Universidad de Valladolid

ESCUELA DE INGENIERÍAS INDUSTRIALES

DEPARTAMENTO DE INGENIERÍA QUÍMICA Y

TECNOLOGÍA DEL MEDIO AMBIENTE

Fractionation process of surplus biomass by autohydrolysis in subcritical water obtaining added value products

Presentada por Florencia Micaela Yedro para optar al grado de Doctor por la Universidad de Valladolid

Dirigida por:

Dr. Juan García Serna

Prof. Dra. M. José Cocero Alonso

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Proceso de fraccionamiento de biomasa excedentaria por autohidrólisis en agua subcrítica obteniendo productos de valor añadido

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Memoria para optar al grado de Doctor,

con Mención de Doctorado Internacional

presentada por la Ingeniera Química

Florencia Micaela Yedro

Siendo los tutores en la Universidad de Valladolid

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Valladolid, Mayo de 2015

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Secretaría

La presente tesis doctoral queda registrada en el folio número _____ del correspondiente libro de registro número _____.

Valladolid, a ___ de _____ de 2015

Fdo. El encargado de registro

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Certifican:

Que la Ingeniera Química FLORENCIA MICAELA YEDRO ha realizado en el Departamento de Ingeniería Química y Tecnología del Medio Ambiente de la Universidad de Valladolid, bajo nuestra dirección, el trabajo que, para optar al grado de Doctorado Internacional, presenta con el título "*Fractionation process of surplus biomass by autohydrolysis in subcritical water obtaining added value products*", cuyo título en castellano es "*Proceso de fraccionamiento de biomasa excedentaria por autohidrólisis en agua subcrítica obteniendo productos de valor añadido*", siendo el Ak. Tapio Salmi su tutor durante la estancia realizada en Åbo Akademi University (Finlandia).

Valladolid, a _____ de _____ de 2015

Fdo. Juan García Serna

Fdo. María José Cocero Alonso

Reunido el tribunal que ha de juzgar la tesis doctoral titulada “Fractionation process of surplus biomass by autohydrolysis in subcritical water obtaining added value products” presentada por la ingeniera Florencia Micaela Yedro y en cumplimiento con lo establecido en el Real Decreto 1393/2007 de 29 Octubre ha acordado conceder por _____ la calificación de _____.

Valladolid, a _____ de _____ de 2015

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1er VOCAL 2º VOCAL 3er VOCAL

A mis padres

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Abstract

Fractionation process of surplus biomass by autohydrolysis in subcritical water obtaining added value products

In the last years, the challenge is a society based on the concept of bioeconomy. This term refers to the sustainable production and conversion of biomass into a range of food, health, fibre, industrial products and energy developed in futures biorefineries. In order to reach this challenge, new policies have been promoted to use the renewable resources and new policies will be necessary to develop a decentralized local-scale production to produce bio-products according to the biomass availability in each area. The decentralized local-scale production results in a major flexibility and versatility in the process due to the use of different biomass generating diversification of products. However, it is required a better knowledge of chemical and structural properties of biomass which would be obtained from research works in science and technology of biomass.

The hydrothermal process has been proposed to develop sustainable and efficient conversion of natural resources into fuels and chemicals. This technology is a promising alternative to perform the fractionation of biomass because the reaction medium allows the transformation of the different fractions of biomass by choosing the appropriate conditions. Furthermore, water is a clean, safe and environmentally benign solvent. The use of semicontinuous reactors has been proposed to study the behaviour of hydrothermal processes being the results obtained “acceptable” compared with the investment and equipment required.

The aim of this PhD thesis is to develop a process capable of obtaining added value products from lignocellulosic biomass using the fractionation hydrolysis process and using subcritical water as solvent. A semicontinuous reactor was designed and constructed. The maximum working temperature is 400ºC and pressure is 25 MPa.

In Chapter 1, the hydrothermal hydrolysis of grape seeds focused in the production of bio-oil was studied. The grape seeds composition in terms of lignin, sugars, ash, extractives and bio-oil was determined. The composition of grape seeds was: 17.0% wt. of extractives; 36.8% wt. of sugars (hemicellulose and cellulose); 43.8% wt. of lignin and 2.4% wt. of ash. The grape seeds were hydrothermally treated using three different temperatures: 250ºC, 300ºC and 340ºC employing a semi-continuous reactor. The solid residue varied from 25.6 - 35.8% wt. depending on the hydrolysis temperature. The maximum yields of Light (15.7% wt.) and Heavy Bio-oil (16.2% wt.) were achieved at

340ºC. The Arrhenius parameters for the kinetic of grape seeds hydrolysis in our system were k_0 = 0.995 g·min^{-1} and E_a=13.8 kJ·mol^{-1}. The increment of the flow rate favoured the mass transfer in the system and so, the hydrolysis rate. However, the maximum hydrolysis rate was found at a water surface velocity of 2.3 cm·min^{-1}.

In Chapter 2, the fractionation of grape seeds as a model biomass was studied using a combination of two processes: solvothermal extraction and hydrothermal fractionation-hydrolysis process in a semicontinuous reactor. First, grape seeds were subjected to an extraction process with ethanol/water (70/30% wt.) at 90ºC during 60 min obtaining ca. 13.0% wt. of oil and extractable components with 4.46% wt. of polyphenols (66% of the maximum). Afterwards, the solvent was water and the biomass was treated in steps at different temperatures (150ºC to 340ºC). During the hydrolysis the pH decreased from 5.5 down to 3.0 due to acetyl group liberation. The total quantity of recovered sugars varied around 20.0 to 23.1% wt. The best experimental condition for obtaining the maximum amount of pentoses + hexoses + oligosaccharides was 180ºC (45min) + 250 to 265ºC (45 min) + 330 to 340ºC (45 min).

In Chapter 3, the hydrolysis of Holm oak (*Quercus ilex*) using subcritical water conditions was investigated. The experiments were carried out in a semicontinuous reactor using different temperatures (from 175 to 207ºC), flow rates (from 3 to 34 ml·min^{-1}) and particle sizes (3 and 6 mm). The behaviour of pH, Total Organic Carbon and sugars by HPLC were measured and studied. The current results provided an interesting relation between these parameters. The minimum pH was located at the same time as the Total Organic Carbon and sugars by HPLC presented a maximum. The pH can be used to follow on-line the hydrolysis process reducing the analytical and time expenditures to the minimum possible and at the same time understanding the behaviour of the system.

In Chapter 4, the hydrothermal treatment of Holm oak (*Quercus ilex*) separating a liquid fraction rich in hemicelluloses was studied. The experiments were carried out using a five reactors connected in series to form a cascade reactor. The temperatures were between 130 and 170ºC. The effects of temperature and reaction time on the conversion and molar mass of hemicelluloses were investigated. The results show that the maximum hydrolysis rate of hemicelluloses depends strongly on the temperature and the biomass used. The maximum conversion (approximately 60%) was obtained at 170ºC during 20

min. After this time, the decreases of conversion can be attributed to the presence of degradation products. On the contrary, the conversion at 130ºC and 140ºC did not exhibit a maximum value indicating that the reaction time was not long enough for complete the hydrolysis. The major component extracted at lower temperatures was glucose (130 and 140ºC) and at higher temperatures (150, 160 and 170ºC) was xylose. The deacetylation was accompanied by a reduction in the molar mass. The average molar mass of the carbohydrates from hydrolysis of Holm oak decreased with increasing reaction temperature. The average molar mass decreased from 12.9 to 1.75 kDa at 170 ºC during 60 min of hydrolysis. At higher temperatures the hemicelluloses had a pronounced lower average molar mass after a few minutes of reaction. Compared to Norway spruce (softwood), the average molar mass in Holm oak (hardwood) was lower under the same reaction conditions suggesting that the deacetylation is higher due to a higher content of acetyl groups.

In Chapter 5, subcritical water was employed to fractionate woody biomass into carbohydrates and lignin. Nine urban trees species (hardwood and softwood) from Spain were studied. The experiments were carried out in a semi-continuous reactor at 250ºC for 64 min. The hemicellulose and cellulose recovery yields were between 30% wt. and 80% wt. while the lignin content in the solid product ranged between 32% wt. and 92% wt. It was observed that an increment of solubilized lignin disfavored the hydrolysis of hemicelluloses. It was determined that the maximum extraction of hemicellulose was achieved at 20 min of solid reaction time while the extraction of celluloses not exhibited a maximum value. The hydrolysis of hemicellulose and cellulose would be governed by the hydrolysis kinetic and the polymers accessibility. In addition, the extraction of hemicellulose was negatively affected by the lignin content in the raw material while cellulose hydrolysis was not affected by this parameter.

Aims and contents

Fractionation process of surplus biomass by autohydrolysis in subcritical water obtaining added value products

In the last years, the situation and future reports indicate that the challenge is a society based on the concept of Bioeconomy. Bioeconomy refers to the sustainable production and conversion of biomass into a range of food, health, fibre, industrial products and energy [1]. In the same way, bioeconomy refers to the set of economic activities related to the invention, development, production and use of bio products and processes [2]. Its emergence and postulates are related with the safety food and the climate change. In the integrated bioeconomy the agriculture plays an important role providing food security and biomass as a renewable raw material for the production of energy, fuel and added value products. The industry will play a crucial role in the development towards a bioeconomy. Several climatic problems related mainly with the emissions of CO_2 due to the use of fossil resources can be reduced using a sustainable bioeconomy. The bioeconomy will provide bio-based products and biofuels using biomass as raw material. The biomass will be the resource that drives the bioeconomy. The huge amount of available biomass represents an excellent potential feedstock. From an economically viewpoint, the investigations suggest that the producing cost from renewable carbon sources may be competitive to the non-renewable carbon source [3]. From a sustainability viewpoint, the biomass is one of the most abundant renewable resources of carbon in nature. The global primary production of biomass rounds $1X10^{17}$gC/y [4]. In the same way, the biomass has a complex structure and the separation into its components followed by conversion through a variety of technologies can lead to a variety of products. In 2004 the U.S. Department of Energy and Renewable Energy prepared a comprehensive report including a series of compounds chosen as candidates to be produced from hemicelluloses, celluloses and lignin [5-7]. World Economic Forum (2010) has identified the main technological challenges of the biorefining processes [8]. Arai et al. provide an excellent review which shows a biorefinery scheme [9]. The biorefinery concept is developed from the petroleum refinery, but some differences can be observed (see Figure 1). In the petrochemical industry, key compounds are very simple molecules such us ethane, propene and benzene. From these compounds several petrochemical products can be produced but the synthesis of these products is through the sequential addition of functional groups and consequently multiple chemical processes are required. These chemical processes include the use of multiple steps, various solvents, catalysts, excessive consume of energy, making the process not

"environmentally friendly" and requiring to centralized production to achieve lower costs. Biorefinery refers to a facility that integrates biomass conversion process and equipment to produce fuels, power and chemical from biomass. In contrast with the petroleum refinery, the decentralized local-scale production system developing compact equipment is desired with the aim of producing products and energy from different raw materials. In the biomass chemical industry, the starting materials are complex molecules with highly functional groups and consequently the elimination of redistribution of functional groups will be necessary to produce the new biomass products. Consequently, the chemical processes can be decentralized to local scale production and compact process can be obtained. After that, the biomass products, when their life ends, can be decomposed to CO_2 and H_2O providing energy.

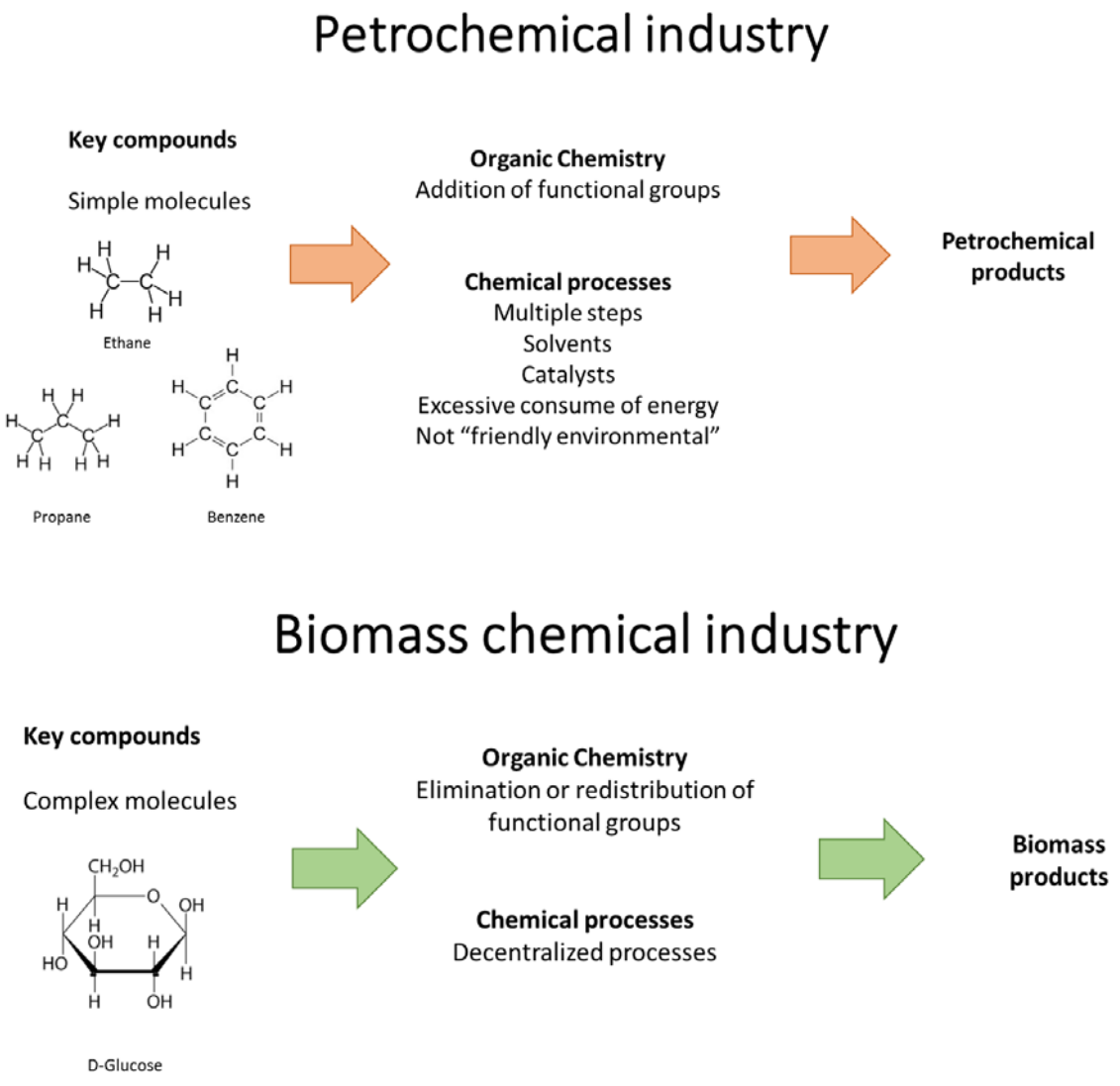

Figure 1. Petrochemical industry versus biomass chemical industry

In Figure 2 it can be observed a diagram of a biorefinery. This diagram includes the main raw materials, processing methods and products. The biomass can be divided by edible and non-edible components. The most important use of edible biomass should be as food. The residue obtaining from food, forestry, industry, etc., not suitable for human consumption, can be used as main source of hemicelluloses, cellulose and lignin. The main processing methods to obtain these three components from biomass is separation.

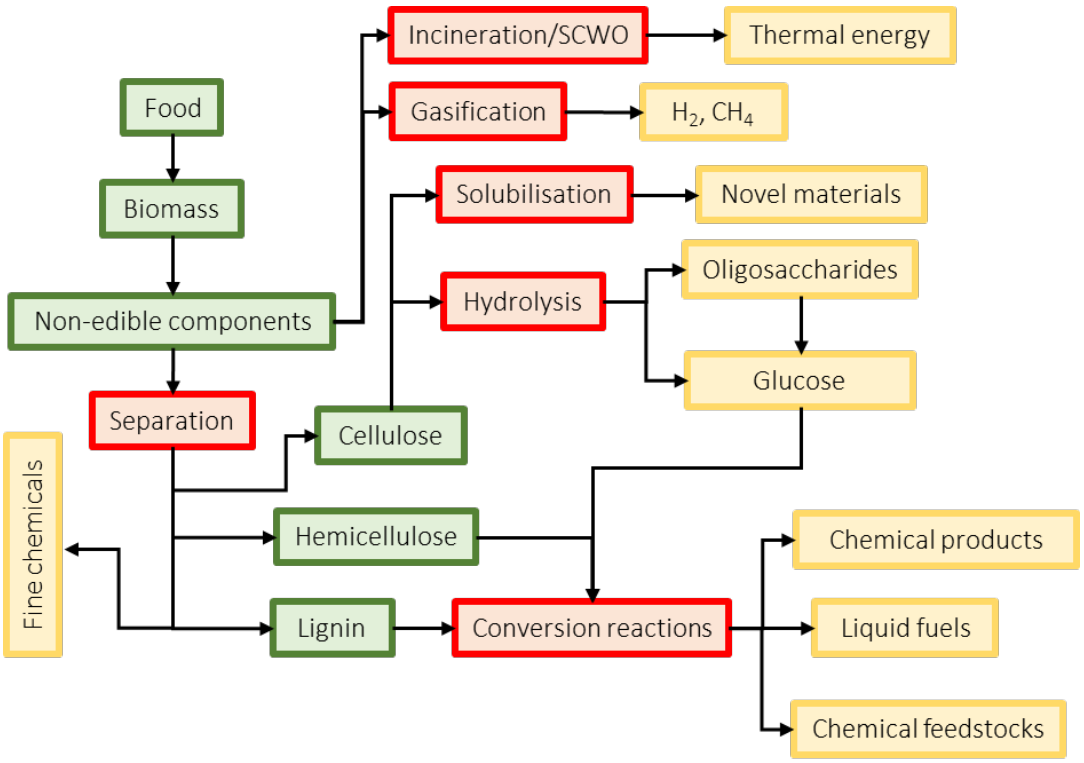

Figure 2. Diagram of biorefinery. Green: main raw materials. Red: main processing methods. Yellow: main products.

Other methods can be applied to valorise the biomass: incineration using supercritical water, gasification, solubilisation, hydrolysis and conversion reactions. The selection of processing method is crucial in the biochemical conversion into added value products, energy or fuels. For example, through gasification, hydrogen and methane can be produced. In contrast, through celluloses hydrolysis, oligosaccharides and glucose can be obtained and these components can be used as raw materials for the production of chemicals products, liquid fuels or chemical feedstocks through conversion reactions. These main methods have been intensively studied and will be reviewed in this manuscript.

1. Incineration/SCWO

The incineration in supercritical water conditions is a promising green technology to convert hazardous wastewaters to innocuous products, producing thermal energy. The innocuous products are clean liquid (pure water), clean solid (metal oxides) and clean gases (CO_2 and N_2). In this process, called as oxidation in supercritical water, the combustion is in aqueous phase, working to temperatures and pressures above a mixture's thermodynamic critical point. The reaction takes place at about 450–600ºC and pressure range of 24–28 MPa [10].Under these conditions the water acts as no-polar solvent being miscible with organic substances and gases as O_2, N_2 or CO2 while inorganic salts are almost insoluble in it. There is a homogeneous reaction medium where there are no mass transfer limitations achieving high destruction levels (approximately 100%) in a short reaction time (lower than 1 min) [11]. The operation under hydrothermal flames is observed when the temperature reaction is higher than autoignition temperature. The role of the high pressure is reduce the autoignition temperature. Some advantages respect to process without flame are: (1) the oxidation takes place in a reaction residence time in milliseconds, consequently the reactor volume is reduced; (2) the process efficiency is approximately to 100% being the total organic carbon at the outlet lower than 2 ppm; (3) secondary reactions are not observed; (4) reactants can be directly injected in the flame, so corrosion in a preheating system is avoided; (5) the organic salts can be separated as solid; (6) the effluent reactor is vapour of H_2O or CO_2 to high temperature and pressure and N_2 in the case of using air as oxidant. From this effluent, the CO_2 to high pressure and temperature can be separated and expanding a turbine for energy production increasing the energy efficiency up to 40%. However, a specific design reactor is needed to work with hydrothermal flames. The application of hydrothermal flames opens a wide field for the production of energy from wastes and the studies of the conditions to produce hydrothermal flame from biomass is a challenge to develop decentralized biorefineries.

2. Gasification

Gasification is an endothermic reaction that converts organic or fossil fuel based carbonaceous materials into gaseous products composed of CO, CO_2, H_2, CH_4 and H_2O with a limited oxygen atmosphere. The biomass, in a conventional process, should be dried to reach higher efficiency. If the biomass has high water content, a previous step to evaporate the water is necessary. The gasification of solid biomass can be carried out using supercritical water operating at high temperatures and pressures (600ºC and 25 MPa) and using catalysts to obtain a gaseous stream rich in H_2 and CO_2 [12] and small quantities of tar, char and ash [13]. The parameters such as temperature, pressure, residence time and gasifying agent play an important role in the hydrothermal gasification process. The reaction takes place at about 350–750ºC and pressure range of 22.5–25 MPa. The advantages of hydrothermal gasification are: (1) production of hydrogen as fuel; (2) high efficiency (the reaction is a single phase); (3) the production of H_2 could be produced without dioxins; (4) wet biomass can be used (up 35%); (5) high molar fraction of H_2 in the gaseous phase; (6) small volume of reactors; (7) clean gas obtained [14].

The gasification provide new opportunities for the conversion of the biomass residue to clean fuel with no environmental impact. Biomass gasification is one of the most promising low carbon emission technologies.

3. Conversion reactions

In order to convert biomass into fuels or valuable products, the chemistry of biomass at higher temperatures and pressure has been studied by a number of authors and institutions [15-21]. A simple example, which the use of water in supercritical conditions can be used to reduce the number of the reaction steps is the synthesis of glycolaldehyde from glucose by retro-aldol condensation [22].

Dehydration, retro-aldol condensation and isomerization are the main reactions of carbohydrates in a water medium. The glycolaldheyde is the main product from glucose conversion in a hydrothermal process [22]. The glucose can follow an isomerization to produce fructose. The dehydration of fructose produces 5-HMF while the dehydration of glucose produces 1,6-anhydro glucose [2].

The knowledge in organic chemistry allows to large impact on the development of future biorefineries.

4. Biomass fractionation

The extraction and fractionation of components from biomass using solvents in supercritical and subcritical conditions have been intensively studied in the last decades [23-27]. The most popular solvents are CO_2 and water. The CO_2 is inexpensive solvent, non-toxic and environmentally friendly. The properties such us density, viscosity and diffusivity can be changed by varying the temperature and pressure. The relatively low temperature to reach the supercritical conditions for CO_2, 31ºC and 7.3 MPa, allows to use this solvent to purify, extract, fractionate, etc. a wide range of raw materials. The CO_2 in supercritical conditions can dissolved non-polar and volatile components but a polar organic co-solvent can added for processing polar compounds.

The subcritical water is defined as liquid water at temperature between 100 and 374ºC (critical temperature). The pressure is applied to keep the water in liquid phase, consequently the pressure takes place between 1.6 MPa and 22.1 MPa. As mentioned above, in the same way that CO_2, the main properties of water can be changed by varying the temperature and pressure. As the temperature rises, the permittivity, viscosity and surface tension decrease and increase the diffusion rate. The reduction of dielectric constant can be systematic reduced increasing the temperature and the pressure (water in liquid phase). So, more polar materials can be extracted, most efficiently, at lower temperatures while less polar materials can be extracted at elevated temperatures.

The water is an excellent reaction medium for the separation-conversion-extraction of lignocellulosic biomass. In general, lignocellulosic biomass include agricultural residues, herbaceous, hardwood, softwood, cellulose wastes and industry co-products [28]. Lignocellulosic biomass is a complex mixture of polymers and it exhibits a big variations in its composition from one resource to another. As mentioned above, the main polymers are hemicelluloses, cellulose and lignin and small amount of extractives and oil can be observed. Hemicelluloses are known to form covalent bonds with functional groups in lignin and interact with cellulose via hydrogen bonds. Familiarity with the structure and chemical composition of these molecules is an important task because all constituent are

responsible for the properties exhibited by biomass products as well as the behaviour showed when biomass is exposed to different process and operational conditions. The most relevant information about each component is described below under the subtitles: Extractives, Hemicelluloses, Cellulose and Lignin.

