a split injector (250 ºC, split ratio 1:25) into the column coated with dimethyl polysiloxane (HP-1, Hewlett Packard). The column length, internal diameter and film thickness were 25 m, 200 µm, and 0.11 µm, respectively. The following temperature programme was applied: 100 ºC during 1 min, 100 ºC to 170 ºC at 4 ºC/min; 170 ºC to 300 ºC at 12 ºC/min and 300 ºC during 7 min. Hydrogen was used as a carrier gas with a flow rate of 45 ml/min. The identification and quantification of sugars were accomplished through the injection of standard samples. The yield of hemicellulose products was determined by dividing the amount of extracted hemicelluloses and the initial content of hemicelluloses in the raw material.

2.3.3 Molar mass

The weight-average and number-average molar mass of hemicelluloses were determined by high-performance size-exclusion chromatography (HPSEC) equipped with multiangle laser-light scattering (MALLS) and a refractive index (RI) detectors. The columns employed were Ultrahydrogel TM Column, Linear, 10 µm, 7.8 mm X 300 mm, 500 – 10M. The eluent was 0.1M NaNO3 at a flowrate of

0.5 mL/min at 40 ºC. Data were collected and the calculations were performed with the software Astra, Wyatt Technology.

3. Results and Discussion

Holm oak was hydrothermally treated in the cascade reactor using only water as a solvent. In order to study the extraction and hydrolysis of hemicelluloses from Holm oak, the influence of temperature and extraction time on the yield and molar mass were studied. After the hydrothermal treatment, two main fractions were obtained: liquid and solid. The liquid phase was analysed to determine the enrichment in hemicelluloses.

3.1 Change in pH

The pH values of the liquid phase samples are shown in Figure 3 as a function of time, at different extraction temperatures. The change in pH was strongly influenced by the temperature, however, the trend was similar in all the experiments. The pH was initially about 5.5 corresponding to the pH of distilled water. Then, the pH decreased during the experiment from 5.5 to about 4-4.3, depending on the reaction temperature.

The decrease of pH was mainly due to the release of acetyl groups from the hemicelluloses resulting in the formation of acetic acid, which increased the hydronium ion concentration in the reaction medium. The acetic acid can catalyse the hydrolysis of the extracted hemicellulose and then, pentoses and hexoses, can be further degraded (see Figure 4). The hemicellulose is mainly composed of mannose, xylose, arabinose, galactose and glucose. The xylose and arabinose can be transformed into furfural through dehydration [15] and the glucose can be transformed into 1,6-anhydroglucose through a dehydration, into glycolaldehyde by retro-aldol condensation or into fructose by isomerization. The fructose can further be transformed into 5-hydroxymethylfurfural (5-HMF) by dehydration reactions and glyceraldehyde by retro-aldol condensation [16]. The 5-HMF can be degraded into levulinic and formic acid [17], while the furfural can be transformed into formic acid.

The pH can also be influenced by the presence of degradation products, mainly carboxylic acids [18]. At 130 ºC, the temperature was not high enough to detach the

hemicelluloses efficiently and the extraction was very slow i.e. low temperatures led to slow reaction kinetics and consequently more basic pH values (4-7), and practically no degradation products [19].

The largest difference was observed when the temperature was increased from 130 to 140 ºC suggesting that the structure of biomass was altered and the hemicelluloses were more available for the extraction process. Increasing the temperature to 160-170 ºC resulted in faster deacetylation, and the pH stabilized after about 20-30 min of extraction. The stabilizing indicated that the hemicelluloses extraction had slowed down. Degradation products were obtained from the hydrolysis of the pentoses and hexoses and 5-hydroxymethylfurfural, furfural, levulinic acid, etc was observed in the analysis [17]. To minimize the formation of degradation products, the rapid removal of the extracted products and the addition of sodium carbonate are two options [1, 20]. Shorter reaction times lead to less consecutive reactions and, the addition of sodium carbonate increased the pH hindering degradation.