Extractives

Extractives are a heterogeneous group of compounds that represent only a small part of wood. They include terpenes (phenols, hydrocarbons), fatty acids (fats, oil, waxes, resins, resin acids, sterols), colouring matter (phlobaphenes, tannins, stilbenes), inorganic material (calcium, potassium, magnesium, sodium). These compounds that can be dissolved in organic solvent, mixtures of organic solvents, water and CO_2 [29].

Hemicelluloses

The hemicelluloses represent the second most abundant polymers. The hemicelluloses content of wood varies from about 18 to 40%. This compound is a hetero-polysaccharides composed by hexoses, pentoses sugars, acetic acid and uronic acids [7]. The pentose sugars comprise mainly xylose and arabinose while the hexose sugars comprise galactose, mannose and glucose. The uronic acids is composed by galacturonic, glucuronic and methylglucuronic acids. The hemicelluloses are divided into four groups: xylans, mannans, mixed linkage β-glucans, and xyloglucans [30]. Xylans are the hetero-polysaccharides that consist of a homopolymeric backbone of β-1,4- linked D-xylopyranos units with random side chains of arabinose, glucuronic, ferulic or acetic acid. Glucomannans are hetero-polysaccharides that consist of a copolymeric backbone of β-1,4-linked D-glucopyranose and D-mannopyranose units with random side chains of galactose. The major hardwood hemicelluloses are xylans while that galactoglucomannans are observed in softwood. The hardwood xylans contain a large amount of acetyl groups.

The hemicelluloses have a degree of polymerization between 100 and 200 [31]. The amorphous structure (without crystalline structure) of hemicelluloses, due the presence of acetyl groups connected to the polymer chain, together with a low degree of polymerization make it easier to hydrolyse hemicellulose than cellulose.

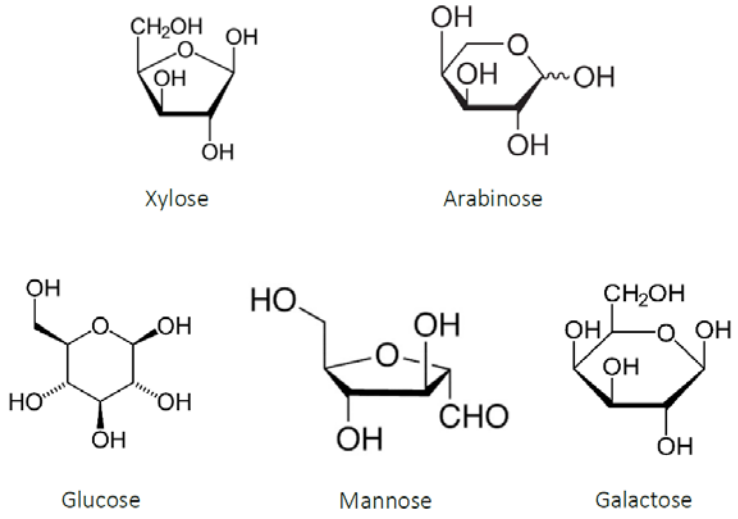

Figure 3. Main components of hemicelluloses

Cellulose

The cellulose represents the first most abundant bio polymer on the world. The cellulose content of wood varies from about 40 to 50%. This compound is a homo-polysaccharide formed by D-gluco-pyranose linked through glycosidic bonds in C-1 and C-4 [32] and its chemical formula is $(C_6H_{10}O_5)_n$. The hydroxides are uniformly distributed on both side of the glucose monomers generating the formation of hydrogen bonds between the cellulose chains. This results in a linear molecule and the presence of hydrogen bonds allow the formation of compounds that contains several parallel chains attached. The molecules of cellulose are associated to form the microfibril. The microfibril can have crystalline and amorphous regions depending on the intra and intermolecular hydrogen bonds between cellulose molecules. The crystalline structure is relatively difficult to the break compared to other polysaccharides. The glucose is a product from cellulose hydrolysis (see Figure 4). The glucose and hexose are the basis of many applications like ethanol for biofuels.

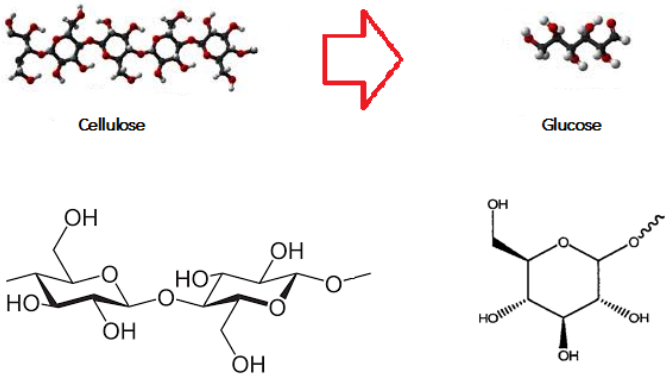

Figure 4. Constituent unit of cellulose: glucose.

Lignin

The most abundant source of aromatic compounds in nature is lignin [33]. This component has a sophisticated structure: it is formed by a complex and three-dimensional polymer composed of phenylpropane units with a molecular weight in excess of 10000 units. Lignin contains three aromatic alcohols: coniferyl alcohol, sinapyl alcohol and p-coumaryl alcohol [34]. These aromatic alcohols are called monolignols. Monolignols differ only in the number of methoxy groups attached to the phenolic unit. The structures of these alcohols are shown in Figure 5. Softwood lignin contains more coniferyl units than hardwood lignin, consequently is more difficult to degrade. To increase the breakage of lignin it is necessary to work at high water densities [35].

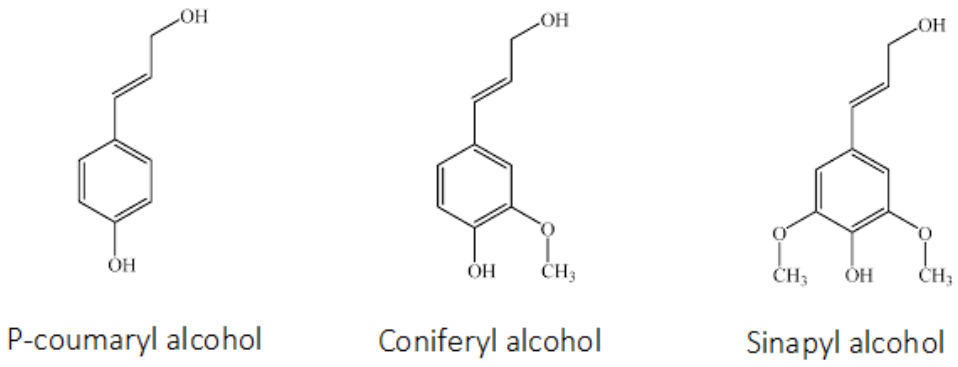

Figure 5. The buildings blocks of lignin.

5. Hydrolysis

Hydrolysis is a reaction in which a molecule is split into two or more new compounds by reacting with water. The general equation is shown in equation 1:

$$A\text{-}B + H2O \longrightarrow AH + BOH \quad (1)$$

There are several pretreatment technologies and solvents for removal of carbohydrates from biomass such as steam explosion, organic solvents, alkali, dilute acid, enzyme treatment and water extraction. In general, the goal is remove the lignin and hemicelluloses and increase the digestibility of cellulose decreasing the degree of polymerization and crystallinity. The water extraction process is based on the use of pressure to keep water in liquid phase at elevated temperatures. The process use only water as solvent offering advantages such us: (1) no mineral acids are added, consequently, there are not equipment corrosion problems; (2) no neutralization and recovery of chemicals from water are necessary; (3) low economic cost and; (4) no toxic and inexpensive medium is used, which make it an environmentally friendly process [36, 37]. The variations in the properties of water manipulating the temperature and pressure make it an excellent reaction medium to obtain desirable products. For example, the ionic reactions are favoured using liquid water below the critical point (374ºC and 22.1 MPa) as solvent while the radical reactions are favoured at supercritical water conditions.

Hot water extraction/hydrolysis of lignocellulosic biomass is a well-known method for separating hemicelluloses, cellulose and lignin, being the first step the fractionation. This treatment is not only interesting for the separation of the main components of biomass. Also, the biomass subjected to this process can undergo chemical and structurally changes; consequently increasing its reactivity in other further process. The extractives are the first ones that can be extracted from biomass, generally in a previous pretreatment. Hydrothermal fractionation can be carried out at soft conditions (T<180ºC) to solubilize hemicelluloses, yielding a solid phase enriched in lignin and cellulose and a liquid phase enriched in hemicelluloses (oligomers and monomers) or it can be carried out at hard conditions to remove hemicelluloses and celluloses to obtain a solid phase enriched in lignin and a liquid phase enriched in hemicelluloses and cellulose (oligomers and monomers). The total biomass recovered during the hydrothermal process is between 40 and 60% [28]. As mentioned above, the structure of hemicelluloses, cellulose

and lignin is different, so the hydrolysis kinetics should be different too. In the same way, the temperature is an important parameter in the hydrolysis of hemicelluloses, cellulose and lignin. The kinetics of hemicelluloses, cellulose and lignin are faster when increasing temperature. High temperature will favour high yields but, if the reaction time is high, a big amount of degradation products can be produced. One of the challenges of biomass fractionation is the coordination between high yields of carbohydrates, low reaction time (small volume reactor or high flow rate) and low degradation products yields. The reduction of analytical methods employed to understand the behaviour of the system is another crucial task.

Interest in the isolation of hemicelluloses and celluloses from biomass has greatly increased in recent years. The hemicelluloses fraction is recovered between 150 and 220ºC [38], but a small amount of hydrolysed hemicelluloses was observed at 120ºC [39]. The deacetylation ocurrs due to presence of acetyl groups from hemicelluloses, producing acetic acid in presence of water and decreasing the pH in the liquid fraction. The acetic acid acts as a catalyst [40] increasing the hydrolysis speed of polysaccharides as well as the formation of degradation products. The control of pH is an important task to obtain long chain carbohydrates and to avoid the formation of by-products. To prevent the presence of degradation products, the pH should be kept in the range 4-7, minimizing the monomer form of hemicelluloses [38].

Cellulose hydrolysis and degradation become significant above 210 ºC and some lignin is removed at about 200ºC [41].

The reaction time is closely related with the reaction temperature and the type of reactor used. The fractionation of biomass has been intensively studied using batch, semi-continuous and continuous reactor. The decision of the operation modes strongly depends on the precision and rate of results required as well as the operational scale. The batch reactors are used for small-scale producing fast results with a poor control of temperature, pressure and reaction time. In general the heating ramp and cooling ramp are difficult to determine. The main advantage is that the equipment is a simple reactor, consequently the obtation of results is cheaper but the precision of results is lower than semicontinuous and continuous reactors .

Contrary, the continuous reactor allows a major control over the process and can provide a more reasonable basis for process design and scale-up for commercialization but the results are "more expensive". The high selectivity in the hydrolysis of products can be obtained varying the residence time changing the flowrate or volume of reactor. The main disadvantage is that the pumping is a complicated task because the concentration of suspension should be limited and the pump should be able to pump solids; consequently the biomass requires a pre-treatment.

The semicontinuous reactor operates with a batch solid phase and a continuous liquid phase. In this type of reactor it can be observed two residence times: one related with the solid residence time and other related with the liquid residence time. The main advantage is that the extraction/hydrolysis of carbohydrates and its subsequently production of degradation products from biomass depends on the liquid residence time.

Biomass surplus in Spain

In the last decade, in Spain, the production of grapes was close to 6 million tons produced annually [42]. Approximately 72% of the grape production is used in the manufacture of wine, but the wine industry produces a lot of waste did not use: 28% of stalks and grape seeds are generated. As an example of great interest to the recovery of such waste is the LIFE +, the Financial Instrument for the Environment of the European Union, which has recently funded the HAproWINE project whose objective is the comprehensive management of waste from the wine industry. The grape seeds have a big interest because contain one of the highest composition of lignin in nature, higher than 40% wt. [43, 44] and the skin and the seeds contain a big amount of polyphenols. The lignin and polyphenols are components of interest in the chemical industry. The grape seed has been selected as source of mainly lignin due to the land area destined to production of wine in Spain has remained stable over the last decade, around 71000 hectares, with a production between 3300 and 4800 Mton/year [45], generating between 660 and 1340 Mton/year very stable over the last 10 years [46]

Agriculture is an important activity in Spain, producing a massive amount of residues that can be used as raw material in the fractionation/hydrolysis process. High amount of biomass are generate from the pruning. The pruning of urban ornamental trees in

Valladolid, Spain, can be a good option to obtain a big amount of raw materials to further submit to hydrothermal process. The decentralized local-scale production system can be applied.

Outlook

A century after industrial revolution, now, problems generated from non-renewable fossil fuel sources, such us, increasing of oil and gas prices, increasing the global warming (caused by greenhouse gases emission) and the progressive depletion of fossil sources (e.g. peak oil) have increased the world demand of renewable resources for the production of added value products, bio oils and energy. The forthcoming big challenge is a society based on the concept of bioeconomy.

Lignocellulosic biomass is a potential feedstock for the production of carbon-based materials, energy and fuel. One of the biggest challenges of converting biomass into these added value products is to fractionate its structure into main components such us extractives, hemicellulose, cellulose and lignin. One of the promising methods is hydrothermal process. Water used as solvent is a good alternative because it is a clean, safe and environmentally benign.

The overall aim of this work is to develop a process capable of obtaining fuels and added value products from lignocellulosic biomass using the fractionation hydrolysis process. The hydrothermal treatment will be used for a double fold, first to produce bio-oils and second to produce bio-based fractions (C5's, C6's and lignin).

In order to achieve the aim of this thesis, the following partial objectives are defined:

- Design, construction and optimization of a feasible lab scale plant in order to study the hydrothermal fractionation process using water as solvent in subcritical conditions. The maximum temperature and pressure required were 400ºC and 25 MPa.
- Study of hydrothermal hydrolysis of grape seeds
    - Study of the effect of temperature on heavy bio oil and light bio oil yield as well as the amount of sugars and solid obtained
    - Study of the effect of a three-stage temperature profile on recovered sugars and solid yield
    - Study the solvothermal extraction using ethanol/water as solvent on polyphenols and oil yield

- Study of hydrothermal hydrolysis of Holm Oak (hardwood)
    - Study of the temperature, flow and particle size on carbohydrates hydrolysed and solid yield
    - Monitoring alternatives to select an `easy-cheap´ parameter that may simplify the following of the hydrothermal process behaviour
    - Study of the temperature and reaction time on hydrolysis of hemicelluloses and average molecular weight obtained
- Study of hydrothermal hydrolysis of different hardwood and softwood species
    - Analysis of the influence of the composition of raw material on carbohydrates and solid yield

In order to achieve these objectives, the work was structured in 5 chapters in which challenges, objectives and partial objectives are collected. The main contents of each chapter are described below.

Chapter 1, *Hydrothermal hydrolysis of* grape *seeds to produce bio-oils*, studied the production of bio oil from grape seeds using a semicontinuous reactor. The influence of the reaction temperature was analysed in order to determine its influence on bio-oil yield. It was observed that the highest amount of heavy and light bio oil was achieved at the highest temperature studied.

Chapter 2, *Hydrothermal fractionation of grape seeds in subcritical water to produce oil extract, sugars and lignin,* investigated a solvothermal extraction follow by a hydrothermal fractionation-hydrolysis. A mixture of ethanol/water was used as solvent to determine the polyphenols and oil yields. It was observed that the extraction efficiency was higher than 60%. The study included three stages of temperature to hydrolyse firstly the maximum amount of hemicelluloses (C5's), secondly the maximum amount of cellulose (C6's) and finally the hydrolysis of remaining carbohydrates. The results showed that a temperature profile can be selected to obtain a maximum carbohydrates yield and high lignin purity in solid product.

In general, the analysis of the product samples to know the behaviour of the system is an expensive task which required longer time consumed. Consequently, it is essential to find

a parameter (the best cost-option techniques) which reduces the costs to follow the hydrolysis process.

Chapter 3, *Monitoring alternatives and main sugar products for the autohydrolysis of Holm Oak hemicelluloses using pressurized hot water,* studied the selection of one parameter that may simplify the analysis of the process. It was observed that the parameter selected (pH) can help to identify the correct time to collect the samples which allows to know when the sugars are being extracted and consequently, reduces the number of samples. It was studied the incorporation of heat exchangers to recover energy makes the process more efficient from an economically and energetically viewpoint. In the same way, the effects of reaction operating parameters such as temperature, flow rate and particle size on the yield of hemicelluloses, celluloses and degradation products was investigated.

Chapter 4, *Obtaining hemicelluloses from hardwood Holm Oak (Quercus ilex) using subcritical water in a pilot plant,* investigated the effect of temperature and reaction time on yield and average molar mass using a five reactors connected in series to form a cascade reactor. The results showed that the deacetylation, strongly dependent of reaction temperature, was accompanied by a reduction in the molecular weight. Furthermore, the average molecular weight obtained was lower than the obtained from softwood by other authors.

Finally, Chapter 5*, Hydrothermal fractionation of woody biomass: lignin effect over sugars recovery*, screened nine urban trees from Spain, including hardwood and softwood species. The investigation included the effect of the composition of the raw materials on hemicelluloses, celluloses, degradation products and solid yield. It was observed that the hydrolysis of hemicelluloses and celluloses was influenced by the composition and structure of the lignocellulosic source employed. However, the kinetics of the hydrolysis were independent of the raw material.

Summarizing, in this thesis we have developed the hydrolysis and fractionation process of surplus biomass in subcritical water in a laboratory scale and pilot plant.

References

[1] M. Yamamoto, M. Iakovlev, S. Bankar, M.S. Tunc, A. van Heiningen, Enzymatic hydrolysis of hardwood and softwood harvest residue fibers released by sulfur dioxide–ethanol–water fractionation, Bioresource Technology 167 (2014) 530-538.

[2] D.A. Cantero, A. Álvarez, M.D. Bermejo, M.J. Cocero, Transformation of glucose into added value compounds in a hydrothermal reaction media, The Journal of Supercritical Fluids 98 (2015) 204-210.

[3] J.J. Bozell, Feedstocks for the future - Biorefinery production of chemicals from renewable carbon, Clean - Soil, Air, Water 36 (2008) 641-647.

[4] C.B. Field, M.J. Behrenfeld, J.T. Randerson, P. Falkowski, Primary production of the biosphere: Integrating terrestrial and oceanic components, Science 281 (1998) 237-240.

[5] T. Werpy, G. Petersen, Top Value Added Chemicals from Biomass. Volume I - Results of Screening for Potential Candidates from Sugars and Synthesis Gas, Pacific Northwest National Laboratory (PNNL) and the National Renewable Energy Laboratory (NREL) 1 (2004) 1-76.

[6] J.E. Holladay, J.J. Bozell, J.F. White, D. Johnson, Top Value Added Chemicals from Biomass. Volume II - Results of Screening for Potential Candidates from Biorefinery Lignin, Pacific Northwest National Laboratory, University of Tennessee and National Renewable Energy Laboratory 1 (2004) 1-87.

[7] P. Mäki-Arvela, T. Salmi, B. Holmbom, S. Willför, D.Y. Murzin, Synthesis of sugars by hydrolysis of hemicelluloses- A review, Chemical Reviews 111 (2011) 5638-5666.

[8] D. King, The future of industrial biorefineries, Worl Economic Forum (2009).

[9] K. Arai, R.L. Smith Jr, T.M. Aida, Decentralized chemical processes with supercritical fluid technology for sustainable society, Journal of Supercritical Fluids 47 (2009) 628-636.

[10] B. Veriansyah, J.-D. Kim, Supercritical water oxidation for the destruction of toxic organic wastewaters: A review, Journal of Environmental Sciences 19 (2007) 513-522

[11] V. Vadillo, J. Sánchez-Oneto, J.R. Portela, E.J. Martínez de la Ossa, Problems in Supercritical Water Oxidation Process and Proposed Solutions, Industrial & Engineering Chemistry Research 52 (2013) 7617-7629

[12] M. Ni, D.Y.C. Leung, M.K.H. Leung, K. Sumathy, An overview of hydrogen production from biomass, Fuel Processing Technology 87 (2006) 461-472.

[13] A. Demirbaş, Hydrogen Production from Biomass by the Gasification Process, Energy Sources 24 (2002) 59-68

[14] Y.J. Lu, L.J. Guo, C.M. Ji, X.M. Zhang, X.H. Hao, Q.H. Yan, Hydrogen production by biomass gasification in supercritical water: A parametric study, International Journal of Hydrogen Energy 31 (2006) 822-831.

[15] V.C. Ghantani, S.T. Lomate, M.K. Dongare, S.B. Umbarkar, Catalytic dehydration of lactic acid to acrylic acid using calcium hydroxyapatite catalysts, Green Chemistry 15 (2013) 1211-1217.

[16] T. Rogalinski, T. Ingram, G. Brunner, Hydrolysis of lignocellulosic biomass in water under elevated temperatures and pressures, The Journal of Supercritical Fluids 47 (2008) 54-63.

[17] A. Corma, G.W. Huber, L. Sauvanaud, P. O'Connor, Biomass to chemicals: Catalytic conversion of glycerol/water mixtures into acrolein, reaction network, Journal of Catalysis 257 (2008) 163-171.

[18] R.S. Assary, T. Kim, J.J. Low, J. Greeley, L.A. Curtiss, Glucose and fructose to platform chemicals: understanding the thermodynamic landscapes of acid-catalysed reactions using high-level ab initio methods, Physical chemistry chemical physics: PCCP 14 (2012) 16603-16611.

[19] B.M. Kabyemela, T. Adschiri, R.M. Malaluan, K. Arai, Glucose and Fructose Decomposition in Subcritical and Supercritical Water: Detailed Reaction Pathway, Mechanisms, and Kinetics, Industrial & Engineering Chemistry Research 38 (1999) 2888-2895

[20] T.M. Aida, A. Ikarashi, Y. Saito, M. Watanabe, R.L. Smith Jr, K. Arai, Dehydration of lactic acid to acrylic acid in high temperature water at high pressures, The Journal of Supercritical Fluids 50 (2009) 257-264.

[21] A. Corma, S. Iborra, A. Velty, Chemical routes for the transformation of biomass into chemicals, Chemical Reviews 107 (2007) 2411-2502.

[22] M. Sasaki, K. Goto, K. Tajima, T. Adschiri, K. Arai, Rapid and selective retro-aldol condensation of glucose to glycolaldehyde in supercritical water, Green Chemistry 4 (2002) 285-287.

[23] P.C. Veggi, R.N. Cavalcanti, M.A.A. Meireles, Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction: Experimental data and economic evaluation, Journal of Food Engineering 131 (2014) 96-109.

[24] M.A. Rostagno, J.M. Prado, R. Vardanega, T. Forster-Carneiro, M.A.A. Meireles, Study of recovery of fermentable sugars with subcritical water and carbon dioxide from palm fiber and grape seed, Chemical Engineering Transactions, 2014, pp. 403-408.

[25] J.M. Prado, L.A. Follegatti-Romero, T. Forster-Carneiro, M.A. Rostagno, F. Maugeri Filho, M.A.A. Meireles, Hydrolysis of sugarcane bagasse in subcritical water, Journal of Supercritical Fluids 86 (2014) 15-22.

[26] S.P.M. Da Silva, A.R.C. Morais, R. Bogel-Łukasik, The CO2-assisted autohydrolysis of wheat straw, Green Chemistry 16 (2014) 238-246.

[27] F.P. Cardenas-Toro, T. Forster-Carneiro, M.A. Rostagno, A.J. Petenate, F. Maugeri Filho, M.A.A. Meireles, Integrated supercritical fluid extraction and subcritical water hydrolysis for the recovery of bioactive compounds from pressed palm fiber, Journal of Supercritical Fluids (2014).

[28] A. Saeed, M.S. Jahan, H. Li, Z. Liu, Y. Ni, A. van Heiningen, Mass balances of components dissolved in the pre-hydrolysis liquor of kraft-based dissolving pulp production process from Canadian hardwoods, Biomass and Bioenergy 39 (2012) 14-19.

[29] D. Fengel, G. Wegener, Wood: chemistry, ultrastructure, reactions, Walter de Gruyter 1983.

[30] S. Liu, H. Lu, R. Hu, A. Shupe, L. Lin, B. Liang, A sustainable woody biomass biorefinery, Biotechnology Advances 30 (2012) 785-810.

[31] C. Sattler, N. Labbé, D. Harper, T. Elder, T. Rials, Effects of Hot Water Extraction on Physical and Chemical Characteristics of Oriented Strand Board (OSB) Wood Flakes, CLEAN – Soil, Air, Water 36 (2008) 674-681

[32] X. Lü, S. Saka, Hydrolysis of Japanese beech by batch and semi-flow water under subcritical temperatures and pressures, Biomass and Bioenergy 34 (2010) 1089-1097.

[33] A. Toledano, A. García, I. Mondragon, J. Labidi, Lignin separation and fractionation by ultrafiltration, Separation and Purification Technology 71 (2010) 38-43.

[34] L. Tock, M. Gassner, F. Maréchal, Thermochemical production of liquid fuels from biomass: Thermo-economic modeling, process design and process integration analysis, Biomass and Bioenergy 34 (2010) 1838-1854.

[35] A.A. Peterson, F. Vogel, R.P. Lachance, M. Fröling, M.J. Antal Jr, J.W. Tester, Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies, Energy and Environmental Science 1 (2008) 32-65.

[36] G. Garrote, H. Domínguez, J.C. Parajó, Kinetic modelling of corncob autohydrolysis, Process Biochemistry 36 (2001) 571-578.

[37] O.Y. Abdelaziz, M.A. Gadalla, M.M. El-Halwagi, F.H. Ashour, A hierarchical approach for the design improvements of an Organocat biorefinery, Bioresource Technology 181 (2015) 321-329.

[38] S. Kumar, U. Kothari, L. Kong, Y.Y. Lee, R.B. Gupta, Hydrothermal pretreatment of switchgrass and corn stover for production of ethanol and carbon microspheres, Biomass and Bioenergy 35 (2011) 956-968

[39] J.V. Rissanen, H. Grénman, S. Willför, D.Y. Murzin, T. Salmi, Spruce Hemicellulose for Chemicals Using Aqueous Extraction: Kinetics, Mass Transfer, and Modeling, Industrial & Engineering Chemistry Research 53 (2014) 6341-6350

[40] S. Liu, Woody biomass: Niche position as a source of sustainable renewable chemicals and energy and kinetics of hot-water extraction/hydrolysis, Biotechnology Advances 28 (2010) 563-582.

[41] O. Bobleter, Hydrothermal degradation of polymers derived from plants, Progress in Polymer Science 19 (1994) 797-841

[42] FAOSTAT, Production of grapes from 2003-2013, Food and Agriculture Organization of the United Nations, 2014.

[43] D. Moldes, P.P. Gallego, S. Rodriguez Couto, A. Sanroman, Grape seeds: The best lignocellulosic waste to produce laccase by solid state cultures of Trametes hirsuta, Biotechnology Letters 25 (2003) 491-495.

[44] M. Spanghero, A.Z.M. Salem, P.H. Robinson, Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces, Animal Feed Science and Technology 152 (2009) 243-255.

[45] JCyL, Agricultura y Ganadería - Producción y Sanidad Agropecuaria - Producción y sanidad vegetal - Producción vegetal - Viñedo, www.jcyl.es (2010).

[46] MARM, Anuario de Estadística - MAPA - Capitulo 13. SUPERFICIES Y PRODUCCIONES DE CULTIVOS, www.mapa.es (2010).

Chapter 1

Hydrothermal hydrolysis of grape seeds to produce bio-oils

Abstract

In the present work, the hydrothermal hydrolysis of grape seeds focused in the production of bio-oil was studied. The grape seeds composition in terms of lignin, sugars, ash, extractives and bio-oil was determined. The composition of grape seeds was: 17.0% wt. of extractives; 36.8% wt. of sugars (hemicellulose and cellulose); 43.8% wt. of lignin and 2.4% wt. of ash. The grape seeds were hydrothermally treated using three different temperatures: 250ºC, 300ºC and 340ºC employing a semi-continuous reactor. The solid residue varied from 25.6 - 35.8% wt. depending on the hydrolysis temperature. The maximum yields of Light (15.7% wt.) and Heavy Bio-oil (16.2% wt.) were achieved at 340ºC. The Arrhenius parameters for the kinetic of grape seeds hydrolysis in our system were k_0 = 0.995 g·min^{-1} and E_a=13.8 kJ·mol^{-1}. The increment of the flow rate favoured the mass transfer in the system and so, the hydrolysis rate. However, the maximum hydrolysis rate was found at a water surface velocity of 2.3 cm·min^{-1}.

Keywords: Grape seeds, biorefinery, lignin, hydrolysis

1. Introduction

The industry based on fossil raw materials will not be sustainable for many decades, and it is necessary to start the shift towards natural materials [1]. This novel economic paradigm is known as bioeconomy [2]. Vegetal biomass is a renewable source of carbon material that can be used as feedstock for the production of chemicals and fuels. Nowadays, vegetal biomass is one of the most abundant resources of carbon on the planet, being the primary production around $1 \cdot 10^{14}$ tons C/year [3]. The three main products that are considered "bio-based products" are: bio-fuels, bio-energy and bio-chemicals, which would be produced in biorefineries [4, 5].

In recent decades, the burning of fossil fuels has contributed to increase the level of CO_2 in the atmosphere generating the global warming observed. Because of this and the uncertainty in the petroleum market, the governments have been promoted the production of biofuels. To achieve this change in the production philosophy, it is necessary to develop new technologies for the sustainable and efficient conversion of natural resources into fuels and chemicals. In the last years the use of shale gas in USA and other countries provoked a significant reduction in the use of bioresources for energy production. However, the use of bioresources was focused in the production of added-value products.

The main advances in the production of liquid fuels from biomass was achieved converting corn and sugar cane crops into ethanol. The main disadvantage of these first-generation biofuels was the competition with the production of food, which has caused an increase of food prices under several conditions. For this reason the second-generation biofuels produced from surplus lignocellulosic feedstock can be more feasible. Lignocellulosic biomass has three major components: cellulose, hemicellulose and lignin with minor contents of extractives and ash. The relative quantity of hemicellulose, cellulose and lignin depend of biomass used and as well as variations in methods of cultivation, climatic factors, physiographic variability, vintage year, harvest date, etc [6, 7].

There are a large number of methods to fractionate biomass using ionic liquids, organic solvents, supercritical fluids, compressed water, steam pre-treatment, several alkaline

treatments, etc [8-11]. One of the methods used to fractionate the three lignocellulosic components is the hydrothermal treatment using pressurised hot water as solvent. Water is a non-toxic, environmental friendly and inexpensive reaction medium [12]. In addition, the hydrothermal biomass conversion require lower temperatures than other processes such as pyrolysis or gasification [13-15].

Viticulture is an important agricultural activity in Spain, producing a massive amount of stalks and grape seeds as by-product. In the last decade, the production of grapes was close to 6 million tons produced annually [16]. In the wine production approximately 72% of grape is used. The waste from wine industry is constituted mainly for two components: 23% of grape seeds and 5% of stalks. This trend consolidated Spain to be the top wine worldwide producer in 2014, together with Italy and France.

Grape seeds contain an important concentration of lignin (43.8% wt.), extractives (17% wt.) and cellulose and hemicellulose (36.8% wt.).

The production of biofuels from different raw materials have been studied by several authors. Tekin and Alkalin et al. have studied the production of Light Bio-oil (LBO) and Heavy Bio-oil (HBO) from beech wood and cornelian cherry stones with and without catalysts (i.e. colemanite) [17, 18]. Huang et al. maximized the LBO production by recycling the HBO to the hydrolysis process obtaining also, higher yields of char [19]. The pyrolysis of coconut shells [20]; rice hulk [21] and corn stover [22] was studied as alternative to hydrothermal treatment for the production of LBO and HBO [20-22].

The use of supercritical CO_2 not alter significantly the microscopic morphology of biomass. The best advantage is that it can be easily removed by depressurization without generating by-products. The CO_2-assisted process can be carried out at similar temperatures than the common autohydrolysis process [23, 24]. Thus, Magalhães da Silva et al. have demonstrated that CO_2 may assist the autohydrolysis via formation of carbonic acid promoting the production of xylo-oligosaccharides. They tested the autohydrolysis of wheat straw from 180 to 210°C and initial CO_2 pressures of 60 bar. The water/biomass ratio was 10:1 $w \cdot w^{-1}$ [23, 25].

In this work, grape seeds were hydrolysed at different temperatures in liquid phase using the autohydrolysis process. The production of HBO, LBO, total amount of sugars

and solid residue were determined. The extraction of the oil and the recovery of the liquid bio-oil and solid residue after the treatment was the aim of this work.

2. Materials and methods

2.1. Materials

Grape seeds from Vitis vinifera L (Tempranillo) were provided by Matarromera S.A. winery (Valbuena de Duero, Spain) campaign 2011. The raw seeds were crushed and sieved and a size 0.5-1.0 mm was selected for experiments.

The reagents used were: sulphuric acid (96%), acetone (99.5%) purchased from Panreac and diethyl ether (+99%) purchased from Sigma-Aldrich. Distilled water and Milli-Q water were used in the experiments.

2.2. Experimental setup

A diagram of experimental setup is shown in Figure 1. The experimental apparatus consisted of a pump (model: PU-2080), a preheater (E-01, 200 cm of 1/8' AISI 316 piping) and a reactor (R-01, 20 cm length, 1/2´ O.D. SS316 piping). The reactor was set inside a former chromatographic oven HP5680 (F-01). The outlet of the reactor was connected to a heat exchanger in order to cool down the sample to 20ºC (E-02, 15 cm of concentric tube heat exchanger 1/4´-3/8´ counter current operation). The fluid used in E-02 was cold water in the outer tube of the heat exchanger. The pressure of the system was controlled by a Go-backpressure valve (BPV-01).

The operation mode was semi-continuous. (i.e. batch for the solid and continuous for the liquid). The reactor (R-01) was filled with 4.00 ± 0.06 g of grape seeds (not dried before extraction, moisture 6.00 ± 0.20% wt.) and then the reactor was closed with two metallic filters (0.1 mm) to avoid material losses. The preheater and the reactor were heated inside an oven to 100ºC. This moment was set to time zero (i.e. reaction started). Heating time of the system was between 5-10 min.

Once the reactor was tightened the flow was fixed at 5 $mL \cdot min^{-1}$ and the pressure was set to 10 bar over the water bubble pressure at the desire temperature. After the reaction time has elapsed, the reactor was gradually cooled down to room temperature by changing the set point in the F-01. Finally, the pumping was stopped and the reactor

was untightened. The remaining solids in the reactor after the hydrolysis process were collected for later analysis of lignin and heavy bio oils content. The liquid obtained during the experiments were collected to determine the light bio oil production.

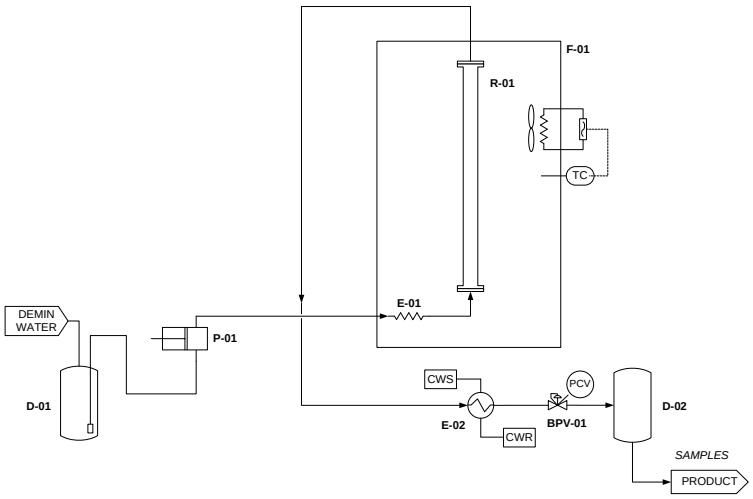

Figure 1. Schematic flow diagram of the experimental system. Equipment: D-01 Feeder, P-01 Pump, E-01 Preheater, R-01 Reactor, F-01 Chromatographic oven, E-02 Heat exchanger, BPV-01 Go-backpressure valve, D-02 exit.

Three trials were performed at each experimental condition to check repeatability of the results.

2.3. Bio-oil Analysis

The sequence of grape seeds analysis after hydrothermal treatment are schematised in Figure 2. The remaining solid inside the reactor after extraction, was oven-dried for 24 hours at 100ºC (SR0). Then, it was subjected to solid liquid extraction with acetone (approximately 25 ml) at 25ºC. This mixture was filtered and the solid was dried for 24 hours at 100ºC (SR1). On the other hand, the acetone was removed from the liquid sample with a rotary evaporator under reduced pressure (-0.8 barg) at 80ºC. This fraction was called heavy bio-oil 1 (HBO1).

An aliquot from the liquid samples collected during the hydrothermal treatment was acidified using H_2SO_4 (96%) to improve the precipitation of solids (if any). They were filtered using a cellulose membrane with a pore diameter of 100 µm. The solids were dried at 100ºC (SRL0). Then they were subjected to a solid-liquid extraction with acetone to recover the heavy bio-oil trapped (HBO2). Finally, these solids were dried in an oven at 100ºC (SR2). On the other hand, the acidified liquid was exposed to a liquid-

liquid extraction with diethyl ether (DEE) in equal volumes. The two phases obtained were then separated in a separation funnel. The organic phase was dosed with Na_2SO_4 and then, it was filtered. The DEE was removed in a rotary evaporator and the soluble light bio-oil (LBO) from the organic phase was recovered. The aqueous phase was dried to compute the sugars (SG).

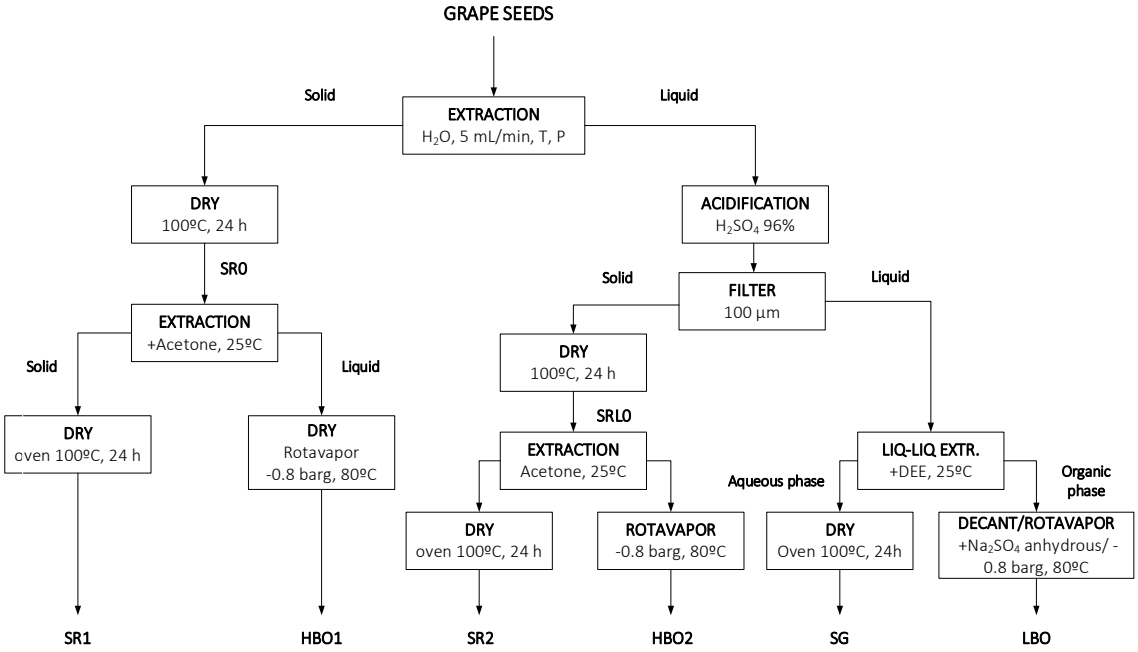

Figure 2. Procedure for the analysis of solid and liquid products from hydrolysis.

2.4. Analytical methods

The analytical methods applied in the analysis procedure presented in section 2.3 are reported below.

2.4.1. Acid insoluble lignin (Klason lignin)

Klason lignin was determined according to the protocol developed by the National Renewable Energy Laboratory [26] in agreement with Liu and Wyman [27], Toledano et al. [28, 29] and Wang et al. [30].

The Klason lignin content of the raw material and the solid residue inside of reactor after hydrolysis was determined as follows. An amount of solid aprox 300 mg was weighted and placed into a hydrolysis flask with 3.00 ± 0.01 mL of sulphuric acid (72%). The mixture was incubated for 30 ± 5 min at 30 ± 3ºC in a convection oven. After this time, the mixture was taken out from the oven and it was diluted with 84.00 ± 0.04 mL

of deionized water. Then, the mixture was kept at 121ºC for 1 hour in a convection oven. After the incubation, the solution was cooled to room temperature and it was filtered under vacuum. Hot deionized water was used to wash any particles adhered to the bottle and also to wash the filter residue in order to remove the remaining acid until pH neutral. The solid was dried at 105 ± 3ºC for at least 24 hours or until a constant weight and then it was weighted. Then, the crucible residue was introduced in a calcination oven at 550 ± 25ºC for 24 hours or until constant weight, and it was then weighted. The quantity of Klason lignin in each sample was determined using the equation 1, where 'KL' is Klason lignin in % wt.; 'AIR' is the acid insoluble residue after acid hydrolysis in mg; 'A' is the ash content in mg and; 'S' is the mass of the sample in mg.

$$KL = (AIR - A) * 100 * S^{-1} \quad (1)$$

2.4.2. Thermogravimetric methods

Thermogravimetric Analysis (TGA) was carried out in a TGA/SDTA RSI analyser of Mettler Toledo. The TGA analysis indicate how the hydrothermal treatment has hydrolysed the oil, hemicelluloses, celluloses and lignin. This analysis consist in analyse the behaviour of a solid sample under gasification conditions over an inert atmosphere (N_2). The samples of approximately 10 mg were heated from 50ºC to 800ºC at a rate of 20ºC·min^{-1} under a N_2 atmosphere (60 mL·min^{-1} flow) to determine the carbonization. In order to determine the final ash content, the sample was heated from 650ºC to 800ºC under an air atmosphere (60 mL·min^{-1} flow) to promote to oxidation of the remaining organic carbonaceous forms.

2.4.3. Spectroscopy FT-IR

Fourier Transform Infrared FT-IR is a quick method that could be used almost in situ to determine functional groups in lignin after the treatment. The FT-IR experiments were conducted using a Bruker Tensor 27. Samples were recorded in the range of 4000-600 cm^{-1} at 4 cm^{-1} resolution and 32 scans per sample. The scanner velocity was 10 KHz and interpherogram size was 14220 points.

2.4.4. Microscopy, SEM

Scanning electron microscopy (SEM) experiments were conducted to identify the physical morphology of the samples. A JSM-820 (JEOL, Japan) operated at a 20 kV accelerating voltage and gold evaporator Balzers SCD003 were used. Gold Thickness used was 25-30 nm.

3. Results and discussion

Grape seeds were hydrothermally treated using water as a solvent for one hour at constant temperature. The experiments were performed at three different temperatures (see Table 1): 250ºC, 300ºC and 340ºC. The amount of bio-oil, solid residue and hydrolysed sugars was determined for each experiment.

The analysis (Klason lignin) of the grape seeds gave a total lignin content of 43.8% wt. similar to values reported in literature [31, 32]. Considering that the ash content was 2.4% wt., the extractable/hydrolysable components represented near to 53.8% wt. of the raw material. The maximum amount of grape seed oil to be extracted was 17.0% wt. dry basis (determined by Soxhlet extraction with hexane). The typical amount of oils in the grape seeds is between 11.0-20.0% wt. [33]. This indicates that the hemicelluloses and celluloses were approx. 36.8% wt. that can be hydrolyse into sugars.

Table 1. Experimental conditions for one-step fractionation of grape seeds.

Experiment	Nº	#01	#02	#03
Flow	$mL \cdot min^{-1}$	5.0	5.0	5.0
Temperature	*ºC*	250	300	340
Pressure	*barg*	50	95	155
Time	*min*	60	60	60
Initial mass grape seeds	*g*	4.002	4.006	4.005
Moisture	*% wt.*	6.00	6.00	6.00

3.1. Hydrolysis products

The solid residue (SR) was determined as the sum of the residues obtained after acetone extraction: SR1 and SR2. The total HBO produced was calculated as the sum of the oil content determined after acetone extraction: HBO1 and HBO2. The total BO produced was obtained as the sum of the LBO and the HBO. The total measured mass

(R) was calculated as the sum of the SR, BO and the rest of the components determined in the hydrolysed liquid phase (SG). Each one of previously explained amounts was divided by the initial quantity of raw material in order to obtain the yield of the produced fraction (Table 2).

Table 2. Yields (% wt.) of the obtained fractions after one-step fractionation of grape seeds.

% wt.	250ºC	300ºC	340ºC
SR1	30.9±1.3	18.6±0.9	12.3±0.9
SRL0	10.7±2	22.6±4.4	28.6±2.3
SR2	4.8±3.1	9.7±1.6	13.3±2.5
SR	35.8±4.3	28.3±2.5	25.6±3.4
LBO	8.1±1.6	15.6±3.1	15.7±3.2
HBO1	7.9±2	0.5±0.4	0.3±0.2
HBO2	5.3±1.3	10.1±0.8	15.9±8.3
HBO	13.2±3.3	10.6±1.3	16.2±8.5
BO	21.3±4.9	26.1±4.4	32±11.7
SG	23.2±1.5	26.8±1.5	28.8±1.7
R	80.2±10.7	81.2±8.4	86.3±16.8

The amount of solid residue inside the reactor (SR1) decreased exponentially when the temperature was increased. The analysis of the solid obtained after the treatment at 250ºC revealed that more than 98% wt. was Klason lignin, so all the hemicellulose and cellulose fractions were hydrolysed. Although lignin was slightly degraded in water, it was observed that the total recovery of lignin as solid decreased with increasing the temperature. Under hydrothermal treatment, the residual lignin has an increased active area that may enhance the decomposition, as it can be inferred later from the carbonization photographs [34]. The amount of solid in the liquid phase (SR2) increased along with temperature (see Figure 3). The total amount of solids decreased with increasing temperature, suggesting that at higher temperatures the kinetics of hydrolysis are faster. Similarly, Mehmet et al. found that the solid residue yields decreased when the residence time was increased at all experimental temperatures [17].

Similarly, the total obtained amount of Bio-oil (BO=LBO+HBO) increased along with temperature. The same behaviour was observed by Mehmet et al. The highest yield of BO was 32.0% wt. at 340ºC. Cellulose and hemicellulose are firstly hydrolysed into

sugars and then, the sugars can be converted into different compounds such as ketones and aldehydes via retro-aldol condensation and dehydration reactions [35]. These reactions are favoured at higher temperatures (increment of HBO) improving the yield of BO. It was also observed that the highest yield of HBO1 (bio-oil retained inside the solid) was obtained at the lowest experimental temperature. The hydrolysis process produced LBO yield between 8.1% and 15.7% wt. Tekin et al. studied the hydrothermal liquefaction of beech wood without and with colemanite. They reported that the amount of LBO increased with increasing temperatures (from 250ºC to 300ºC). The results of our study are in good agreement with this previous research [18].