The minimum pH value was observed at the same point in time as the conversion of hemicelluloses was at a maximum. This behaviour was observed at temperatures exceeding 150 ºC. The pH can be used as an indicator for following the hydrothermal process and identifying the reaction time necessary for achieving the maximum conversion [21].

3.2 Hemicelluloses extraction kinetics

The hemicelluloses extraction rate was strongly affected by temperature (Figure 5). At higher temperatures, a higher concentration of hemicelluloses was obtained. The concentration profile as a function of time at 130 ºC and 140 ºC was approximately linear, indicating that maximum conversion was not reached during the experiments. At temperatures exceeding 150 ºC two different stages were observed (Figure 5).

The first stage corresponds to the extraction and hydrolysis of hemicelluloses. The second stage (negative slope) indicates the presence of degradation products, indicated by the behaviour of pH discussed previously as well as the decrease in the carbohydrate concentration with time. The higher acidity could also indicate that a more severe deacetylation took place during extraction resulting in more severe

hydrolysis of the hemicelluloses as well as the formation of degradation products. The time necessary for achieving the maximum concentration of hemicelluloses was 80 min at 150 ºC, but only 20 min at 170 ºC. At 170 °C, after 20 min, the concentration of hemicelluloses decreased indicating that the reaction time should be shorter to avoid the formation of undesired products. The errors in the data were lower than 10% in mass, indicating good reproducibility in the experiments.

The yield of hemicelluloses depends strongly on the reaction time and temperature (Figure 6) but the maximum yield depends on the type of biomass treated (Figure 7). The time required for obtaining a 30% hemicellulose yield was 110 min at 140 ºC, while it was 50 min at 150 ºC and only 8 min at 170 ºC. This time is closely related to the behaviour of the pH: the deacetylation enhances the hydrolysis of hemicelluloses lowering the pH and increasing the acid hydrolysis rate. The autocatalytic hydrolysis is a very interesting process because the solubilisation of hemicelluloses can be performed without the addition of any solvent other than water. The choice of operational conditions plays an important role in the production of the desired products. The maximum conversion achieved depended on the reaction temperature. The ionic product of water increases with temperature [10] (until 374ºC) increasing the reaction kinetics and consequently the hydrolysis of hemicelluloses is faster.

The yield obtained at different temperatures was compared to the data of Rissanen et al. who studied the extraction of hemicelluloses from Norway spruce (softwood) using subcritical water [13]. As shown in Figure 7, the yield obtained for the softwood species was higher than for the hardwood species, and larger differences in the extraction rate of the hemicelluloses were observed at higher temperatures. Hardwood has a higher content of acetyl groups than softwood [22], but hardwood has a lower content of lignin [23]. Three reasons may explain the higher extraction rate of softwood hemicelluloses under similar experimental conditions: a) less deacetylation results in lower hydronium ion concentration in the liquid decreasing the formation of degradation products and increasing the amount of hemicelluloses in the liquid phase or/and b) the low content of lignin in the raw material increases the accessibility of hemicelluloses increasing the hydrolysis of hemicelluloses, too, or/and c) the hardwood hemicelluloses (mainly xylan) are more susceptible to degradation which is observed as lower yields.

As shown in Figure 8, the final chemical composition of the liquid phase was influenced by temperature and time. The major component extracted from hemicelluloses was xylose at 150, 160 and 170 ºC [6]. Between 130 and 140 ºC the extraction of glucose was predominant, probably because it was a free glucose, as it remained mostly constant increasing temperature further. The xylose concentration increased when the acidity was higher [3] and it exhibited a linear behaviour (r^2 between 0.998 and 0.97) when the reaction temperature was under 160 ºC, as did glucose (r^2 between 0.97 and 0.95) at less than 150 ºC indicating that the reaction time was not long enough to reach maximum yields. Xylose and glucose accounted for 49.7 and 65.0% wt. of the extracted sugars respectively.

3.3 Molar mass distribution in hemicellulose extract

Deacetylation was accompanied by a reduction in the molar mass. The molar mass exhibited similar behaviour in all the experiments: the largest hemicelluloses were extracted at the shortest reaction times and it decreased as the temperature was increased. Figure 9 depicts the evolution of the average molar mass during the autohydrolysis process at different temperatures.

A mix of lower molar mass hemicelluloses was produced during the extraction process. The highest average molar mass obtained was 12.9 kDa (~72 DP) at 170 ºC (5 min), while the lowest molar mass 1.8 kDa was obtained at 170 °C after 60 min, as also indicated by the pH value. At 160 and 170 ºC, the hemicelluloses had a significantly lower molar mass compared to the initial value already after a few minutes of extraction. The final molar mass varied from 3.83 to 1.75 kDa, depending on the temperature and reaction time.

The molar mass as a function of time was previously reported by Rissanen et al. for softwood extraction (Norway spruce) [14]. The molar mass of carbohydrates depends on the wood species. As shown in Figure 10, it is clear that the spruce molar mass was higher than for the Holm oak under the same reaction conditions, suggesting that the deacetylation is more pronounced in hardwood species due to a high content of acetyl groups. Hydrolysis most likely occurred even inside the particles, as has been demonstrated previously by Rissanen et al., although in this article a small particle size was used to minimize the effect [13]. The results indicate that if high molar mass is desired, it is better to use softwood.

4. Conclusions

A hydrothermal extraction process can be used to recover carbohydrates and lignin from lignocellulosic biomass. Holm oak is an important biomass in south-western Spain, mainly in dehesas, and the information on its possible uses for hydrothermal treatments is almost null. In this work, holm oak was fractionated using subcritical water at temperatures between 130 and 170 ºC employing a cascade reactor comprising five Parr reactors in series. The reaction times were between 60 and 220 min and the particle size was 1.25-2 mm. The conversion was significantly influenced by the reaction temperature. The final conversion of hemicelluloses varied from 21.1% at 130 ºC to 55.9 % at 170 ºC, mostly constituted by glucose and xylose. The concentration profile at lower temperatures was linear indicating that the time was not long enough to extract all the hemicelluloses. When the temperature was increased to 150 ºC, two stages were observed in the concentration profile. The first stage (positive slope) corresponded to the extraction/hydrolysis of hemicelluloses and the second stage (negative slope) suggested that significant degradation of the sugars occurred. The yield of hemicelluloses from Norway spruce (softwood) was observed to be higher than from Holm oak (hardwood), when the experimental data was compared to data previously reported in literature. This difference can be due to the lower content of acetyl groups in softwood, which results in lower hydronium ion concentration in the liquid phase and the lower content of lignin in hardwood, which increases the `accessibility´ to hemicelluloses and consequently increases the formation of degradation products.

The pH was strongly influenced by temperature, reaching levels of about 4-4.3. The largest difference was observed between 130 ºC and 140 ºC, suggesting that the structure of hardwood was altered, which lead to a better `accessibility´ to hemicelluloses. At higher temperatures, a faster deacetylation was observed together with a faster decreased in the molar mass. The yield and molar mass obtained after the extraction process was affected by the temperature and the reaction time as well as the structure and composition of the raw material. Holm oak (hardwood) can be a good option for obtaining hemicelluloses of low molar mass, the highest average molar mass achieved being 12.9 kDa and the lowest 1.75 kDa. If high molar mass is targeted it would recommendable to use a softwood. The results showed that a diversified mix of lower molar mass hemicelluloses can be

obtained in high yield from Holm oak and that modifying the experimental conditions can be used to influence the molar mass.

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Figure Captions

Figure 1. Hemicelluloses composition in the raw material

Figure 2. Schematic flow diagram of the experimental system. Equipment: V-01 Collector vessel, P-01 Pump, R-01/R-05 Reactors.