The aqueous-soluble products were obtained by filtration and subsequent drying (fraction SG). The yield of SG was increased from 23.2% wt. to 28.8% wt. when temperature was increased from 250ºC to 340ºC. Considering that, the aqueous products (SG) were mainly composed of sugars, and the yield obtained was close to the cellulose and hemicellulose content in the raw material. The mass balance of the experiments (R) was between 80-86% wt. of the initial product (see R in Table 2 and Figure 3). These mass balance values are acceptable considering the small scale used (ca. 4.0 g of grape seeds) and the difficulty of accounting for the sugars provided: a) only an aliquot was dried, b) it was difficult to homogenise the hydrolysed product.

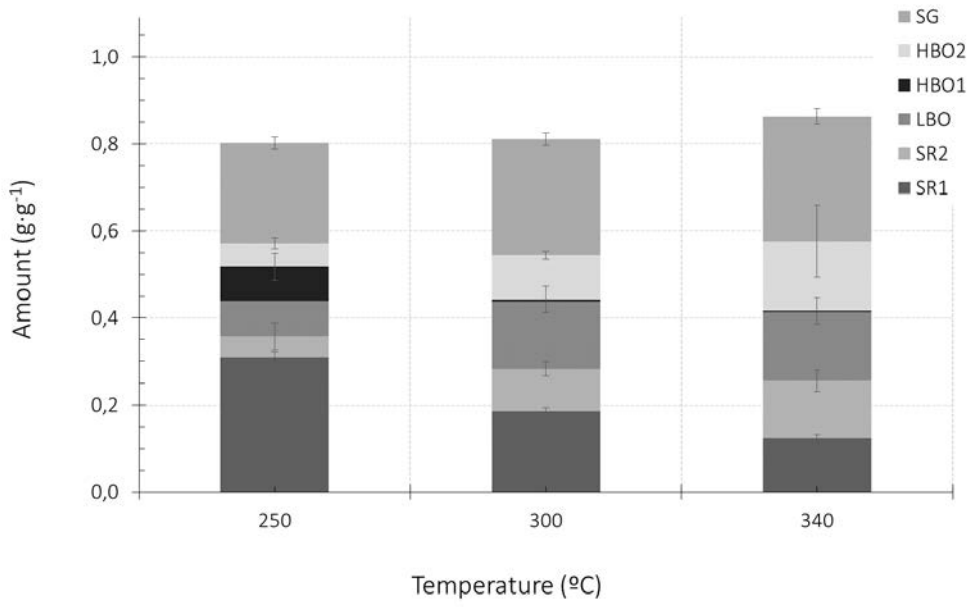

Figure 3. Variation of hydrolysis products with the temperature.

The solid samples obtained in SR1 as well as the raw material were analysed by TGA (Figure 4 and 5). Thermogravimetric Analysis (TGA) provides an idea of how the hydrolysis process has extracted the oil, hemicelluloses, celluloses and lignin, by analysing the behaviour of the sample under gasification conditions under inert atmosphere (N2). The TGA of grape seeds is labelled as 'Grape seeds' and the TGA for the obtained solids at 250ºC, 300ºC and 340ºC are labelled 'SR 250ºC', 'SR 300ºC' and 'SR 340ºC' respectively.

The analysis in Figure 4 is referred to the mass of the sample after the treatment, for this reason all the curves start from 100% (the values of the final solid residue are listed in Table 2) while that in Figure 5 the TGA is referred to the initial mass of grape seeds. The curves have a sigmoidal shape and the most degraded samples by hydrothermal treatment were less gasified in the TGA. At about 625ºC all the curves exhibited a plateau (see 'Grape seeds' curve) indicating that the remaining biomass have produced char which was non-degradable by gasification. The ash content was determined by totally oxidising the sample. For this, the gas phase was switched from N2 (inert) to air. The approx. ash content was 2.4% wt. The TGA results of the samples SR 250ºC and SR 300ºC were similar and the curves overlap (Figure 4). This phenomenon suggests that the solid composition after hydrolysis may not be altered at temperatures between 250ºC and 300ºC. On the contrary, when the hydrolysis was carried out at 340ºC, almost all the extractable and hydrolysable components were gone, and the remaining material accounts for ca. 12% wt. of the initial seeds. This value was low compared to the previous lignin contents in grape seeds reported in literature (43% wt.) [31, 32] and also the value obtained in this work of 43.8% wt. This indicates that part of the lignin was probably extracted or hydrolysed too.

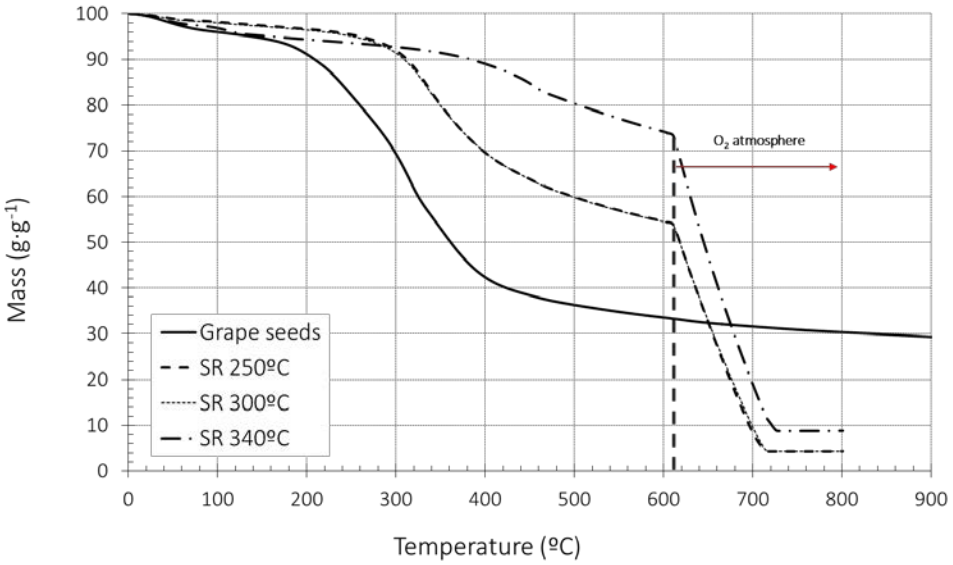

Figure 4. TGA analysis of the solids from hydrolysis and the raw material.

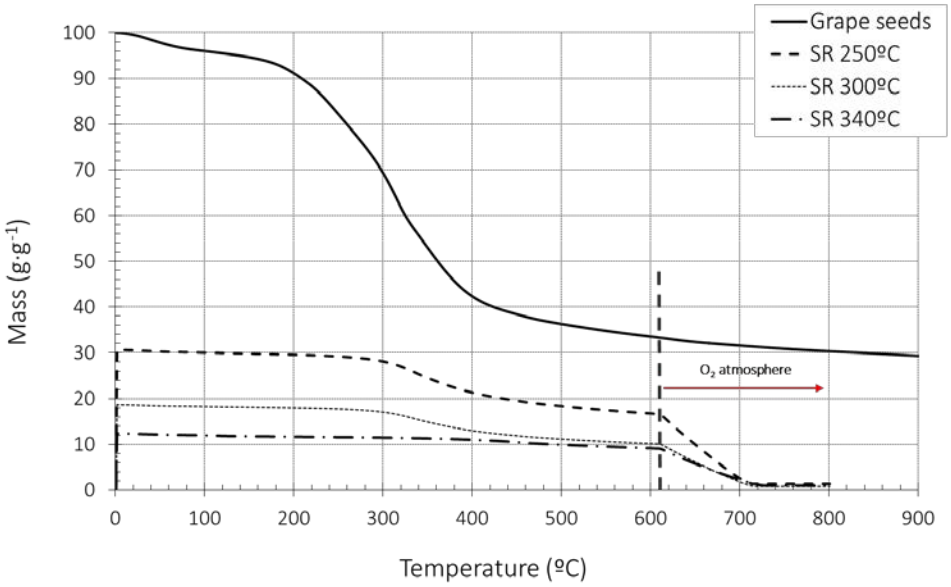

Figure 5. TGA analysis of the solids from hydrolysis and the raw materials referred to the grape seed raw material.

The grape seeds exhibited a plateau around 400ºC during gasification with a yield between 43-45% wt. The inflexion point observed near 400ºC is related to the lignin content of the grape seeds. The extraction with water might modify the structure of the grape seeds so that the final char produced in TGA varied from ca. 54% wt. relative (Figure 4) and 17% wt. absolute (Figure 5) at 250ºC and to around 74% wt. relative (Figure 4) and 10% wt. absolute (Figure 5) at 300ºC - 340ºC. The HBO1 was 7.90% wt. at 250ºC and less than 0.10% wt. in both 300ºC and 340ºC. This can be detected also in

the TGA curve at 250ºC, in the values of temperatures between 275ºC and 420ºC, where the mass loss was ca. 8% - 10% wt. (Figure 5), similar to the HBO1 value. At 340ºC, less than 1% wt. (Figure 5) of mass loss was observed in the same range of temperature. This does not mean that the TGA step indicates directly the HBO trapped in the solid residue, but they are related. At 300ºC the HBO content was almost negligible. This was probably because the HBO was dissolved by the flowing water. There are not specific studies in the solubility of bio-oil in water at elevated temperatures presented in literature up to the best of our knowledge. Nevertheless, as an estimation from works related to fatty acids, the solubility at 250ºC may round 2-20 $g \cdot L^{-1}$ (0.20%), while at 300ºC will be close to 30-100 $g \cdot L^{-1}$ (3.00-10.0%) and at 340ºC would be completely soluble if they have not been hydrolysed yet [36, 37].

Figure 6 shows the SEM images of the solid residue obtained after the hydrothermal treatment. The hydrothermal carbonization (HTC), with and without catalysts (such as KOH, etc.), is a process in which nanostructures are created in the carbonized material increasing its adsorption capacity considerably [34]. Unur et al. have recently demonstrated the effectiveness of the hydrothermal treatment to produce high capacity adsorbents for batteries at temperatures up to 600ºC [38].

The samples obtained at 250ºC presented structures similar to those reported and illustrated by other authors [39, 40]. The micrographs of the samples did not show sugar crystals probably because most of the sugars were hydrolysed and dissolved. Thus, a solid residue with a high purity in Klason lignin was obtained. In all cases shown in Figure 5, it can be observed a disorganization in the fibres, indicating that almost all the hemicellulose was removed. These results indicated that the solid residue was depleted in hemicellulose, which agrees with FTIR and TGA analyses. Also, the formation of carbon spheres in the solid residue was observed at 340ºC. Sevilla and Fuertes indicated that the presence of carbon spheres depend on the temperature of hydrolysis process, the reaction time, the concentration of the saccharides solution, etc [41]. Recently, Reddy et al. have presented in a conference that these spheres may come from the dissolved and re-precipitated lignin [42].

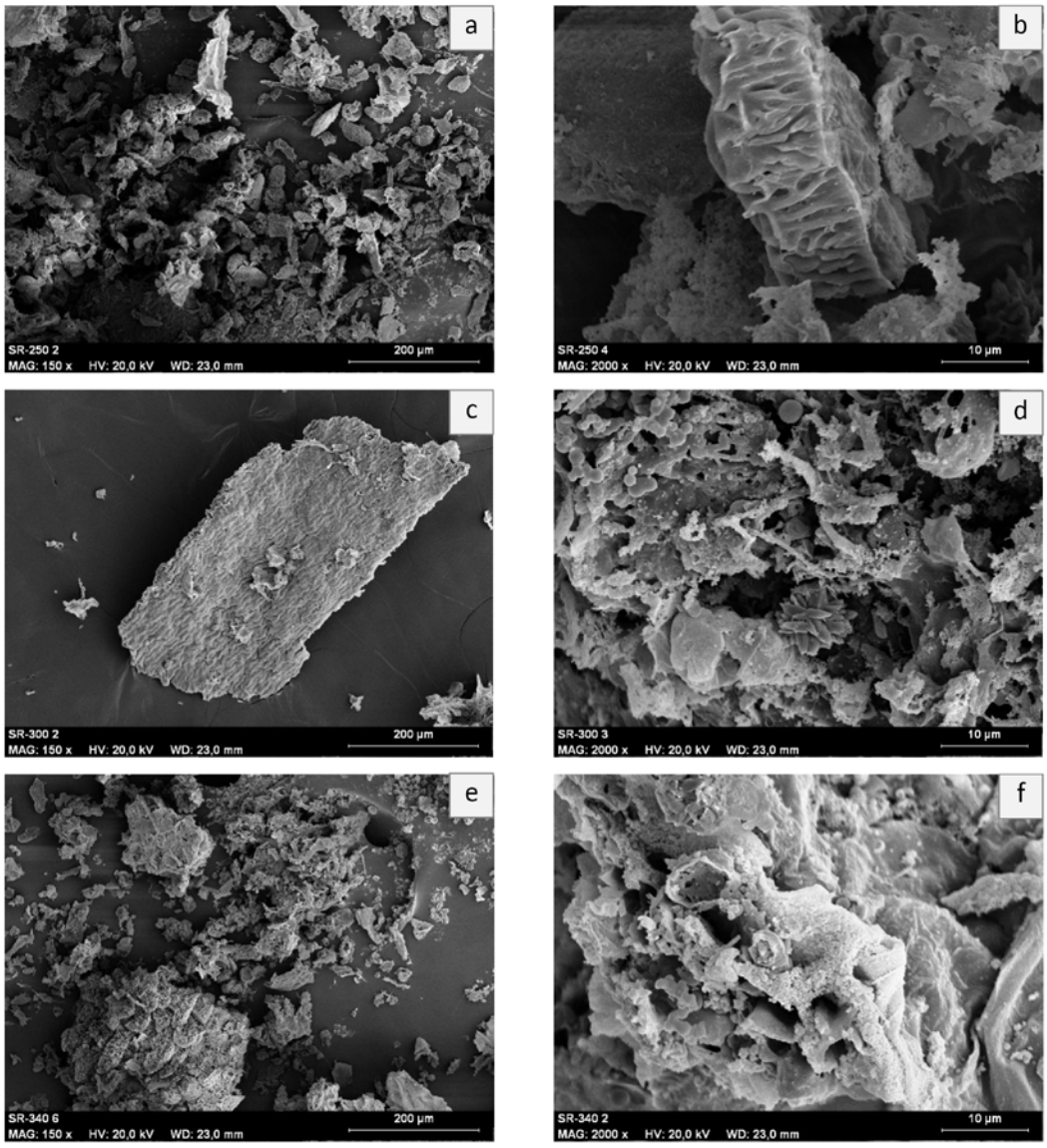

Figure 6. SEM images of the solid residue when grape seeds were treated to different temperatures for one hour. Solid residue (SR) with reaction at 250ºC (a-b), SR at 300ºC (c-d) and SR at 340ºC (e-f).

The effect of the treatment over the solid samples was also analysed by FT-IR essays. The FT-IR spectrum of the raw material as well as the SR 300ºC and SR 340ºC are shown in Figure 6. The main FT-IR bands detected are listed in Table 3.

As shown in Figure 7, the spectra display several absorption peaks indicating the complex nature of the raw material and solid residue. Significant differences can be distinguished in the three samples. The bands in the region 2882-2942 cm^{-1} are associated with u(C-H) stretch in methyl and methylene groups. A reduction in the absorbance intensity of these bands located between in the spectra of the sample SR 340ºC with respect to the others samples were noted. The band at 1747 cm^{-1} is

characteristic of ester-linked acetyl, feruloyl and p-coumaroyl groups between hemicellulose and lignin [43, 44]. The lack of this band in the treated samples (it was only observed in the untreated grape seeds) suggest that the links between hemicellulose and lignin were broken during the hydrothermal treatment.

The band at 1717 cm^{-1} is characteristic of u(C=O) of ketone, carboxyl and ester groups of hemicellulose. Thus, the band at 1717 cm^{-1} was observed with more intensity in the grape seeds that in SR 300ºC and 340ºC indicating that the linkage between lignin and hemicellulose was broken, similarly.

The typical bands at 1515, 1465, 1424 and 1375 cm^{-1} are characteristics of lignin [45] and they were observed in the three cases.

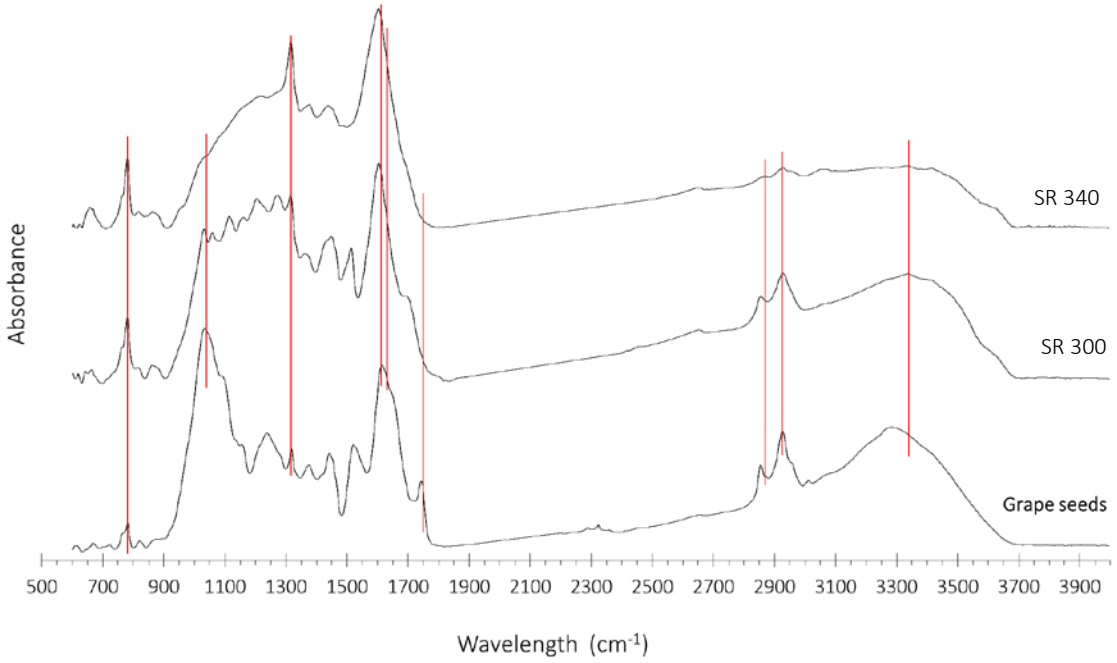

Figure 7. FTIR analysis of the raw material and solid product.

The bands at 1608 cm^{-1} and 1632 cm^{-1} are associated with the presence of lignin [46]. The presence of these bands was observed in all cases. Similarly, the aromatic ring bands at 777 cm^{-1} were also identified.

The band at 2924 cm^{-1} is associated with aliphatic -CH_2- which is a typical band of cellulose. This band can be observed in the three solid residual samples, but it is observed with less intensity as the operating temperature was increased [17].

Table 3. Assignment of Bands in FT-IR Spectra of the grape seeds and solid residues at 300ºC and 340ºC.

Wavelength, cm^{-1}	Assignment[43, 46]
777	C–H deformation out of plane, aromatic ring
1037	C-O stretching vibration
1317	Aryl ring breathing with C–O stretch
1375	Existence of guaiacyl and syringyl groups
1402	C-H deformation
1424	C-C bounds and aromatic ring vibration of the phenylpropane groups
1465	C-H vibration of CH2 and CH3 groups and deformations and aromatic ring vibrations
1513	C-C bounds and aromatic ring vibrations of the phenylpropane groups
1608	Aromatic skeletal modes
1632	C=C benzene stretching ring
1717	C=O stretch, unconjugated ketone, carboxyl, and ester groups
1747	Ester-linked acetyl, feruloyl and p-coumaroyl
2868	C–H stretch in methyl and methylene groups
2933	C–H stretch methyl and methylene groups
3340	O–H stretch, H-bonded

As it can be seen in Figure 7, reduction in the absorbance of the typical bands of hemicellulose and cellulose at 2868, 2933 and 3340 cm^{-1} after the treatment at 300 and 340ºC suggest that these fraction were hydrolysed.

Similarly, the aromatic ring bands are kept in the treated biomass.

3.2. Determination of Arrhenius parameters

To determine the kinetics in process of hydrolysis of lignocellulosic biomass is common to use simplified models. For instance, many authors use the severity factor [47-50]. Others, as it has been done in this work, use directly the zero or first order kinetics [51-54].

The effect of temperature in the hydrolysis rate was studied by determining the solid after 60 min of hydrothermal treatment. The analysed temperatures were: 150, 175, 200, 275, 300, 325 and 340 ºC.

It was assumed that the observed reaction rate behaved following a zero order reaction (not depending on the concentration of the remaining biomass). Thus, the reaction rate was calculated as the mass of biomass degraded per time (in this case 60 min). The results are depicted in an Arrhenius plot in Figure 8. The pre-exponential factor of Arrhenius relationship was k_0 = 0.995 $g \cdot min^{-1}$ and the activation energy was E_a = 13.8 $kJ \cdot mol^{-1}$. The regression coefficient R^2=0.98 shows a good relation between

experimental data and predicted data, indicating that this model is representing the process behaviour.

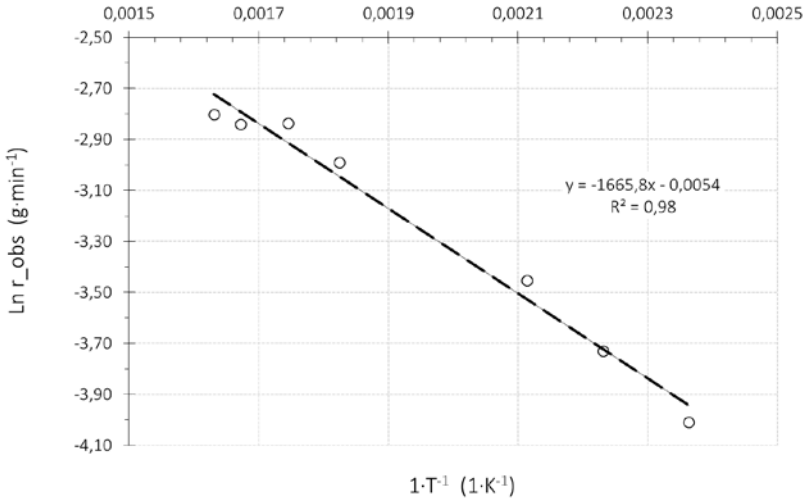

Figure 8. Arrhenius plot for the observed hydrolysis rate.

A direct comparison of the kinetic parameters is difficult due to the differences in substrate materials, kinetics models and differences in the process.

The study of production of xylose from sugar cane bagasse by acid hydrolysis was carried by Aguilar et al. [52] They used temperatures between 100 to 128ºC and concentrations of sulphuric acid between 2% to 6%. The activation energy average values reported were between 110.9 to 159.6 $kJ \cdot mol^{-1}$. Those values are similar to others authors for other lignocellulosic biomass [51, 53, 54]. The difference of values of energy activation between the literature and this study is significant, however the difference in the biomass used and the presence of acid in the process can modify considerable the behaviour. Also, the existence of big amount of extractives in the raw material can influence in the reduction of activation energy, as in the case of grape seeds.

3.3. Effect of the flow rate

The effect of the flow rate was analysed at 250ºC and 50 barg varying the flow rate from 2 to 10 $mL \cdot min^{-1}$. The reaction time was 60 min. The results for the observed reaction rate (r_obs) is shown in Figure 9.

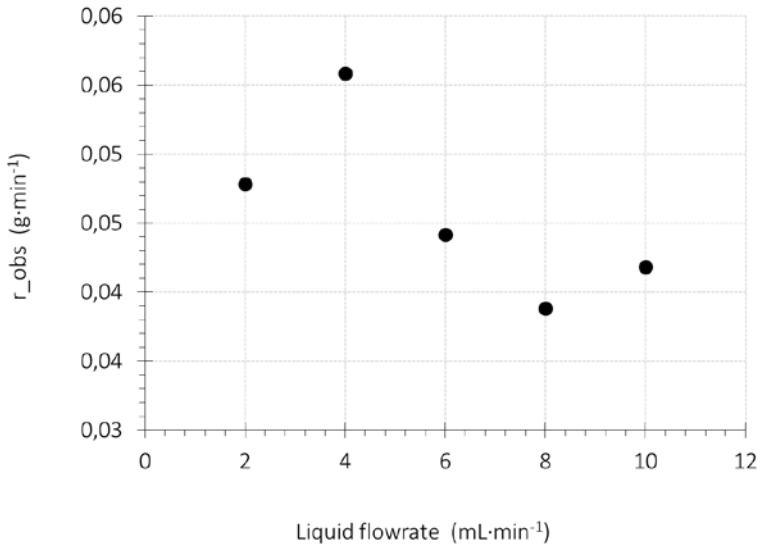

Figure 9. Effect of the flowrate in the hydrolysis rate and final solid residue at 250ºC.