Figure 3. The pH behaviour in function of time at different temperatures

Figure 4. Reaction pathway for the hydrolysis of hemicellulose and formation of degradation products

Figure 5. Concentration of hydrolysed hemicelluloses as a function of time at different temperatures

Figure 6. Yield of hemicelluloses as a function of time at different temperatures

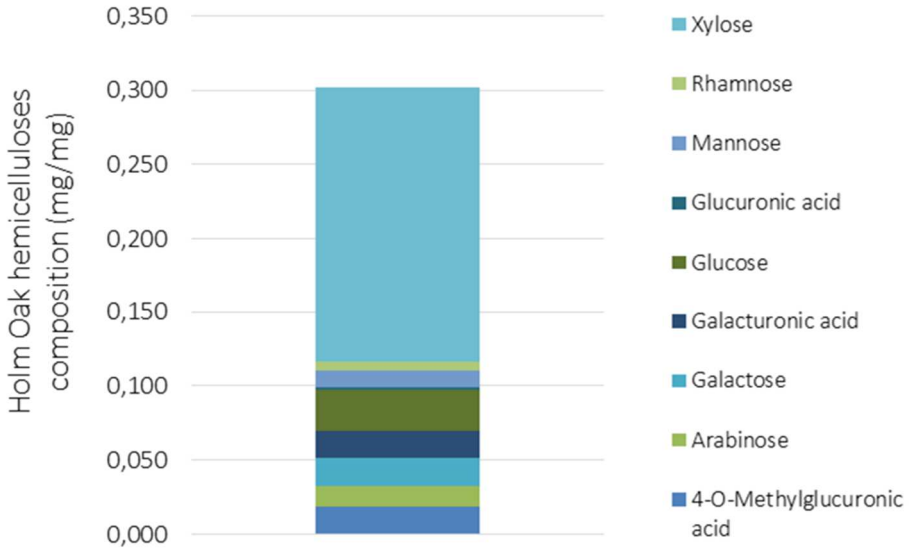
Figure 7. Yield of hemicelluloses as a function of the time at different temperatures for Holm oak and Norway spruce

Figure 8. Accumulate concentration of hemicelluloses and composition in hydrothermal process

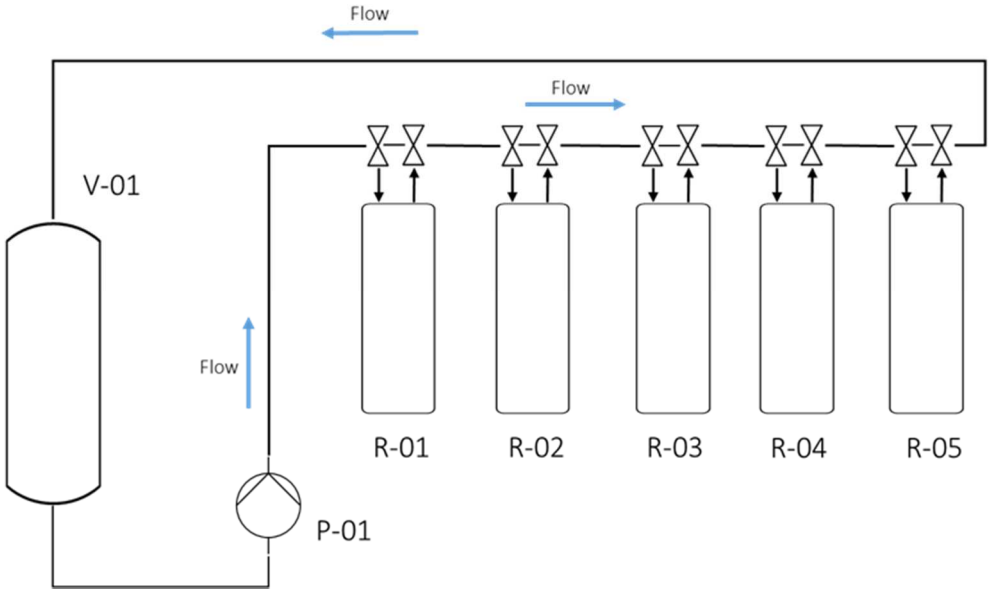
Figure 9. Change in the average molar mass during autohydrolysis at different temperatures

Figure 10. Molar mass along time for softwood and hardwood species

Figure 1.


Holm Oak hemicelluloses composition (mg/mg)
0,350
0,300
0,250
0,200
0,150
0,100
0,050
0,000
Xylose
Rhamnose
Mannose
Glucuronic acid
Glucose
Galacturonic acid
Galactose
Arabinose
4-O-Methylglucuronic acid

420 **Figure 2.**



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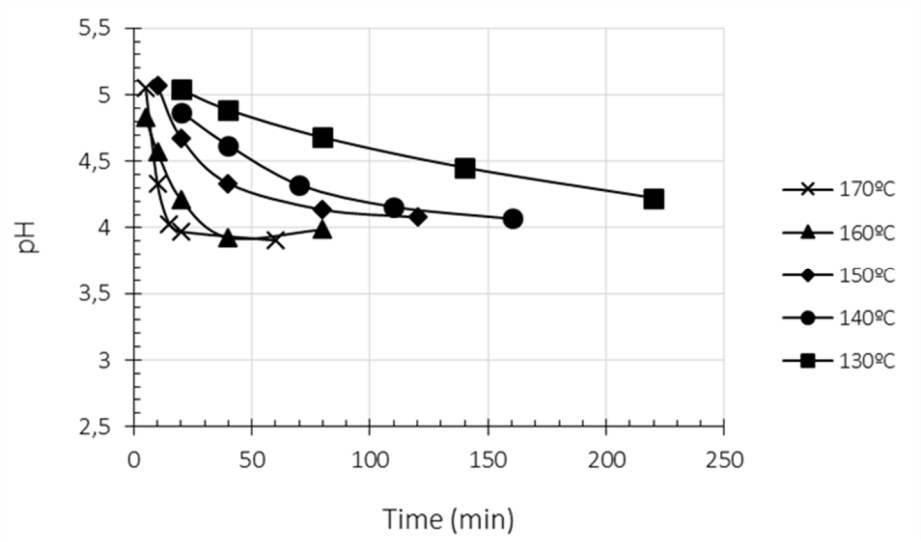
423 **Figure 3.**



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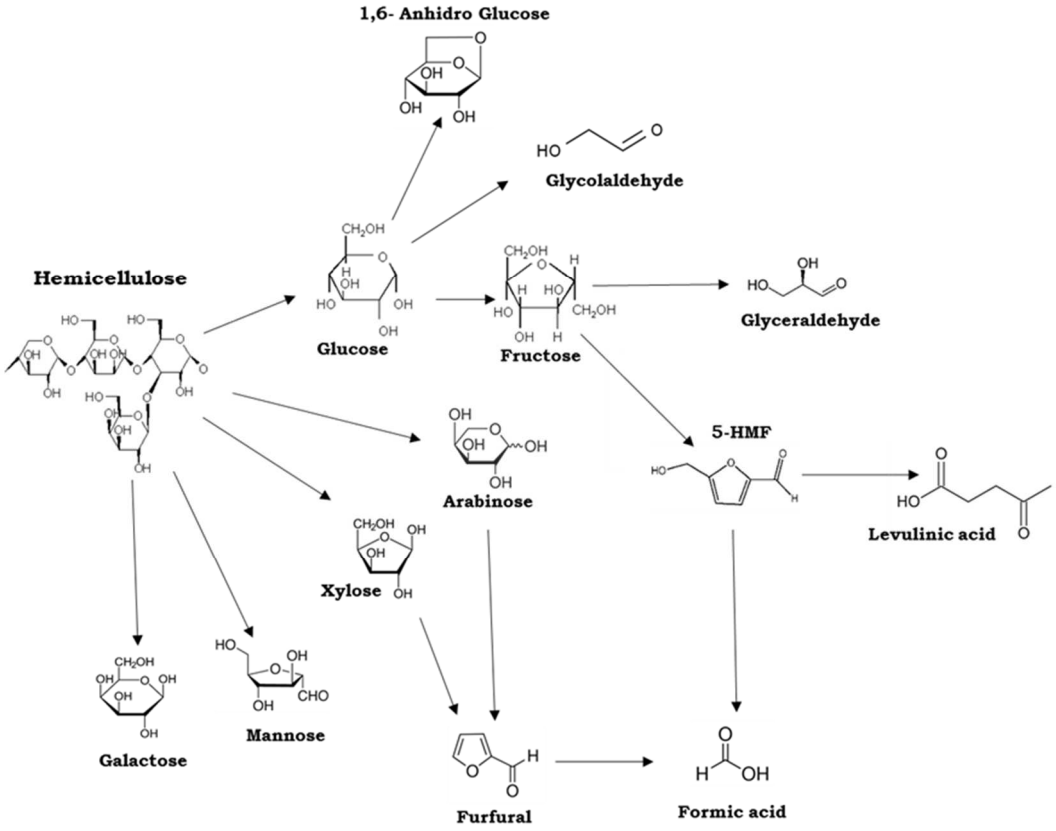
426 **Figure 4.**