The effect of flow on hydrolysis of hemicellulose from corn stover at 180, 200 and 220ºC and at flow rates of 0, 1 and 10 mL·min^{-1} in a tubular flowthrough reactor was studied. In this study, the authors concluded that the solubilisation of hemicellulose increased with flow [27].

The effect of flow rate in reaction kinetic is related to the mass transfer. It was found that a flow rate of 4 mL·min^{-1} is the best alternative to maximize the reaction rate of hydrolysis. The rate of reaction increased when the flow rate was incremented from 2 mL·min^{-1} to 4 mL·min^{-1}. However, the reaction rate decreased when the flow rate was increased at values higher than 4 mL·min^{-1}.

The decrease in the r_obs could be observed because of back-mixing due to the excess of velocity and also due to preferential ways in the fixed bed.

4. Conclusions

The production of bio-oils from grape seeds using a hydrothermal medium was studied at temperatures between 250ºC and 340ºC. Monitoring of yields obtained from grape seeds can be used as indicator on the trends during the process. The hydrolysis process produced LBO yield between 8.1% - 15.7% wt., HBO yield between 10.6% - 16.2% wt. and the solid residue was between 25.6% - 35.8% wt. referred to the mass initial of grape seeds. The mass balance or the system was ca. 80.2-86.3% wt.

The Arrhenius parameters determined for kinetics of hemicelluloses and celluloses hydrolysis between (TT) were k_0 =0.995 g·min^{-1} with an activation energy E_a= 13.8 kJ·mol^{-1}.

The largest amount of extractable and hydrolysable compounds was obtained at 340°C. The HBO obtained from the solid residue inside the reactor decreased as the temperature was increased. It was probably because it was dissolved by the flowing water (solubility increases with temperature). The total amount of solid residue decreased when temperature was increased, this would be because of lignin degradation.

TGA analysis showed that the structure of the grape seeds were modified after the treatment. The FT-IR spectra revealed that the main aromatic groups were preserved in the solid residue, while the linkage between hemicellulose and lignin was broken.

In the next work, the combination of solvothermal extraction with hydrothermal fractionation-hydrolysis using a semicontinuous reactor will be investigated. The effect of the temperature on the amount of hydrolysed C5, C6 and oligomers will be studied.

Acknowledgements

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References

[1] J.H. Clark, V. Budarin, F.E.I. Deswarte, J.J.E. Hardy, F.M. Kerton, A.J. Hunt, R. Luque, D.J. Macquarrie, K. Milkowski, A. Rodriguez, O. Samuel, S.J. Tavener, R.J. White, A.J. Wilson, Green chemistry and the biorefinery: A partnership for a sustainable future, Green Chemistry 8 (2006) 853-860.

[2] T.E.P.S. Organisation, THE EUROPEAN BIOECONOMY IN 2030. Delivering Sustainable Growth by addressing the Grand Societal Challenges, 2011, pp. 1-24.

[3] C.B. Field, M.J. Behrenfeld, J.T. Randerson, P. Falkowski, Primary production of the biosphere: Integrating terrestrial and oceanic components, Science 281 (1998) 237-240.

[4] J.J. Bozell, Feedstocks for the future - Biorefinery production of chemicals from renewable carbon, Clean - Soil, Air, Water 36 (2008) 641-647.

[5] S. Cheng, S. Zhu, Lignocellulosic feedstock biorefinery-the future of the chemical and energy industry, BioResources 4 (2009) 456-457.

[6] J.V. Galván, J.J.J. Novo, A.G. Cabrera, D. Ariza, J. García-Olmo, R.M.N. Cerrillo, Population variability based on the morphometry and chemical composition of the acorn in Holm oak (Quercus ilex subsp. ballota [Desf.] Samp.), Eur J Forest Res 131 (2012) 893–904.

[7] G. Gea-Izquierdo, I. Cañellas, G. Montero, Acorn production in Spanish holm oak woodlands, Invest Agrar: Sist Recur For 15(3) (2006) 339-354.

[8] B. Acharya, I. Sule, A. Dutta, A review on advances of torrefaction technologies for biomass processing, Biomass Conversion and Biorefineries 2 (2012) 349-369.

[9] P. Mäki-Arvela, T. Salmi, B. Holmbom, S. Willför, D.Y. Murzin, Synthesis of sugars by hydrolysis of hemicelluloses- A review, Chemical Reviews 111 (2011) 5638-5666.

[10] A.A. Peterson, F. Vogel, R.P. Lachance, M. Fröling, M.J. Antal Jr, J.W. Tester, Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies, Energy and Environmental Science 1 (2008) 32-65.

[11] L. Tock, M. Gassner, F. Maréchal, Thermochemical production of liquid fuels from biomass: Thermo-economic modeling, process design and process integration analysis, Biomass and Bioenergy 34 (2010) 1838-1854.

[12] L.-P. Xiao, Z.-J. Shi, F. Xu, R.-C. Sun, Hydrothermal carbonization of lignocellulosic biomass, Bioresource Technology 118 (2012) 619-623

[13] Z. Liu, A. Quek, S. Kent Hoekman, R. Balasubramanian, Production of solid biochar fuel from waste biomass by hydrothermal carbonization, Special Issue of The Second International Symposium on Gasification and its Application 103 (2013) 943-949

[14] A.V. Bridgwater, G.V.C. Peacocke, Fast pyrolysis processes for biomass, Renewable and Sustainable Energy Reviews 4 (2000) 1-73

[15] B.M. Wagenaar, W. Prins, W.P.M. van Swaaij, Pyrolysis of biomass in the rotating cone reactor: modelling and experimental justification, Chemical Engineering Science 49 (1994) 5109-5126.

[16] FAOSTAT, Production of grapes from 2003-2013, Food and Agriculture Organization of the United Nations, 2014.

[17] M.K. Akalin, K. Tekin, S. Karagöz, Hydrothermal liquefaction of cornelian cherry stones for bio-oil production, Bioresource Technology 110 (2012) 682-687.

[18] K. Tekin, S. Karagöz, S. Bektaş, Hydrothermal liquefaction of beech wood using a natural calcium borate mineral, Journal of Supercritical Fluids 72 (2012) 134-139.

[19] Y. Huang, S. Kudo, O. Masek, K. Norinaga, J.I. Hayashi, Simultaneous maximization of the char yield and volatility of oil from biomass pyrolysis, Energy and Fuels 27 (2013) 247-254.

[20] U. Hasanah, B. Setiaji, Triyono, C. Anwar, The Chemical Composition and Physical Properties of the Light and Heavy Tar Resulted from Coconut Shell Pyrolysis, J. Pure App. Chem. Res. 1 (2012) 26-32.

[21] X. Guo, S. Wang, Q. Wang, Z. Guo, Z. Luo, Properties of Bio-oil from Fast Pyrolysis of Rice Husk, Chinese Journal of Chemical Engineering 19 (2011) 116-121.

[22] J.A. Capunitan, S.C. Capareda, Characterization and separation of corn stover bio-oil by fractional distillation, Fuel 112 (2013) 60-73

[23] F.M. Gírio, C. Fonseca, F. Carvalheiro, L.C. Duarte, S. Marques, R. Bogel-Łukasik, Hemicelluloses for fuel ethanol: A review, Bioresource Technology 101 (2010) 4775-4800.

[24] C. Schacht, C. Zetzl, G. Brunner, From plant materials to ethanol by means of supercritical fluid technology, Journal of Supercritical Fluids 46 (2008) 299-321.

[25] S.P.M. Da Silva, A.R.C. Morais, R. Bogel-Łukasik, The CO2-assisted autohydrolysis of wheat straw, Green Chemistry 16 (2014) 238-246.

[26] J.B. Sluiter, R.O. Ruiz, C.J. Scarlata, A.D. Sluiter, D.W. Templeton, Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods, J. Agric. Food Chem. 58 (2010) 9043-9053.

[27] C. Liu, C.E. Wyman, The effect of flow rate of compressed hot water on xylan, lignin, and total mass removal from corn stover, Industrial and Engineering Chemistry Research 42 (2003) 5409-5416.

[28] A. Toledano, L. Serrano, J. Labidi, Extraction and revalorization of olive tree (Olea europea) pruning lignin, Journal of the Taiwan Institute of Chemical Engineers (2013).

[29] A. Toledano, L. Serrano, J. Labidi, Process for olive tree pruning lignin revalorisation, Chemical Engineering Journal 193-194 (2012) 396-403.

[30] K. Wang, H. Yang, X. Yao, F. Xu, R.C. Sun, Structural transformation of hemicelluloses and lignin from triploid poplar during acid-pretreatment based biorefinery process, Bioresource Technology 116 (2012) 99-106.

[31] D. Moldes, P.P. Gallego, S. Rodriguez Couto, A. Sanroman, Grape seeds: The best lignocellulosic waste to produce laccase by solid state cultures of Trametes hirsuta, Biotechnology Letters 25 (2003) 491-495.

[32] M. Spanghero, A.Z.M. Salem, P.H. Robinson, Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces, Animal Feed Science and Technology 152 (2009) 243-255.

[33] N.G. Baydar, M. Akkurt, Oil content and oil quality properties of some grape seeds, Bazi Üzüm Çekirdeklerinin Yağ Içeriği ve Yağ Kalite Özellikleri 25 (2001) 163-168.

[34] B. Hu, K. Wang, L. Wu, S.H. Yu, M. Antonietti, M.M. Titirici, Engineering carbon materials from the hydrothermal carbonization process of biomass, Advanced Materials 22 (2010) 813-828.

[35] M. Sasaki, K. Goto, K. Tajima, T. Adschiri, K. Arai, Rapid and selective retro-aldol condensation of glucose to glycolaldehyde in supercritical water, Green Chem. 4 (2002) 285-287.

[36] A.L. Milliren, J.C. Wissinger, V. Gottumukala, C.A. Schall, Kinetics of soybean oil hydrolysis in subcritical water, Fuel 108 (2013) 277-281.

[37] P. Khuwijitjaru, S. Adachi, R. Matsuno, Solubility of saturated fatty acids in water at elevated temperatures, Bioscience, Biotechnology and Biochemistry 66 (2002) 1723-1726.

[38] E. Unur, S. Brutti, S. Panero, B. Scrosati, Nanoporous carbons from hydrothermally treated biomass as anode materials for lithium ion batteries, Microporous and Mesoporous Materials 174 (2013) 25-33.

[39] C. He, A. Giannis, J.-Y. Wang, Conversion of sewage sludge to clean solid fuel using hydrothermal carbonization: Hydrochar fuel characteristics and combustion behavior, Applied Energy 111 (2013) 257-266.

[40] S.M. Heilmann, L.R. Jader, M.J. Sadowsky, F.J. Schendel, M.G. von Keitz, K.J. Valentas, Hydrothermal carbonization of distiller's grains, Biomass and Bioenergy 35 (2011) 2526-2533.

[41] M. Sevilla, A.B. Fuertes, Chemical and structural properties of carbonaceous products obtained by hydrothermal carbonization of saccharides, Chemistry - A European Journal 15 (2009) 4195-4203.

[42] P. Reddy, P. Lekha, W. Hüppop, C. Kirsch, Structural characterization of pretreated solids from liquid hot water treatment of South African sugarcane bagasse in a flow-through reactor, RRB-10, Valladolid (Spain), 2014.

[43] C. Heitner, D.R. Dimmel, J.A. Schmidt, Lignin and lignans. Advances in chemistry., CRC Press, New York, 2010.

[44] K. Bilba, A. Ouensanga, Fourier transform infrared spectroscopic study of thermal degradation of sugar cane bagasse, Journal of Analytical and Applied Pyrolysis 38 (1996) 61-73.

[45] P. Manara, A. Zabaniotou, C. Vanderghem, A. Richel, Lignin extraction from Mediterranean agro-wastes: Impact of pretreatment conditions on lignin chemical structure and thermal degradation behavior, Catalysis Today 223 (2014) 25-34.

[46] K. Bilba, A. Ouensanga, Fourier transform infrared spectroscopic study of thermal degradation of sugar cane bagasse, Journal of Analytical and Applied Pyrolysis 38 (1996) 61-73

[47] S. Mateo, J.G. Puentes, S. Sánchez, A.J. Moya, Oligosaccharides and monomeric carbohydrates production from olive tree pruning biomass, Carbohydrate Polymers 93 (2013) 416-423.

[48] Y. Kim, T. Kreke, N.S. Mosier, M.R. Ladisch, Severity factor coefficients for subcritical liquid hot water pretreatment of hardwood chips, Biotechnology and Bioengineering 111 (2014) 254-263.

[49] P. Khuwijitjaru, A. Pokpong, K. Klinchongkon, S. Adachi, Production of oligosaccharides from coconut meal by subcritical water treatment, International Journal of Food Science and Technology (2014).

[50] I.N. Ahmed, S. Sutanto, L.H. Huynh, S. Ismadji, Y.H. Ju, Subcritical water and dilute acid pretreatments for bioethanol production from Melaleuca leucadendron shedding bark, Biochemical Engineering Journal 78 (2013) 44-52.

[51] M.T. Maloney, T.W. Chapman, A.J. Baker, Dilute acid hydrolysis of paper birch: kinetics studies of xylan and acetyl-group hydrolysis, Biotechnology and bioengineering 27 (1985) 355-361.

[52] R. Aguilar, J.A. Ramírez, G. Garrote, M. Vázquez, Kinetic study of the acid hydrolysis of sugar cane bagasse, Journal of Food Engineering 55 (2002) 309-318.

[53] N. Eken-Saraçoğlu, S.F. Mutlu, G. Dilmaç, H. Çavuşoğlu, A comparative kinetic study of acidic hemicellulose hydrolysis in corn cob and sunflower seed hull, Bioresource Technology 65 (1998) 29-33.

[54] N. Bhandari, D.G. Macdonald, N.N. Bakhshi, Kinetic studies of corn stover saccharification using sulphuric acid, Biotechnology and bioengineering 26 (1984) 320-327.

Chapter 2

Hydrothermal fractionation of grape seeds in subcritical water to produce oil extract, sugars and lignin

Abstract

In this work, the fractionation of grape seeds as a model biomass was studied using a combination of two processes: solvothermal extraction and hydrothermal fractionation-hydrolysis process in a semicontinuous reactor. First, grape seeds were subjected to an extraction process with ethanol/water (70/30% wt.) at 90ºC during 60 min obtaining ca. 13.0% wt. of oil and extractable components with 4.46% wt. of polyphenols (66% of the maximum). Afterwards, the solvent was water and the biomass was treated in steps at different temperatures (150ºC to 340ºC). During the hydrolysis the pH decreased from 5.5 down to 3.0 due to acetyl group liberation. The total quantity of recovered sugars varied around 20.0 to 23.1% wt. The best experimental condition for obtaining the maximum amount of pentoses + hexoses + oligosaccharides was 180ºC (45min) + 250 to 265ºC (45 min) + 330 to 340ºC (45 min).

Keywords: Biomass, biorefinery, hydrothermal, pretreatment, carbonization

1. Introduction

The most abundant source of carbon in the world is biomass. Nowadays, the global primary production of biomass rounds 100 $PgC \cdot y^{-1}$ (P = Peta = 10^{15}) [1], being the global carbon emissions nearly 10 $PgC \cdot y^{-1}$ [2]. The production of fuels and value added products from biomass has been widely investigated in the last years. Like any other lignocellulosic residue, the grape seeds primarily comprises three major fractions: hemicellulose, cellulose and lignin. In addition, low amounts of minerals (ash) and other compounds, such as extractives, can be found in grape seeds (i.e. grape seed oil and polyphenols).

There are several solvents available for performing the biomass fractionation, such as organic solvents [3], ionic liquids [4] or simply water [5-7]. Fractionation is often considered a pre-treatment process, prior to produce sugars and lignin by conversion. After this process, there are many alternatives for conversion to produce value added components by catalytic or non-catalytic transformations [8]. In 2004 the U.S. Department of Energy and Renewable Energy prepared a comprehensive report including series of compounds and building blocks chosen as candidates to be produced from cellulose, hemicellulose [9, 10] and lignin [11].

Hydrothermal treatment is the processing of biomass in water at high pressures and temperatures in liquid phase. This technology is a promising alternative to perform the fractionation of biomass because the reaction medium allows the transformation of the different fractions of biomass by choosing the appropriate conditions [12, 13]. Furthermore, water is a clean, safe and environmentally benign solvent [14]. Hydrothermal fractionation can be carried out at soft conditions (<100ºC) to remove the water-soluble extractives and hydrolyse hemicelluloses (<180ºC), yielding a solid phase enriched in lignin and cellulose. The autohydrolysis process (reactions catalysed by H^+ and OH^- produced by H_2O dissociation [13-15] due to the acetyl group liberation and organic acids produced) can produce oligosaccharides that maintain the polymeric structure. A subsequent hydrolysis at more severe conditions or with enzymes will yield monomeric sugars [16]. On the other hand, hydrothermal carbonization can be an option when the production of a carbon-based nanomaterial is pursued.

The hydrothermal treatment can be used for a double fold, to produce bio-oils or to produce bio-based streams (C5's, C6's and lignin) [17]. Analysing the production of bio-based streams, Requejo et al. demonstrated that the use of olive tree biomass to produce soluble hemicellulose-derived saccharides can be carried out in a hydrothermal medium obtaining near 26% of the original biomass as soluble sugars. In addition, the degree of enzymatic hydrolysis of that product was about 80%, obtaining in this way, a highly fermentable stream (70% conversion in ethanol) [18]. Grenman et al. obtained a rich hemicellulose fraction from spruce in an intensified reactor, determining the kinetics of fractionation. They found that the activation energy was 135 $kJ \cdot mol^{-1}$ at 150-170ºC [19]. Recently, a comprehensive study in the hydrothermal treatment of sugar cane bagasse has been carried out by Prado et al. [20, 21]. The sugar cane bagasse (between 2 g and 11 g) was hydrolysed in a semi-batch reactor (50mL) at flow-rates between 11 $mL \cdot min^{-1}$ and 55 mL/min and temperatures from 213ºC to 290ºC in liquid phase (at 20 MPa). The main products analysed from sugar cane bagasse were arabinose, fructose, galactose, glucose, mannose, xylose and cellobiose as well as some inhibitors such as 5-hydroxymethylfurfural, 4-hydroxybenzoic acid, vanillin, etc. They found that the all hemicelluloses were hydrolysed at 190-230ºC during the first 2 to 15 min of process time.

Recently, one of the first processes for sugar production from second generation biomass using supercritical water has been started-up by the company RENMATIX [22, 23]. They base the fractionation process in the hydrolytic process itself. The process has been studied thoroughly by Cantero et al. using a reactor operating at supercritical conditions and extremely low residence times, below 1 s [24].

The wine industry produces ca. 28% of lignocellulosic residues in weight basis of the used biomass (5% stalks and 23% grape seeds). The grape seeds contain one of the highest composition of lignin in nature, ca. 43% wt. [25, 26], being lignin a component of interest due to its direct applications in the chemical industry. Also, the seeds and the skin contain a great quantity of high value polyphenols that can be extracted using ethanol-water or methanol-water mixtures at low temperatures (from 20 to 60ºC), avoiding in this way the degradation of the polyphenols [27, 28].

The grape marc (mixture of grape seeds, skin and pulp) contains phenolic acids, coloured anthocyanins (important antioxidant activity), simple flavonoids and complex flavonoids, non-flavonoid compounds, flavonols and polyphenolic tannins. At the present time, these components are important in different industries such as food, cosmetic and pharmaceutical, mainly extracted from residual sources [27]. Thus, according to a recent market survey the global polyphenols market was ca. 12200 tons in 2011 (USD 580 million) and is expected to grow up to 21000 tons by 2018 (USD 873.7 million) [29].

The main compounds that can be found in grape seeds are: gallic acid, flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin 3-O-gallate, procyanidin dimers, trimmers and procyanidins [30]. Solýom et al. have demonstrated that the polyphenol compounds are stable at temperatures as high as 70ºC. In addition, they found that the extraction kinetics increased considerably by increasing temperature. Furthermore, it was observed that an ultrasound pretreatment did not make any further improvement in the extraction process. Therefore, the sole effect of the hydrothermal treatment was enough for an efficient extraction [31]. Additionally, the thermal degradation of grape marc was studied at different temperatures and it was concluded that the total phenol content was increased at temperatures above 100ºC. In the same way, the antioxidant activity did not change at 80ºC in the liquid extract, and the degradation was observed at 150ºC. Maier et al. studied yields of phenolic compounds in seven grape seeds with different solvents [32]. The use of pure ethanol resulted in poor polyphenol extraction. The products obtained from the extraction using ethanol or methanol mixtures with water as solvents contain higher quantities of anthocyanins and polyphenols than the extraction with water. In addition, the recovered amount of these components is increased when the extraction time was raised (1, 12 and 24 h).

The objective of this work was to study the influence of temperature in the solvothermal extraction and the hydrothermal fractionation-hydrolysis process of grape seeds. It has been studied the extraction of polyphenols (in gallic acid basis) and the production of pentose and hexose sugars fractions as well as the residual lignin.

2. Materials and methods

2.1. Materials

Grape seeds from Vitis vinifera L (Tempranillo) from Matarromera S.A. winery (Valbuena de Duero, Spain) campaign 2011 were used as raw material. The biomass material for this study was crushed using a mortar to a particle size between 0.5 to 1.0 mm. The chemical composition of the grape seeds was determined according to section 2.3 obtaining the following values: 2.4% wt. ash, 17.0% wt. grape seed oil, 43.8% wt. lignin and 36.8% wt. hemicelluloses and celluloses in dry basis.

The reagents used for HPLC analysis were: cellobiose (+98%), glucose (+99%), fructose (+99%), glyceraldehyde (95%), pyruvaldehyde (40%), arabinose (+99%), 5-hydroxymethylfurfural (99%), lactic acid (85%), formic acid (98%), acrylic acid (99%), mannose (+99%), xylose (+99%) and galactose (+99%) purchased from Sigma and used without further modification.

For the structural carbohydrates and lignin determination sulphuric acid (98%) and calcium carbonate (≥ 99.0%) were used as reagents supplied by Sigma.

For the determination of polyphenols content the following reagents were supplied by Sigma used: ethanol (96%), sodium carbonate (≥ 99.0%), Folin–Ciocalteu reagent (2N), Gallic acid monohydrate (≥ 98.0%). Distilled water and Milli-Q water were used in the experiments.

2.2. Experimental device

The fractionation process was carried out in a semi-continuous reactor. A diagram of experimental setup is shown in Figure 1. The reactor was charged with 4.00±0.06 g of grinded grape seeds (not dried before extraction, moisture 6.00±0.20% wt.). Two metallic filters at the top and the bottom of the reactor were used to keep the fixed bed and avoid the loss of the grape seeds particles. Once the reactor was tightened up, the pump (Jasco model PU-2080) was set to 5 mL/min and the pressure was set using the Go-backpressure valve (BPV-01). The pressure was selected always to 10 bar over the bubble point at the reaction temperature, to assure liquid phase. The system was cold-checked for leaks at this point. The reactor (R-01, 20 cm length, 1/2´ O.D. SS316 piping)

and preheater (E-01, 200 cm of 1/8´ AISI 316 piping) were placed inside a former chromatographic oven HP5680. The oven was then set to the desired reaction temperature and the cooling system was started up (E-02, 15 cm of concentric tube heat exchanger 1/4´-3/8´ counter-current operation).