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429 **Figure 5.**

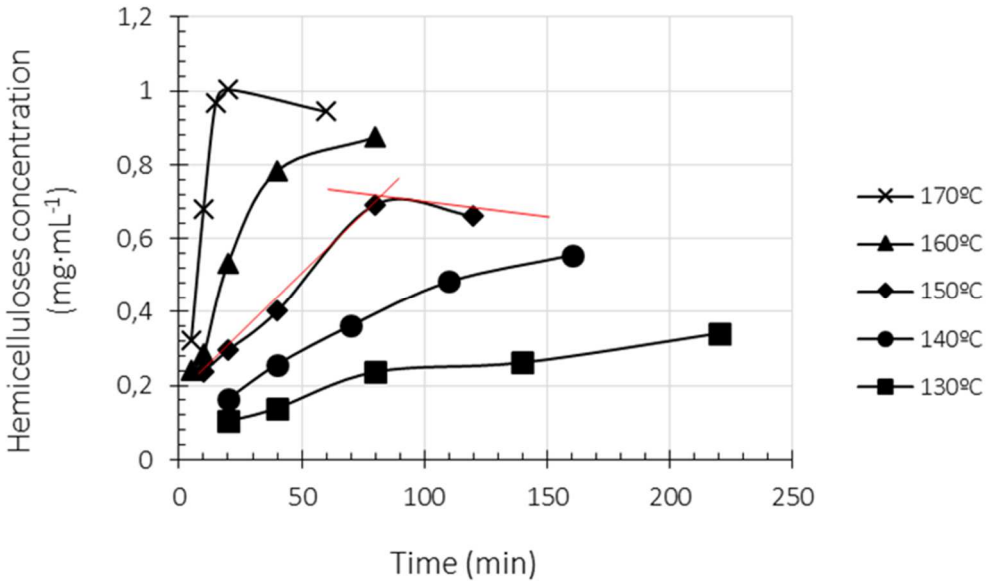

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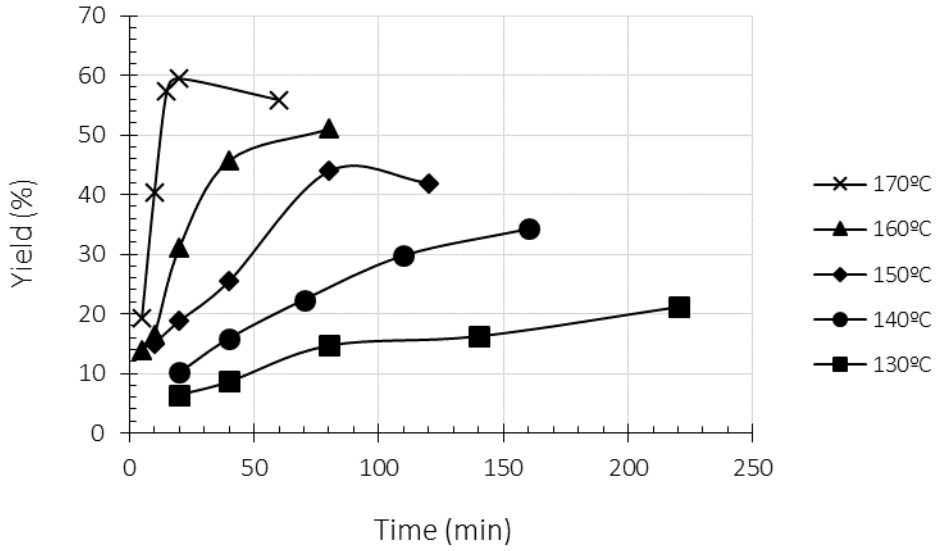
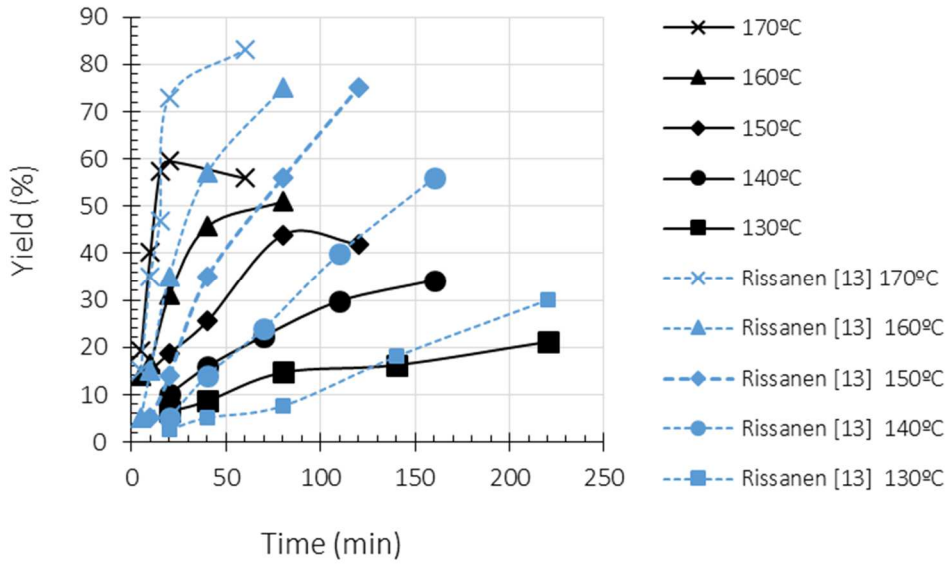
Figure 6.


70
60
50
40
30
20
10
0
Yield (%)
0
50
100
150
200
250
Time (min)
170ºC
160ºC
150ºC
140ºC
130ºC

435 **Figure 7.**



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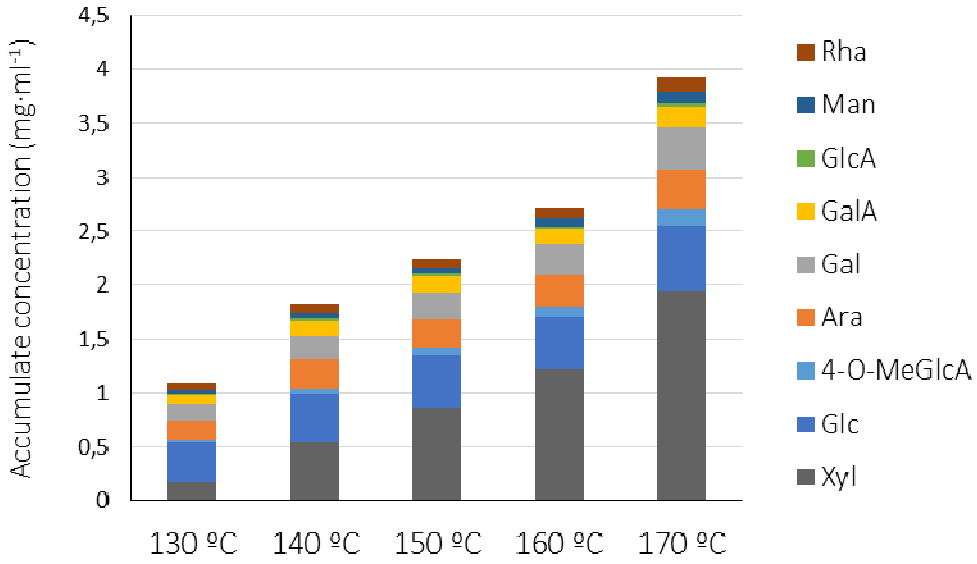
438 **Figure 8.**