The grape seeds were hydrothermally treated, through a multi-step two-solvent and temperature profile fractionation in order to separate the value added components from hemicelluloses, celluloses and lignin. During the first 60 min of treatment, the temperature and pressure were set at 90ºC 15 bar respectively using a mixture ethanol-water (70/30% wt.) as solvent with a flow rate of 5 $mL \cdot min^{-1}$. The aim of this first step was to extract the polyphenols and essential oils from the raw material before the fractionation-hydrolysis process. This step was a solvothermal extraction (organosolv step), the values of temperature and percentage of ethanol were selected taking into account the highest obtained yields in previous works in the field, e.g. Lapornik et al. [27] and the recommendations of Sólyom et al. [31]. Liquid samples of 10-15 mL were taken every 20 min.

After 60 minutes of solvothermal extraction, the solvent was changed to distilled water (for the Tests 1, 2 and 3) or to a mixture water-hydrogen peroxide (91/9% wt.) for the Test 4. The aim of this second treatment was to apply a hydrothermal fractionation-hydrolysis process. In order to set an appropriate reaction medium and temperature to each biomass fraction (hemicellulose, cellulose and lignin), three heating stages were applied to the biomass. Moreover, the temperature of each stage was changed. In the Test 1 the process temperature was set to 150ºC, 250ºC and 320ºC; in Test 2 and Test 4 the temperature was set to 165ºC, 265ºC and 330ºC and; in Test 3 the temperature was set to 180ºC, 280ºC and 340ºC. The treatment time for each temperature was 45 minutes. Thus, the total time for each experiment was 225 min (60min+45min+45min+45min). The pressure was set accordingly, to ensure liquid phase (depending on temperature) and liquid samples of 10-15 mL were taken every 10 min. After finishing the stepwise fractionation of biomass, the pump was set to zero flow and the oven temperature to 20ºC. The system was then slowly depressurized, the reactor was loosened and its contents were collected and washed in a filter with distilled water.

A sample of the final residual product (solid inside of the reactor after fractionation) and the liquid aliquots were analysed further as explained in section 2.3.

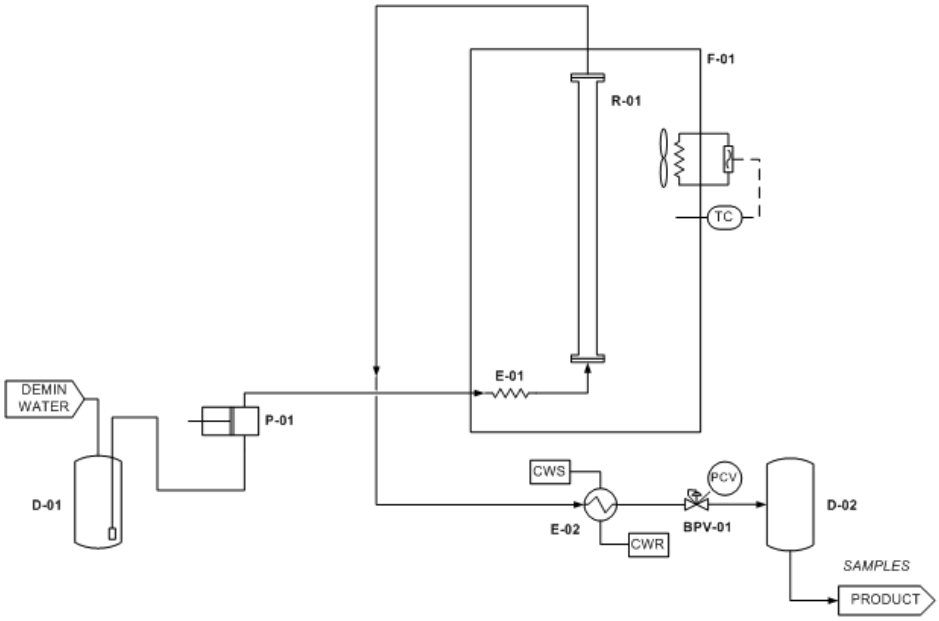

Figure 1. Schematic flow diagram of the experimental setup. Equipment: D-01 Feeder, P-01 Pump, E-01 Preheater, R-01 Reactor, F-01 Chromatographic oven, E-02 Heat exchanger, BPV-01 Go-backpressure valve, D-02 exit.

2.3. Analytical methods

2.3.1. Chemical characterization of the liquid product

During solvothermal extraction, liquid samples were taken to analyse the total polyphenol content in Gallic acid basis. On the other hand, the liquids samples taken during hydrothermal fractionation were analysed to determine the sugar content.

2.3.1.1. Polyphenols content

Total phenolic compound composition was determined as Gallic acid equivalent (GAE). An extract sample of 20 µL was diluted with water (1.5 mL) to which 100 µL undiluted Folin–Ciocalteu reagent were added. After 1 min, 300 µL of a saturated solution of sodium carbonate was added. Then, the sample was incubated at 40 ºC during 30 min, the absorbance was measured at 765 nm and it was compared to a prepared Gallic acid calibration curve.

2.3.1.2. Sugars content

The obtained samples after hydrothermal fractionation may contain oligomers; therefore, it was hydrolysed prior to HPLC analysis in order to determine the total sugar content mainly as glucose, fructose, xylose and arabinose. The samples were hydrolysed adding 3.00±0.01 mL of sulphuric acid (72%) to 15 mL of each aliquot. Each sample was incubated in a forced convection oven for 30±5 min at 30±3ºC. After this time, the samples were taken out from the oven, they were diluted with 84.00±0.04 mL of deionized water and finally they were placed in the oven for 1 hour at 121 ºC. Afterwards, the solution was cooled down to room temperature and it was filtered under vacuum. Before injecting in the HPLC the samples were neutralized to pH=6-7 using calcium carbonate.

The HPLC column used for the separation of the compounds was a SUGAR SH-1011 Shodex at 50 ºC at a flow of 0.8 $mL \cdot min^{-1}$ using a solution of 0.01N of sulphuric acid and Milli-Q water as a mobile phase. A Waters IR detector 2414 and Waters dual λ absorbance detector 2487 (210 nm and 254 nm) were used to identify the sugars and their derivatives. In order to identify the main peaks and calculate the areas of the complex mixture that it was obtained during the experiments, it has been used a band-analysis via Fast Fourier Transform (fft) and band-adjustment by Gaussian functions. The adjustment was done by minimizing the quadratic error using a Nelder-Mead algorithm.

The total content of sugars were quantified as shown in equation (1)

$$ST = C5 + C6 + O \quad (1)$$

The total content of pentoses (C5) and hexoses (C6) and oligosaccharides were quantified as shown in equations (2) and (3) respectively

$$C5 = M + G + X + A \quad (2)$$

$$C6 = C + G + F + Gl + P + LA + FA + AA + 5\text{-}HMF \quad (3)$$

Where ST are sugars total (g/g), C5 are pentose sugars (g/g), C6 are hexose sugars (g/g); M is mannose (g/g); G is galactose (g/g); X is xylose (g/g); A is arabinose (g/g); C: cellobiose (g/g); G is glucose (g/g); F is fructose (g/g); Gl is glyceraldehyde (g/g); P is

pyruvaldehyde (g/g); LA is lactic acid (g/g); FA is formic acid (g/g); AA is acrylic acid (g/g); 5-HMF is 5-hydroxymethylfurfural (g/g) and O are oligosaccharides (g/g).

2.3.2. Solid analysis. Klason lignin determination and sugars attached to the solid

The raw material and the residue inside of reactor were analysed for lignin content using the Klason assay according to the TAPPI standard method T-222 om-98 [33] . To do so, 300 mg of sample was put into laboratory glass bottles, 3 mL of sulphuric acid (72%) was added and it was incubated during 30 min at 30ºC and it was shaken vigorously every 5-10 min. Then, the mixture was diluted with 84 mL of deionized water and it was placed in an oven for 1 h at 121ºC. Then, the sample was taken out from the oven, cooled down to room temperature and the mixture was filtered under vacuum. The obtained solid after filtration was dried at 105ºC for 24 h, it was cooled down in a desiccator and then it was weighted. This solid was introduced in the calcination oven at 550ºC for 24 h to determine the ash content. Considering the weight differences, the Klason lignin content was calculated. The hydrolysis liquid was neutralized with calcium carbonate to pH=6-7, then it was filtered and analysed by HPLC as explained in section 2.3.1.2 Sugars.

2.3.3. Scanning electron microscopy (SEM)

The residue inside in the reactor was investigated by SEM. For scanning electron microscopy (SEM) a Jeol JSM-820 SEM and a gold evaporator Balzers SCD003 were used. Images were done in the high vacuum mode at an accelerating voltage of 20 kV, using secondary electrons.

3. Results and discussion

The grape seeds are composed of several value added compounds, i.e. grape seed oil and polyphenols and the typical biomass components i.e. hemicellulose, cellulose and lignin. Aimed at fractionating the grape seeds into these fractions, a multi-step process based on the polarity of the compounds and the temperature resistance was designed. Thus, a mixture of ethanol/water was used first at 90ºC to extract the oil and polyphenols, and then pure water at three temperature levels during the same experiment were used to hydrolyse hemicellulose, cellulose and lignin.

3.1. Solvothermal extraction

A mixture of ethanol and water (70/30 %wt.) was used to extract the grape seed oil and the polyphenols. Polyphenols are thermo-labile molecules, so it is important to extract them at low temperatures to avoid degradation. As it has been reported before elsewhere, grape seed content in polyphenols is 0.02-1.2 $mg \cdot g^{-1}$ (0.002% to 0.120% wt.) in dry basis [34].

In this work, the polyphenol fraction was extracted from the grape seeds in a semi-continuous way at 90ºC during 60 min. These conditions were chosen in order to maximize the polyphenol extraction, and at the same time minimizing the degradation. Although in the literature other authors were using temperatures up to 80ºC at 2h [31] and 68ºC and 3h [35], we decided to use 90ºC and 1h, at a similar severity factor level. The severity factor is an expression that combine two effects: time and temperature. It is defined by equation (4) and it is normally used for hydrothermal treatments.

$$log\ R_0 = log\ (t * e^{(T - Tref)/14.75}) \quad (4)$$

Where t is time (min) and T is temperature (ºC)

The parameter log R_0 was used to quantify the severity of the solvothermal extraction for our study, choosing the reference temperature as 20ºC. The conventional energy of activation is 14.75 (process hydrolytic and the first order conversion) was maintained. The log R_0 was 3.84 for the conditions used in this study (90ºC and 1h). This matches with the conditions used by Sòlyom et al. [31] (log R_0=3.84 at 80ºC and 2h) and by Szentmihàlyi et al. [35] (log R_0=3.69 at 68.7ºC and 3h).

The results of this solvothermal extraction treatment are shown in Figure 2 and Table 1. The extraction process was modelled using a simple extraction model in order to predict the maximum amount of polyphenols within the fixed grape seeds batch and the velocity of extraction, as per eq. (5).

$$c_{GAE} = c_{GAE0} * e^{-b.t} \quad (5)$$

Where c_{GAE0} = 0.972 $mgGAE \cdot mL^{-1}$ of extract and b = 0.018 min^{-1}

The maximum error of the proposed model was lower than 0.3%. From the simulation value of the maximum polyphenols in the sample batch the total extraction is the

integration of the concentration multiplied by the flow rate (q=5mL·min^{-1} of solvent) yielding 270 mgGAE/batch (every batch was 4 g of grape seeds, see eq. (6) for t=∞). Therefore, the concentration of total polyphenols in the grape seeds can be estimated as 6.75% wt. During the 60 min extraction we extracted a total of 4.46% wt. (66% wt. of the maximum).

$$m_{GAE} = \int_0^t q \cdot c_{GAE_0} \cdot e^{-b \cdot t} = \frac{q \cdot c_{GAE_0}}{b}(1 - e^{-b \cdot t}) \tag{6}$$

Stopping the process at this point the remaining mass of the extracted grapes inside the reactor was 87.0% wt. of the initial raw material, therefore, the oil plus the polyphenols extracted was 13.0% wt. (comparable to the maximum 17.0±0.1% wt. by Soxhlet with hexane and 13.7±0.1% wt. with ethanol). These values were considerably higher than the values previously reported [34, 36, 37] but the authors used a different method to determine the concentration, such as NMR. Considering that, the polyphenols account for a 40% wt. of the concentration in the oil (6.75% wt./17.0% wt. = 0.40).

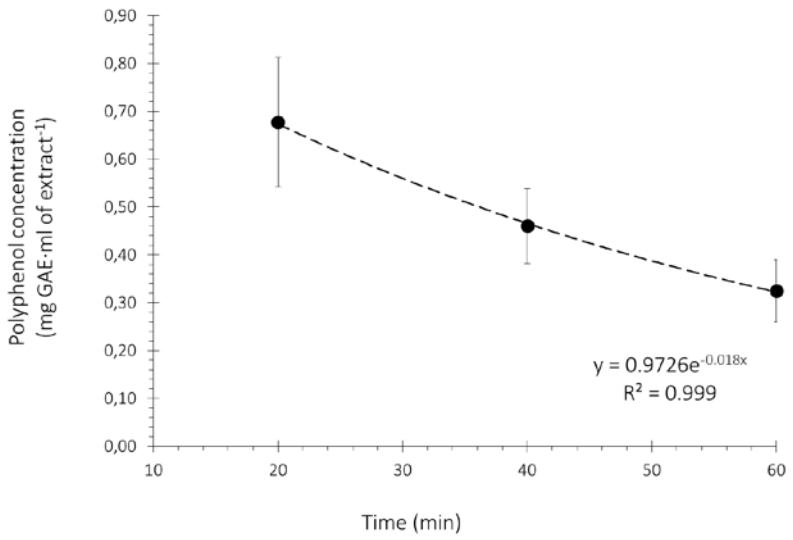

Figure 2. Semicontinuous extraction of polyphenols with ethanol-water (70/30%wt) in solvothermal extraction.

Table 1. Polyphenols analysis from grape seeds in solvothermal extraction.

Time (min)	Concentration ($mgGAE \cdot mL^{-1}$)	Deviation ($mgGAE \cdot mL^{-1}$)	Error (%)	Samples (Nº)
20	0,677	0,135	19,9	5
40	0,460	0,078	16,9	5
60	0,324	0,065	20	5

3.2. Hydrothermal fractionation-hydrolysis process.

The hydrothermal experiments were designed focusing on the treatment of the different biomass fractions in an independent way. To do so, different fractionation temperatures were chosen between 150ºC to 340ºC, as it is shown in Figure 3. In this way, a stepwise fractionation was designed looking for the temperature optimization of the fractionation counting each fraction as product. The experimental temperature profiles are shown in Table 2. The attempt was to separate the extraction-hydrolysis components of the grape seeds using time and temperature.

Table 2. Experimental temperature profiles for hydrothermal fractionation-hydrolysis process.

Time (min)	Test 1 (H_2O) (ºC)	Test 2 (H_2O) (ºC)	Test 3 (H_2O) (ºC)	Test 4 (9% H_2O_2) (ºC)
60	90	90	90	90
105	150	165	180	165
150	250	265	280	265
195	320	330	340	330

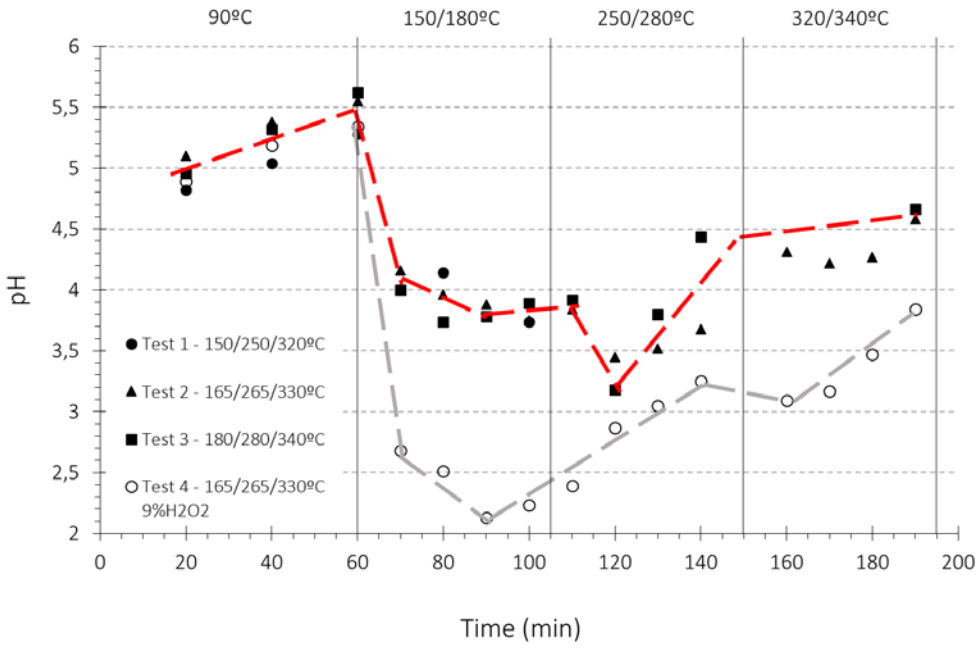

Figure 3. Temperature profile and pH variation during hydrolysis.

Acetyl groups are included within the hemicellulose structure and they can be released when the polymer is hydrolysed. The acetyl groups form acetic acid that dissociates generating hydroxyl ions (H^+) and subsequently reduces the pH of the medium. Following this argument, several authors indicate that a non-catalysed hydrothermal treatment, like in this work, is actually auto-catalysed by the protons released in the hydrolysis [38]. There have been many models considering the effect of protons in a pseudo-first order kinetics, although the results are not always reproducible [39].

Our results confirmed that in the three experiments (Test 1, 2 and 3) carried out, the behaviour of the pH was similar, as depicted in Figure 3. In our system the fresh solvent entered the reactor continuously in the course of the experiment.

During the first 60 min due to the slightly basicity of ethanol/water mixtures [40] the pH increased slowly from 5.0 to 5.5. After this first period, when only distilled water was used (original pH=5.0) the pH decreased suddenly to 3.8-4.0 and it remained at this level during the next 60 min at mild temperatures between 150ºC and 280ºC. There was a second sudden decrease to 3.0 within this period around 120 min, probably when the most inaccessible acetyl groups were hydrolysed. After that, the pH slightly increased to the value of distilled water, indicating that all the hemicelluloses and celluloses have been hydrolysed or extracted. To compare the hydrolytic effect to

oxidation we carried out an oxidation experiment (Test 4). The pH decreased down to 2.6 and to 2.0 at 90 min due to the formation of acids by oxidation. The oxidation was complete, obtaining zero lignin residue inside the reactor.

The final amount of solid in the reactor (SRF) is plotted in Figure 4 (see also Table 3). The values of SRF were similar in the three experiments obtaining between 10.4% wt. and 12.9% wt. The lignin is slightly degraded in subcritical water, but the existence of active area when the lignin is submitted to hydrothermal treatment can be the reason for obtaining between 23.7-29.4% wt. with respect to initial value (43.8% wt.). The quantity of Klason Lignin in the SRF 320ºC, SRF 330ºC and SRF 340ºC was exceeding 90% wt., indicating that high purity in the solid was achieved. The physical properties of the solid residue was characterized. Figure 5 shows SEM images of the solid inside in the reactor obtained from hydrothermal fractionation-hydrolysis process with others experimental conditions. The grape seeds were carried out in a semicontinuous reactor using three different temperatures: 250, 300 and 350ºC. The process time was 1h and the flow (water) was 5 $mL \cdot min^{-1}$. These images were analysed because the grape seeds were subjected to similar temperature conditions in the different stage of this process (solvothermal extraction and hydrothermal fractionation-hydrolysis process).

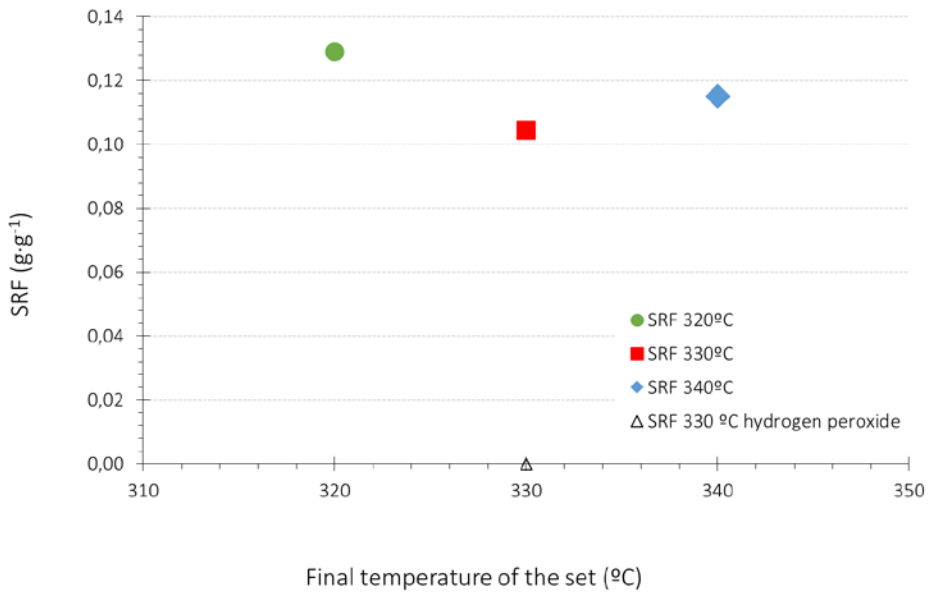

Figure 4. Residual solid inside the reactor after solvothermal extraction and hydrothermal fractionation-hydrolysis process.

Table 3. Final residual solid obtained after solvothermal extraction and hydrothermal fractionation-hydrolysis process.

Test (Nº)	Final temperature (ºC)	Final residual solid ($g \cdot g^{-1}$)	Samples (Nº)
1	320	0.129±0.01	4
2	330	0.104±0.016	3
3	340	0.115±0.014	1
4	330	0	1

The SEM image of solid residue at 250ºC was similar to solid residue at 300ºC, in both cases no presence of microspheres was visible. The formation of carbon spheres with different shapes and sizes in the solid residue at 340ºC was observed. All images at 340ºC showed uneven distribution of size. Sevilla et al. carried out a study of hydrothermal carbonization of saccharides and they found that the carbon spheres contained a high concentration of oxygen functional groups [41]. A more detailed analysis of the solid lignin has been done in a previous work [17].

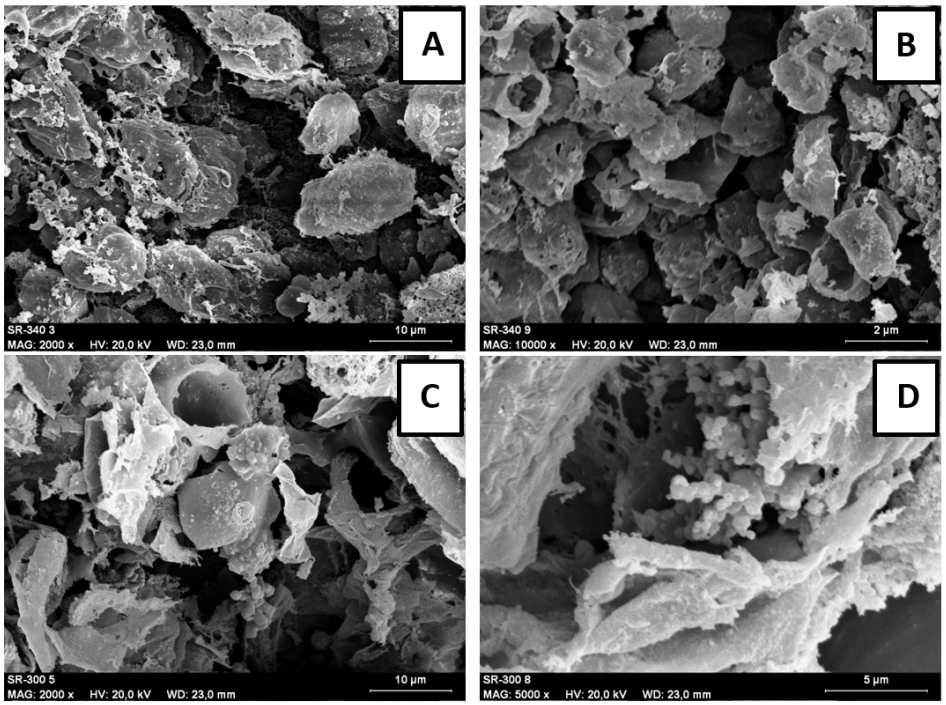

Figure 5. SEM images of solid residue obtained from hydrothermal fractionation-hydrolysis process of biomass at (A) 250ºC, (B)300ºC and (C,D) 340ºC as final temperature and an hour.