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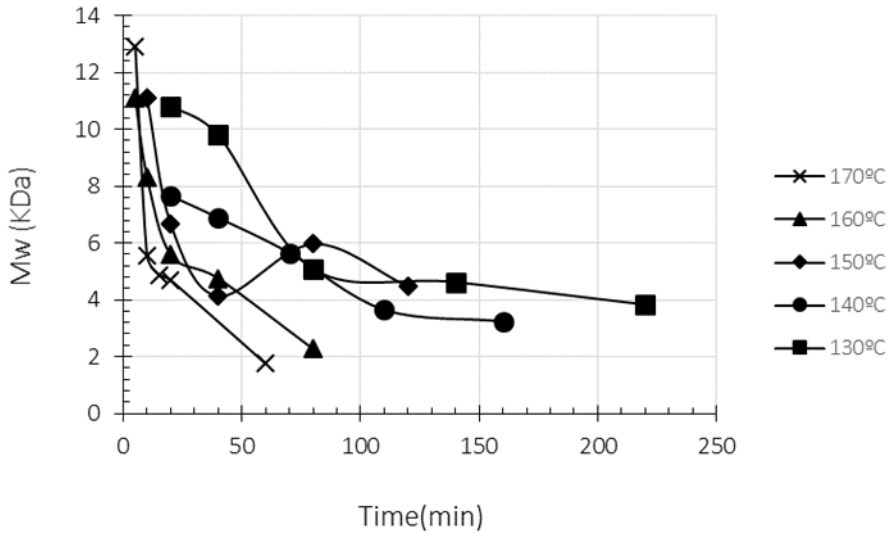
441 **Figure 9.**



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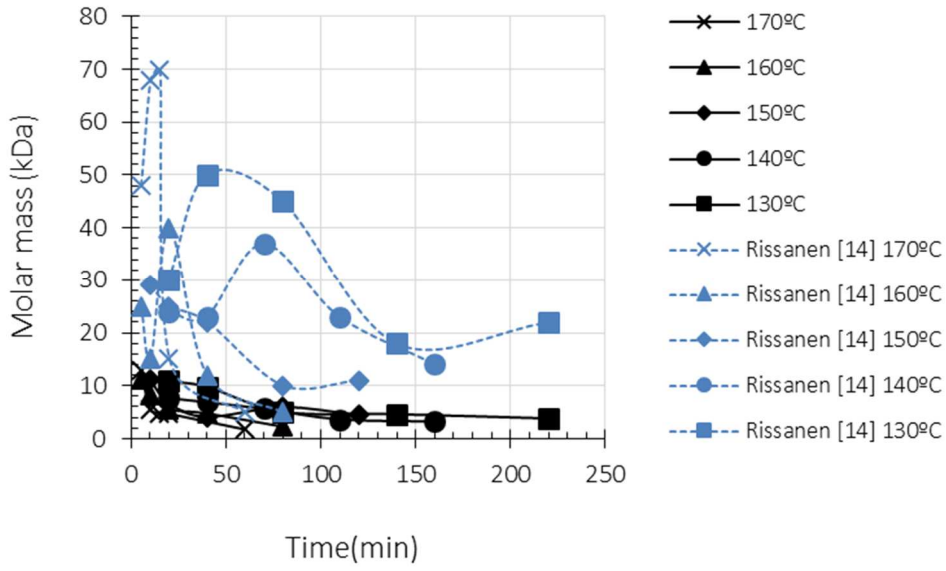
444 **Figure 10.**



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Tables

Table 1. Sampling time for each experimental temperature

450 **Table 1.**

Reactor (Nº)	130 ºC Sampling time (min)	140 ºC Sampling time (min)	150 ºC Sampling time (min)	160 ºC Sampling time (min)	170 ºC Sampling time (min)
1	20	20	10	5	5
2	40	40	20	10	10
3	80	70	40	20	15
4	140	110	80	40	20
5	220	160	120	80	60

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