The main mechanism for cellulose hydrolysis can be found in the work of Cantero et al. who compiled the work of several authors [24]. A simplified version of the reaction pathway is schematized in Figure 6. In this case we have omitted the intermediate step were the cellulose polymer degrades forming oligomers and those finally yield cellobiose and then glucose and other components. According the Xiang [42] glucose can produce 5-hydroxymethylfurfural (5-HMF). The lactic acid can be produced by a rearrangement from glyceraldehyde [43] as it was shown in the work of Eriksen et al. using complex catalysts [44]. The lactic acid can be dehydrated into acrylic acid [45]. For the case of formic acid, the oxidation is the most common process for its production. In the process developed in this work, the free oxygen is relatively low, however, the oxygen can be transferred from the enormous amount of oxygenated molecules that can be hydrolysed [46]. The hemicellulose is hetero-polysaccharide formed by mannose, xylose, arabinose and galactose [47-49]. The dehydration of pentoses, like xylose and arabinose, produce furfural [50].

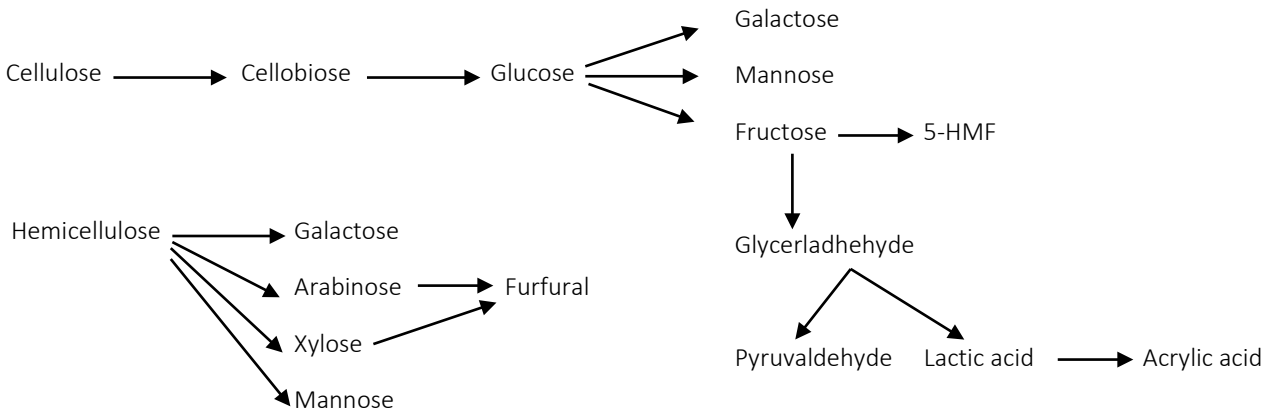

Figure 6. Simplified reaction pathway for hemicelluloses and cellulose and subsequent reactions.

During the experiments several aliquots were collected from the reactor outlet every 10 min in order to measure the instant concentration of sugars and derived products. After that, the samples were hydrolysed using sulphuric acid to avoid to presence of oligomers. This method is necessary to determine the total sugars (C5 and C6 monomers). Table 4 shows the final amount of sugars and sugar degradations after acid hydrolysis. Therefore, it must be considered that the products in Table 4 are the result

of the acid hydrolysis of the liquid effluent obtained from the autohydrolytic pretreatment.

The total amount of C5, C6 and oligosaccharides fractions was 20.0% wt. in Test 1, 23.1% wt. in Test 2 and 22.7% wt. in Test 3 (see Figure 7, Figure 8, Table 4 and Table 5).

The yield of sugars decreased due to their degradation into organic acids, 5-HMF and methylglyoxal, among others. This decrease was observed more strongly at high temperatures and longer times. For this reason, in the Test 2 the quantity of hydrolysis of sugars was 36.7 %wt. lower than in Test 1. On the contrary, the amount of sugar derivatives was higher in Test 2 (as shown in Table 4).

Thus, in this work, the amount of hydrolysis and degradation products increased with temperature and time depending of the type of sugars degradation. In some cases, the value of derivatized products was similar even with an increase of temperature or time (Table 4).

Table 4. Sugars and sugars degradation obtained by solvothermal extraction and hydrothermal fractionation-hydrolysis process of grape seeds followed by sulphuric acid hydrolysis.

Test (Nº)	Test 1			Test 2			Test 3		
Time (min)	100	150	160	100	150	190	100	150	190
Temperature (ºC)	150	250	320	165	265	330	180	280	340
Accumulate sugars and derivatives (% wt.)									
Cellobiose	0.000	0.012	0.012	0.000	0.006	0.006	0.005	0.009	0.009
Glucose	0.001	0.009	0.009	0.000	0.007	0.007	0.005	0.009	0.012
Mannose+Galactose+Xylose	0.000	0.019	0.019	0.001	0.008	0.008	0.006	0.007	0.014
Fructose	0.000	0.000	0.000	0.000	0.004	0.004	0.015	0.020	0.024
Arabinose	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.002	0.002
Glyceraldehyde	0.000	0.002	0.002	0.000	0.020	0.021	0.005	0.011	0.011
Methylglyoxal	0.000	0.030	0.030	0.000	0.024	0.025	0.012	0.013	0.013
Lactic Acid	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.002	0.004
Formic Acid	0.004	0.018	0.018	0.000	0.002	0.002	0.012	0.025	0.025
Acrylic Acid	0.000	0.005	0.005	0.000	0.002	0.003	0.002	0.005	0.006

5-Hydroxymethylfurfural	0.000	0.012	0.012	0.000	0.046	0.047	0.000	0.025	0.026
Oligosaccharides	0.023	0.086	0.091	0.036	0.086	0.097	0.059	0.069	0.082

Table 5. Accumulate total mass referred to initial mass (%) (C5, C6 and oligosaccharides) for Test 1, Test 2 and Test 3.

	Test 1	Test 2	Test 3
Time (min)	Total mass (%)	Total mass (%)	Total mass (%)
70	0.00	0.00	0.00
80	1.01	0.56	7.72
90	1.67	1.12	11.8
100	2.90	3.78	12.1
110	5.03	9.70	13.2
120	13.2	14.0	18.1
130	16.1	18.6	18.7
140	18.9	20.0	19.2
150	19.4	20.5	19.7
160	20.0	22.3	21.3
170	N/A	22.4	21.9
180	N/A	22.4	22.6
190	N/A	23.1	22.7

The maximum amount of sugars in the first temperature range was obtained at 180ºC. On the other hand, the maximum concentration of sugars and derivatives was achieved by working at temperatures between 250 and 265ºC. During the third temperature range only a gently increase in the hydrolysis of sugars was observed, indicating that the extraction of sugars was almost completed in the previous steps. The aim of this last treatment step was to increase the lignin content in the solid removing the remaining sugars.

The arabinose was extracted always at lower temperatures than the others hemicellulose sugars (see Table 4). The maximum amount of arabinose was obtained

close to 80 min for the Test 1, to 100 min for the Test 2 and to 105 min for the Test 3. The cellobiose was detected when the temperature was higher than 180ºC and the mannose, galactose, xylose and fructose when it was higher than 165ºC (see Table 4). Similarly, the maximum extraction for mannose, galactose and xylose was before 110 min in all cases, i.e. in the first extraction step. This behaviour indicated that the maximum quantity of hemicelluloses were extracted at low temperatures and that the best temperature for hydrolysis of C5 sugars was 180ºC suggesting that the hemicellulose is the polymer easier to hydrolyse than cellulose and lignin.

The presence of large amount of oligosaccharides in this step defined the shape of the extraction curve. Considering the maximum of C6 sugars, during this first step a total of 7.65% wt., 24.0% wt. and 47.9% wt. of C6 sugars were obtained respectively in tests #1 to #3.

When the temperature range was 250ºC-280ºC, it can be observed two different slopes in Figure 7: from 105 min to 120 min and from 120 min to 150 min. The first slope was probably caused by the heating-up of the reactor. The second slope was lower than the first slope, indicating that the maximum extraction of sugars was in the first minutes of that step. In the Test 1, 2 and 3 the amount of C6 sugars with respect to its total were 92.3, 65.0 and 44.2% wt. respectively. In this step, the large amount of C6 sugars was extracted. The hydrolysis process was more efficient at temperatures about 250-265ºC.

Finally, the third temperature range was carried out aimed at hydrolysing all the remaining sugars and oligosaccharides and obtaining a solid with a high lignin content. In Test 2 and 3, the amount extracted was similar (about 11.0-13.2% wt.) and in the Test 1 this value was lower than the other experiences, only 2.80%. For this reason, the best temperature was about 330-340ºC.

The total sugars hydrolysis relative to the initial mass were 0.20, 0.23 and 0.23 $g \cdot g^{-1}$ respectively in the three tests. These values represented 54% wt., 63% wt. and 62% wt. of the total amount of sugars in the raw material (36.8% dry basis).

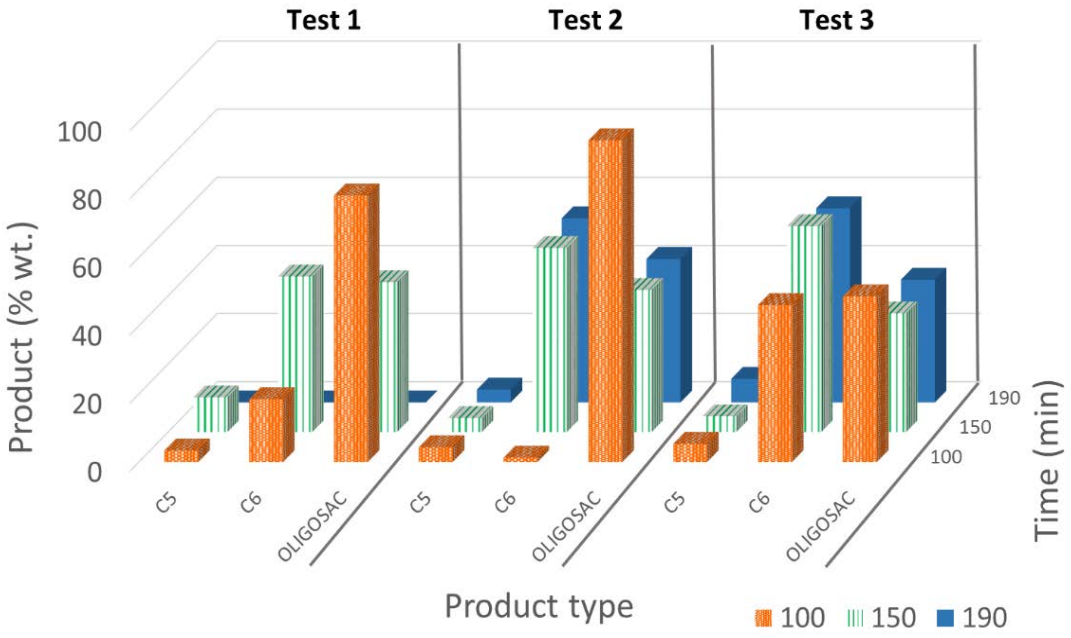

Figure 7. Instant distribution of products (C5, C6 and oligosaccharides) in hydrothermal fractionation-hydrolysis process followed by sulphuric acid hydrolysis.

In view of the results obtained, the best temperature values for performing the hydrolysis of grape seed biomass for the obtaining of sugars would be: step 1 (180ºC), step 2 (250-265ºC) and step 3 (330-340ºC).

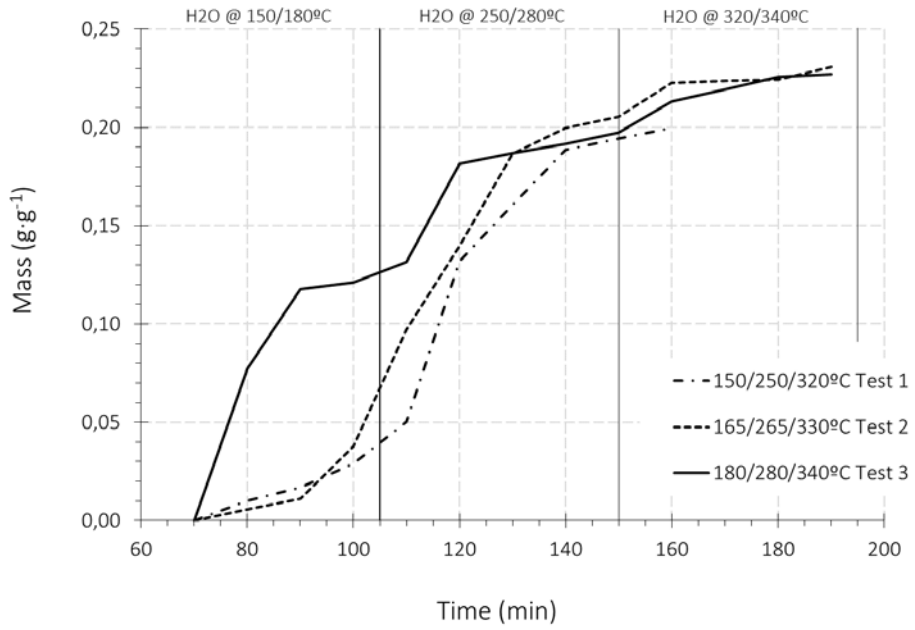

Figure 8. Total mass (g/g) of hydrothermal fractionation-hydrolysis process products for Test 1, Test 2 and Test 3.

During the acidification of the samples, undesirable quantities of 5-HMF were produced at temperatures about of 265ºC. This side reaction would cause troubles in post fermentation processes of the obtained sugars due to yeast intoxication [51]. The used hydrolysis procedure with H_2SO_4 increased the yield of 5-HMF obtained after the hydrothermal treatment. However this method was required to determine the total quantity of C5+C6 fraction. The yield of 5-HMF was determined by analysing the samples after sulphuric acid hydrolysis. Approximately at residence times higher than 110 min, the production of low quantities of 5-HMF was observed.

At 265ºC (Test 2), the production of this component was higher than at 280ºC (Test 3). The amount of 5-HMF was 55.4% wt. higher than at 280ºC as depicted in Figure 9. Also, the yield of 5-HMF remained constant after 140 min.

In a further study it will be investigated how to reduce the 5-HMF production following a similar strategy than Cantero et al. [24] in a previous work, operating at fast residence times and higher temperatures. Also, the analysis procedure should be improved in order to quantify both, the real components after the hydrolysis and also the total quantity of C5 and C6 fraction.

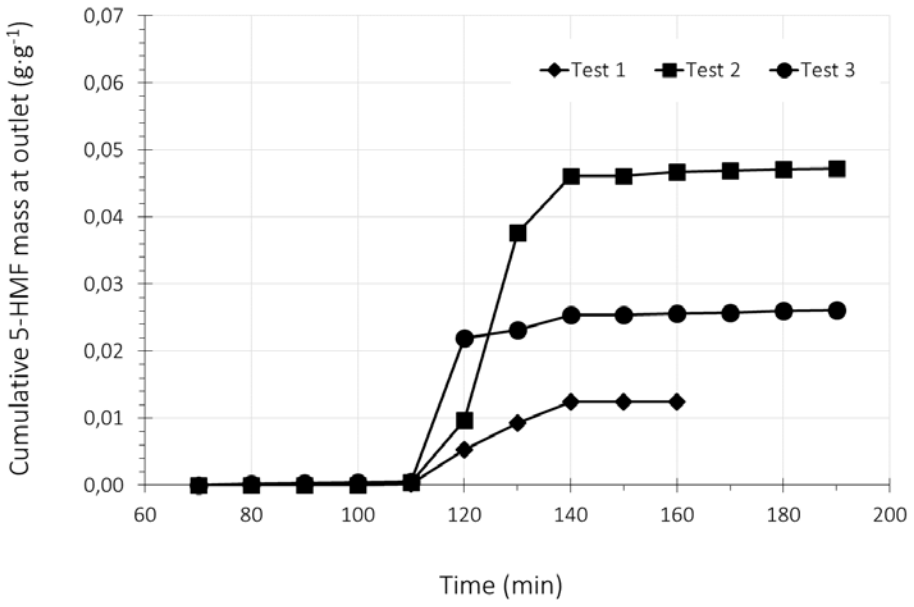

Figure 9. Cumulative 5-HMF mass at outlet (g/g) for Test 1, Test 2 and Test 3.

4. Conclusions

Grape seeds have been used in this work as a model biomass to study the hydrothermal treatment, as their content ca. 17.0% wt. of essential oil (40% of it are valuable polyphenols) and 43.8% wt. of lignin (one of the highest in nature).

The essential oil and polyphenols were extracted through a solvothermal extraction stage with a mixture ethanol/water (70/30% wt.). The efficiency of extraction was 66% wt. at 90ºC and 15 bar using a flowrate of 5 mL/min during 60 min. This previous step of hydrolysis is a promising alternative to extract polyphenols, components that are important in cosmetic and pharmaceutical industries.

In this work, a 3-step variable temperature hydrolysis was applied to study the extraction of sugars in a hydrothermal fractionation-hydrolysis process stage. Due to the autohydrolysis phenomenon, the pH of the effluent changed, and it behaved similarly in the tests. The pH decreased because the acetyl groups were hydrolysed and then it increased to the original value pH=5, showing that all hemicelluloses and celluloses were degraded. The combination of temperature and time profile can lead to a desired combination of products for a subsequent step (e.g. fermentation). From the results analysed in this work, it can be concluded that the best temperature profile for increasing the hydrolysed products concentration would be: step 1 (180ºC - 45 min), step 2 (250-265ºC - 45 min) and step 3 (330-340ºC - 45 min). This time can be reduced to 10-20 min if a faster heating-up rate is used. It has been extracted 50 to 62% of the total sugars (corresponding to 0.20 to 0.23 g-sugars/g-grape seed).

The quantity of Klason Lignin in the SRF 320ºC, SRF 330ºC and SRF 340ºC was exceeding 90% wt., indicating that high purity in the solid was achieved.

Summarizing, with this solvothermal extraction and hydrothermal fractionation-hydrolysis process, the grape seeds can be fractionated into: oil plus polyphenols, sugars and lignin.

In future work, the hydrolysis of Holm oak will be studied. First, the influence of temperature, particle size and flow on yield of hydrolysed carbohydrates will be investigated. Second, the behaviour of three parameters will be analysed to find the best cost-option techniques to follow on line the process.

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References

[1] C.B. Field, M.J. Behrenfeld, J.T. Randerson, P. Falkowski, Primary production of the biosphere: Integrating terrestrial and oceanic components, Science 281 (1998) 237-240.

[2] IEA, CO2 Emissions from fuel combustion. Highlights., International Energy Agency (2012).

[3] J.J. Bozell, S.K. Black, M. Myers, D. Cahill, W.P. Miller, S. Park, Solvent fractionation of renewable woody feedstocks: Organosolv generation of biorefinery process streams for the production of biobased chemicals, Biomass and Bioenergy 35 (2011) 4197-4208.

[4] A. Brandt, J. Gräsvik, J.P. Hallett, T. Welton, Deconstruction of lignocellulosic biomass with ionic liquids, Green Chemistry 15 (2013) 550-583.

[5] M. Sasaki, T. Adschiri, K. Arai, Fractionation of sugarcane bagasse by hydrothermal treatment, Bioresource Technology 86 (2003) 301-304.

[6] R.L. Smith Jr, Water under hydrothermal, supercritical, and high pressure conditions as key to developing green processes and new technologies, 2010, pp. 21-24.

[7] B. Zhang, M. Von Keitz, K. Valentas, Hydrothermal conversion of wood: Reaction kinetics and process development, ACS National Meeting Book of Abstracts, 2007.

[8] P. Mäki-Arvela, B. Holmbom, T. Salmi, D.Y. Murzin, Recent Progress in Synthesis of Fine and Specialty Chemicals from Wood and Other Biomass by Heterogeneous Catalytic Processes, Catalysis Reviews 49 (2007) 197-340

[9] T. Werpy, G. Petersen, Top Value Added Chemicals from Biomass. Volume I - Results of Screening for Potential Candidates from Sugars and Synthesis Gas, Pacific Northwest National Laboratory (PNNL) and the National Renewable Energy Laboratory (NREL) 1 (2004) 1-76.

[10] P. Mäki-Arvela, T. Salmi, B. Holmbom, S. Willför, D.Y. Murzin, Synthesis of sugars by hydrolysis of hemicelluloses- A review, Chemical Reviews 111 (2011) 5638-5666.

[11] J.E. Holladay, J.J. Bozell, J.F. White, D. Johnson, Top Value Added Chemicals from Biomass. Volume II - Results of Screening for Potential Candidates from Biorefinery Lignin, Pacific Northwest National Laboratory, University of Tennessee and National Renewable Energy Laboratory 1 (2004) 1-87.

[12] A.A. Peterson, F. Vogel, R.P. Lachance, M. Fröling, M.J. Antal Jr, J.W. Tester, Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies, Energy and Environmental Science 1 (2008) 32-65.

[13] R.C. Brown, Hydrothermal processing, in: C.V. Stevens, (Ed.), Thermochemical Processing of Biomass. Conversion into Fuels, Chemicals and Power, John Wiley & Sons, Noida (India), 2011, pp. 201-231.

[14] P.E.S. Naoko Akiya, Roles of water for chemical reactions in high-temperature water, Chemical reviews 102 (2002) 2725-2750.

[15] G. Garrote, H. Domínguez, J.C. Parajó, Hydrothermal processing of lignocellulosic materials, Holz als Roh - und Werkstoff 57 (1999) 191-202.

[16] P. Gullón, A. Romaní, C. Vila, G. Garrote, J.C. Parajó, Potential of hydrothermal treatments in lignocellulose biorefineries, Biofuels, Bioproducts and Biorefining 6 (2012) 219-232.

[17] F.M. Yedro, J. García-Serna, D.A. Cantero, F. Sobrón, M.J. Cocero, Hydrothermal hydrolysis of grape seeds to produce bio-oil, RSC Advances (2014) (accepted 21 June 2014, DOI: 2010.1039/C2014RA00429A).

[18] A. Requejo, S. Peleteiro, A. Rodríguez, G. Garrote, J.C. Parajó, Valorization of residual woody biomass (Olea europaea trimmings) based on aqueous

fractionation, Journal of Chemical Technology and Biotechnology 87 (2012) 87-94.

[19] H. Grenman, K. Eranen, J. Krogell, S. Willfor, T. Salmi, D.Y. Murzin, Kinetics of aqueous extraction of hemicelluloses from spruce in an intensified reactor system, Industrial and Engineering Chemistry Research 50 (2011) 3818-3828.

[20] J.M. Prado, L.A. Follegatti-Romero, T. Forster-Carneiro, M.A. Rostagno, F. Maugeri Filho, M.A.A. Meireles, Hydrolysis of sugarcane bagasse in subcritical water, Journal of Supercritical Fluids 86 (2014) 15-22.

[21] J.M. Prado, M.A.A. Meireles, Production of Valuable Compounds by Supercritical Technology Using Residues from Sugarcane Processing, in: C. Bergeron, D.J. Carrier, S. Ramaswamy, (Eds.), Biorefinery Co-Products: Phytochemicals, Primary Metabolites and Value-Added Biomass Processing John Wiley & Sons, Ltd, Kennebunk, Maine, United States, 2012, pp. 133-151.

[22] M.S. Reisch, THE SUGAR MAKERS, Chemical & Engineering News Archive 89 (2011) 20-21.

[23] S. Kilambi, K.L. Kadam, Production of Fermentable Sugars and Lignin from Biomass Using Supercritical Fluids, RENMATIX, Inc., 2011.

[24] D.A. Cantero, M.D. Bermejo, M.J. Cocero, Kinetic analysis of cellulose depolymerization reactions in near critical water, Journal of Supercritical Fluids 75 (2013) 48-57.

[25] D. Moldes, P.P. Gallego, S. Rodriguez Couto, A. Sanroman, Grape seeds: The best lignocellulosic waste to produce laccase by solid state cultures of Trametes hirsuta, Biotechnology Letters 25 (2003) 491-495.

[26] M. Spanghero, A.Z.M. Salem, P.H. Robinson, Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces, Animal Feed Science and Technology 152 (2009) 243-255.

[27] B. Lapornik, M. Prošek, A.G. Wondra, Comparison of extracts prepared from plant by-products using different solvents and extraction time, Journal of Food Engineering 71 (2005) 214-222.

[28] V. Sant'Anna, A. Brandelli, L.D.F. Marczak, I.C. Tessaro, Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts, Separation and Purification Technology 100 (2012) 82-87.

[29] T.M. Research, Polyphenols Market by Product (Grape seed, Green tea, Apple and Others), by Application (Functional beverages, Functional food, Dietary supplements and Others) - Global Industry Analysis, Size, Share, Growth, Trends and Forecast, 2012 - 2018, (2013).

[30] J. Shi, J. Yu, J.E. Pohorly, Y. Kakuda, Polyphenolics in grape seeds-biochemistry and functionality, Journal of medicinal food 6 (2003) 291-299.

[31] K. Sólyom, D. Varga, R.B. Mato, M.J. Cocero, Polyphenol extraction of grape marc after ultrasound pretreatment, Journal of Food Engineering (accepted) (2013).

[32] T. Maier, A. Schieber, D.R. Kammerer, R. Carle, Residues of grape (Vitis vinifera L.) seed oil production as a valuable source of phenolic antioxidants, Food Chemistry 112 (2009) 551-559.

[33] L.Y. Meng, S.M. Kang, X.M. Zhang, Y.Y. Wu, F. Xu, R.C. Sun, Fractional pretreatment of hybrid poplar for accelerated enzymatic hydrolysis: Characterization of cellulose-enriched fraction, Bioresource Technology 110 (2012) 308-313

[34] C.P. Passos, R.M. Silva, F.A. Da Silva, M.A. Coimbra, C.M. Silva, Supercritical fluid extraction of grape seed (Vitis vinifera L.) oil. Effect of the operating conditions upon oil composition and antioxidant capacity, Chemical Engineering Journal 160 (2010) 634-640.

[35] K. Szentmihályi, P. Vinkler, B. Lakatos, V. Illés, M. Then, Rose hip (Rosa canina L.) oil obtained from waste hip seeds by different extraction methods, Bioresource Technology 82 (2002) 195-201.

[36] N.G. Baydar, M. Akkurt, Oil content and oil quality properties of some grape seeds, Bazi Üzüm Çekirdeklerinin Yağ Içeriği ve Yağ Kalite Özellikleri 25 (2001) 163-168.

[37] S. Bail, G. Stuebiger, S. Krist, H. Unterweger, G. Buchbauer, Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity, Food Chemistry 108 (2008) 1122-1132.

[38] S.E. Jacobsen, C.E. Wyman, Xylose monomer and oligomer yields for uncatalyzed hydrolysis of sugarcane bagasse hemicellulose at varying solids concentration, Industrial and Engineering Chemistry Research 41 (2002) 1454-1461.

[39] C.E. Wyman, S.R. Decker, M.E. Himmel, J.W. Brady, C.E. Skopec, L. Viikari, Hydrolysis of cellulose and hemicellulose, in: S. Dumitriu, (Ed.), Polysaccharides: Structural Diversity and Functional Versatility, CRC Press, 2005, pp. 1023-1062.

[40] D.G. Lee, R. Cameron, Basicity of ethanol. Acidity function for alcohols, Journal of the American Chemical Society 93 (1971) 4724-4728.

[41] M. Sevilla, A.B. Fuertes, Chemical and structural properties of carbonaceous products obtained by hydrothermal carbonization of saccharides, Chemistry - A European Journal 15 (2009) 4195-4203.

[42] Q. Xiang, Y.Y. Lee, R.W. Torget, Kinetics of glucose decomposition during dilute-acid hydrolysis of lignocellulosic biomass, Applied biochemistry and biotechnology 113-116 (2004) 1127-1138.

[43] Z. Srokol, A.G. Bouche, A. Van Estrik, R.C.J. Strik, T. Maschmeyer, J.A. Peters, Hydrothermal upgrading of biomass to biofuel; studies on some monosaccharide model compounds, Carbohydrate Research 339 (2004) 1717-1726.

[44] J. Eriksen, O. Mønsted, L. Mønsted, Mechanism of lactic acid formation catalyzed by tetraamine rhodium(III) complexes, Transition Metal Chemistry 23 (1998) 783-787.

[45] V.C. Ghantani, S.T. Lomate, M.K. Dongare, S.B. Umbarkar, Catalytic dehydration of lactic acid to acrylic acid using calcium hydroxyapatite catalysts, Green Chemistry 15 (2013) 1211-1217.

[46] J. Yun, F. Jin, A. Kishita, K. Tohji, H. Enomoto, Formic acid production from carbohydrates biomass by hydrothermal reaction, Journal of Physics: Conference Series 215 (2010).

[47] L. Faba, B.T. Kusema, E.V. Murzina, A. Tokarev, N. Kumar, A. Smeds, E. Díaz, S. Ordóñez, P. Mäki-Arvela, S. Willför, T. Salmi, D.Y. Murzin, Hemicellulose hydrolysis and hydrolytic hydrogenation over proton- and metal modified beta zeolites., Microporous and Mesoporous Materials (2013).

[48] G. Garrote, H. Domínguez, J.C. Parajó, Kinetic modelling of corncob autohydrolysis, Process Biochemistry 36 (2001) 571-578.

[49] S. Wang, Y. Zhou, T. Liang, X. Guo, Catalytic pyrolysis of mannose as a model compound of hemicellulose over zeolites, Biomass and Bioenergy 57 (2013) 106-112.

[50] B. Danon, W. Hongsiri, L. van der Aa, W. de Jong, Kinetic study on homogeneously catalyzed xylose dehydration to furfural in the presence of arabinose and glucose, Biomass and Bioenergy 66 (2014) 364-370

[51] J.M. Lee, R.A. Venditti, H. Jameel, W.R. Kenealy, Detoxification of woody hydrolyzates with activated carbon for bioconversion to ethanol by the thermophilic anaerobic bacterium Thermoanaerobacterium saccharolyticum, Biomass and Bioenergy 35 (2011) 626-636.

Chapter 3

Monitoring alternatives and main sugar products for the autohydrolysis of Holm oak hemicelluloses using pressurized hot water

Abstract

The present work investigate the hydrolysis of Holm oak (*Quercus ilex*) using subcritical water conditions. The experiments were carried out in a semicontinuous reactor using different temperatures (from 175 to 207ºC), flow rates (from 3 to 34 ml·min^{-1}) and particle sizes (3 and 6 mm). The behaviour of pH, Total Organic Carbon and sugars by HPLC were measured and studied. The current results provided an interesting relation between these parameters. The minimum pH was located at the same time as the Total Organic Carbon and sugars by HPLC presented a maximum. The pH can be used to follow on-line the hydrolysis process reducing the analytical and time expenditures to the minimum possible and at the same time understanding the behaviour of the system.

Keywords: Hydrolysis, hydrothermal process, biomass, Holm oak (Quercus ilex), pH, Total Organic Carbon

1. Introduction

A number of research groups, SMEs and companies have intensively studied the hydrolysis and autohydrolysis process of biomass, targeting at the production of bio-fuels or the production of value added products, such as monomers (e.g. reduced sugars, aldehydes, acids or aromatics) or biopolymers (i.e. hemicellulose, cellulose and lignin). Analysing the products in both cases is a tedious and extremely expensive task. Examples of typical analyses found in the research literature are chromatography, spectrometry and elemental analysis among others. These techniques, from a practical point of view, can be divided by the time required:

On-line

- pH of the liquid effluent [1]
- Colorimeter

Relatively simple and fast

- Total Organic Carbon of the liquid and solid [2]
- FTIR and RAMAN to identify the functional groups in the remaining solid [3, 4]

Complex and slow

- Hydrolysis with strong acid followed by HPLC or methanolysis followed by GC to determine the sugar content, lignin, ashes, etc. [5]
- NMR to identify the functional groups in the remaining solid [6]
- Proximate analysis of the solid determining C, H, O and S followed by an estimation of High Heating Value using an equation [7]

The hydrolysis using subcritical water can be carried out in batch, semi-continuous or continuous mode [2, 8-11]

For the case of the continuous reaction, the value of the properties of the liquid and solid at the reactor outlet will be constant under steady state conditions. On the other hand, for the batch and semicontinuous process, the values of the properties will be continuously changing and the production engineer will require an on-site decision criterion to stop the reaction or to change into the different biomass beds. Thus, it is

essential to have an 'easy' and 'cheap' parameter to follow the hydrolysis process as on-line as possible (real monitoring) cutting unnecessary costs due to the abundance of sampling.

Hemicelluloses are linked to lignin and account for approximately 15 to 25% of the biomass material. A number of processes have been proposed to utilize the hemicelluloses, which are currently degraded during the chemical pulping process [12-14]. An extensive previous work has been done, mainly focused in the study of the results from the autohydrolysis in order to understand the mechanisms and the differences between raw materials [15-17].

During the autohydrolysis process, the hemicelluloses are extracted and depolymerized in water between 120 and 170ºC. In many cases deacetylation occur, producing acetic acid that decreases the pH down to 3 to 4 [18]. Tunc and Van Heiningen studied the extraction of hemicelluloses from hardwood, determining the number of acetyl and uronic acid groups (e.g. glucuronic acid) that remain in the solid [19]. During the extraction at 150ºC the number of acetyl groups reduced 17% from 5.1 down to 4.2 (per 10 of xylose groups), while the uronic acid groups decreased 54% from 2.2 to 1.0 (per 10 of xylose groups). The acidity of acetic acid (pKa=4.76) is lower than the acidity of glucuronic acid (pKa=3.03 to 3.51) [20, 21]. This pKa values explain well the pH obtained during the hydrolysis process by the different authors, between 5.5 and 3.0 [19, 22-25].

Garrote et al. studied the deacetylation of hemicelluloses from Eucalyptus wood, concluding that up to 90% of the initial acetyl groups can be removed from the substrate by the hydrothermal treatment between 145 (after 7.5h) and 190ºC (after 0.5h). Then, they indicated that the formation of acetic acid occurred only at higher temperatures [25]. This acetic acid will decrease the pH of the system.

The autohydrolysis of the sugar cane bagasse between 213 and 290ºC in a 50 mL semicontinuous reactor produced up to 95% liquefaction degree, as reported by Prado et al. [22]. They studied the process at different flowrates between 11 and 55 $mL \cdot min^{-1}$ observing that the flowrate did not affect the liquefaction results. They included cumulative curves of the reduced sugars produced and the pH of the effluent. The pH

decreased from 5.5 down to 3.5 during the first 15 min and then slightly increased up to 4.0-4.5. The same research group studied thoroughly the autohydrolysis assisted by compressed CO_2, concluding that there is no clear beneficial effect of the CO_2 [26, 27].

On the other hand, Silva and Morais et al. claim that CO_2 reduces the pH of the effluent due to the formation of carbonic acid, improving considerably the hydrolytic process of wheat straw and other agricultural surplus biomass. In fact, between 201 and 250ºC they found the xylose production almost doubled by using 60 bar of CO_2 [28, 29]. At the same time, 5-HMF and furfural production increased under the same conditions.

For this research, the authors have selected Holm oak to study the autohydrolysis process in-deep, shedding light on the pH behaviour. Holm oak is the main tree, with ca. 70%, of the 'dehesas' of the southwestern Iberian Peninsula [30]. The 'dehesas' are man-made ecosystems planted, managed, and regularly pruned in Europe, with approximately 2.9 million hectares in Spain and 0.5 million hectares in Portugal. The sweetness of the Q. ilex acorns and their high content in unsaturated fatty acids, make this tree perfect to feed the valuable Iberian pig. The rule of thumb indicates that 9 kg of Q. ilex acorns can produce of 1 kg of pork meat [31]. The use of Holm oak pruning for direct fire heating is common, with a heating capacity of 18.5-19.8 $MJ \cdot kg^{-1}$ [32]. Instead of that, the most common current approach must be said, the authors integrate the surplus Holm oak into the biorefinery concept by studying the autohydrolysis process as a feasible pretreatment step.

This paper takes a new look at the autohydrolysis solution reexamining the results from other authors and including new data of the autohydrolysis of Holm oak, it can be of help in a forthcoming industrial application of the technology.

In this work, the hydrolysis of carbohydrates from Holm oak was studied, focusing on the effect of temperature, flow and particle size on yield of hydrolysed carbohydrates and the temperature and flow on yield of degradation products. The main objective in this study was to identify an 'easy-to-measure' and 'cheap' parameter that may simplify the on-line tracking of the reaction. The behaviour and interaction between three parameters (pH, total organic carbon and amount of hydrolysed carbohydrates) were

investigated. The correct selection and use of one of these parameters may lead to a comprehensive knowledge of the process with a manageable analytical expenditure.

2. Materials and methods

2.1. Raw material and materials

The Holm oak (*Quercus ilex*) was dried, milled and sieved to select the particle size with average diameters between 3 and 6 mm. The extractives were determined gravimetrically using n-hexane (96%) by Soxhlet according to the 'Determination of Extractives in Biomass'[33]. All chemicals used in this study were purchased from Sigma and used without further purification. The reagents used for HPLC analysis were: cellobiose (+98%), glucose (+99%), fructose (+99%), glyceraldehyde (95%), pyruvaldehyde (40%), arabinose (+99%), 5-hydroxymethylfurfural (99%), lactic acid (85%), formic acid (98%), acrylic acid (99%), mannose (+99%), xylose (+99%), levulinic acid (+99%) and galactose (+99%). For the analysis of carbohydrates and lignin we used sulphuric acid (98%) for acidification and calcium carbonate (+99.0%) for neutralization. For the analysis of extractives n-hexane (95%) was used. Distilled water was used for all the hydrolysis experiments.

2.2. Experimental setup

All hydrothermal fractionation processes were carried out in a semi-continuous reactor system as depicted in Figure 1. In order to perform the tests, approximately 5 gr of dry biomass was charged into the reactor. Two average particle sizes were used (3 and 6 mm). To keep the raw material in place two metallic filters were used (located in the top and bottom of the reactor). The pump (Jasco model: PU-2080) was set to 3, 10, 19 and 34 $mL \cdot min^{-1}$ using distilled water and the pressure was set using a go-backpressure valve. The pressure was fixed to 10 MPa in all runs and the total process time was 94 min. The reactor (R-01, 38 cm length, 1/2´ O.D. SS316 piping) and pre-heater (E-02, 200 cm of 1/8´ AISI 316 piping) were placed inside an oven HP5680 (H-01) to keep the temperature. The pre-heater (E-02) was used to assure at working temperature. Heat recovery was performed in a counter-current concentric tube heat exchanger (E-01, 70 cm of concentric tube heat exchanger 1/4´-3/8´). The out flow of the reactor was used to heat up the feed flow (E-01). The second heat exchanger (E-03, 15 cm of concentric

tube heat exchanger 1/4´-3/8´counter current operation) was used to cool the products to room temperature (approximately 25-30ºC). Liquid samples were taken every 10 min (at operational flows lower than 10 mL·min^{-1}) or every 5 min (at operational flows higher than 10 mL·min^{-1}). Heating time of the system was between 4 and 10 min. After the total process time (normally 94 min), the reactor was gradually cooled down back to room temperature, the system was depressurized and the pump was set to zero flow. The solid inside of the reactor was collected and quantified. These samples together with the liquid samples were analysed as explained below.

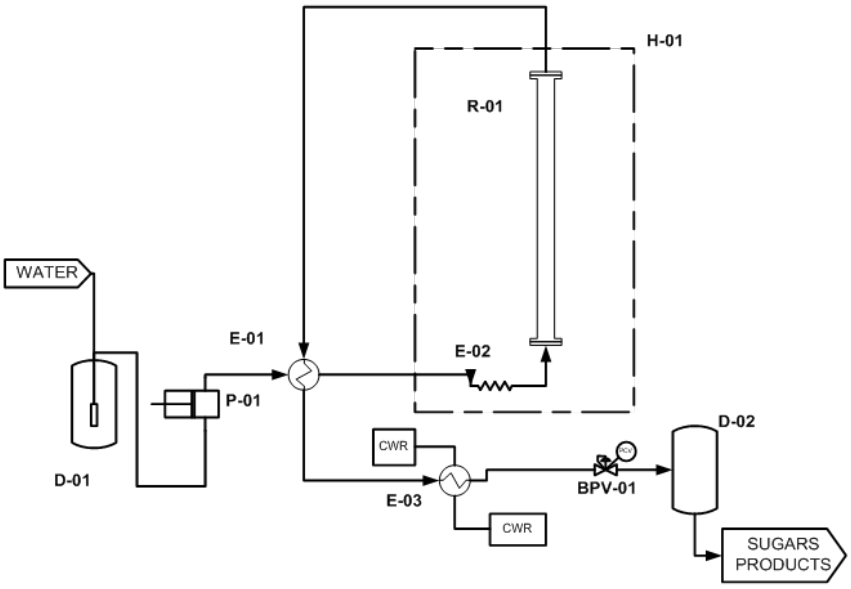

Figure 1. Schema of the hydrolysis process. Equipment: D-01 Feeder, P-01 Pump, E-01 Heat exchanger, E-02 Preheater, R-01 Reactor, H-01 oven, E-03 Heat exchanger, BPV-01 Go-backpressure valve, D-02 Liquid sampling vessel.

2.3. Chemical analysis

The liquid and solid samples taken during hydrothermal fractionation were analysed to determine the content of hemicelluloses, cellulose and degradation products in the liquid samples and the content of lignin (soluble and insoluble acid), hemicelluloses and cellulose in the solid samples. The procedure is explained in sections 2.3.1 and 2.3.2.

2.3.1. Sugar and lignin content in the solid samples

The samples were characterized according to the National Renewable Energy Laboratory (NREL) – Determination of Structural Carbohydrates and Lignin in Biomass [34, 35]. For this purpose, 300 mg of solid sample (Wi) was hydrolysed adding 3 mL of

sulphuric acid (72%) and it was incubated during 30 min at 30ºC. Then, it was added 84 mL of distilled water and it was incubated for one hour at 121ºC. The solid was separated from the liquid by filtration under vacuum, washed with distillated water, dried at 105ºC for 24 h and weighted (W1). Then, the solid was treated at 550ºC for 24 h in a muffle and then weighted (W2) to determine, by gravimetric methods, the content of acid insoluble lignin (AIL) and ash (A). The amount of acid insoluble lignin was calculated using the equation 1:

$$AIL=\frac{W_1-W_2}{W_i}100 \quad (1)$$

The amount of ash was calculated using the equation 2:

$$A=\frac{W_2}{W_i}100 \quad (2)$$

The content of acid soluble lignin was determined by spectrophotometry, measuring the absorbance at 320 nm and using an absorptivity at recommended wavelength of 30 $L \cdot g\text{-}1 \cdot cm^{-1}$ [36]. Also, 30 mL of liquid was neutralized with calcium carbonate to pH=6-7, then it was filtered using a 0.2 µm filters and analysed by high pressure liquid chromatography (HPLC). The HPLC column was SUGAR SH-1011 (Shodex) at 50ºC at a flow of 0.8 ml/min using a solution of 0.01 N of sulfuric acid and Milli-Q water as a mobile phase. To identify the hemicelluloses, cellulose and reduced sugars the authors used two detectors: Waters IR detector 2414 (210 nm) and Waters dual λ absorbance detector 2487 (254 nm). To calculate the amount of carbohydrates the authors used a band-analysis via Fast Fourier Transform (fft) and band-adjustment by Gaussian functions. The adjustment was done by minimizing the quadratic error using a Nelder-Mead algorithm.

The total content of hemicelluloses, cellulose and degradation products were calculated as shown in equations (3), (4) and (5) respectively.

Hemicelluloses (g/g)= xylose(g/g)+arabinose (g/g)+galactose(g/g)+mannose(g/g) (3)

Celluloses (g/g)= glucose(/gg)+fructose (g/g)+cellobiose(g/g) (4)

Derived-products (g/g)= glyceraldehyde(g/g)+pyruvaldehyde(g/g)+lactic-acid(g/g)+formic-acid(g/g)

+acrylic-acid(g/g)+5-hydroxymethylfurfural(g/g)+acetic-acid(g/g) (5)

2.3.2. Sugar content in the liquid samples

During the hydrothermal fractionation the hydrolysates may content oligomers that need to be cleavage for a proper sugar analysis. To this end, the liquid samples were post-hydrolysed adding 4 mL of sulphuric acid to 10 mL of each aliquot and it was incubated during 30 min at 30ºC. Then, it was added 86 mL of distilled water for one hour at 121ºC. Then, it was neutralized with calcium carbonate to pH between 6 and 7. Before injecting in the HPLC, the samples were filtered using a 0.2 µm filters. For this analysis, it was used the same column as the one mentioned above.

The pH was measured by a Nahita model 903 pH-meter. The electrode was Glass-Body ElectroJelly PH5101-3B. Total Organic Carbon (TOC) was measured by a Shimadzu equipment model TOC-VCSH. The carbon concentration of the standard solutions corresponds to 500 $mgC \cdot L^{-1}$.

2.4. Physicochemical characterization of biomass

Scanning electron microscopy (SEM) analysis for residue inside of the reactor and raw material was performed using a Jeol JSM-820 SEM and a gold evaporator Balzers SCD003. Gold Thickness used was 25-30 nm. Fourier Transform Infrared (FT-IR) spectra of the residue inside of the reactor and raw material were recorded on a Bruker Tensor 27 in the wavelength range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans number per sample with a velocity of 10 KHz.

2.5. Heat integration and energy saving

One of the limitations of the pressurized hot water hydrolysis is the intensive use of energy for heating up and cooling down of the water. In this research, heat integration to reduce the energy demand was studied using a counter-current heat exchanger between water inlet and product outlet. A simplified energy balance was computed considering the energy equation for steady state. The main assumptions were:

- Constant overall heat transfer coefficient and mass flow rates along the heat exchanger

- Constant thermo-physical properties of fluids
- Negligible radial effects

The heat load can be calculated as function of mass flow and the enthalpies of the different streams, as shown in equation (6)

$$m_h * (h_{he} - h_{ho}) = m_c * (h_{co} - h_{ce}) \quad (6)$$

Where m_h is hot water mass flow ($kg \cdot s^{-1}$), mc is cold water mass flow ($kg \cdot s^{-1}$) and h represents the enthalpies of the different streams ($kJ \cdot kg^{-1}$).

Assuming the specific heat (Cp, $kJ \cdot ºC \cdot kg^{-1}$) of the two fluids was constant, the equation 7 is obtained as:

$$Q = m_h * Cp_h * (T_{he} - T_{ho}) = m_c * Cp_c * (T_{co} - T_{ce}) \quad (7)$$

Where Q is heat load (W) and T is temperature of the different streams.

The logarithmic mean temperature difference is calculated as shown in equation 8:

$$\Delta T_{lm} = \frac{\Delta T_1 - \Delta T_2}{\ln \frac{\Delta T_1}{\Delta T_2}} \quad (8)$$

Where ΔTlm is logarithmic mean temperature difference (ºC).

The area inside of the reactor is evaluated by equation 9.

$$A = \pi * d_i * L \quad (9)$$

Where A is internal exchanger area (m^2), di is inside diameter of the heat exchanger (m), L is length of the heat exchanger (m).

The overall heat transfer coefficient is calculated as shown in equation 10:

$$U = \frac{Q}{A * \Delta T_{lm}} \quad (10)$$

Where U is overall heat transfer coefficient ($W \cdot ºC \cdot m^2$)

Finally, the efficiency of a counter flow heat exchanger is calculated as shown in equation 11:

$$\eta = \frac{Q}{Q_{max}} \quad (11)$$

Where η is the efficiency of the system and Q_{max} (W) is the maximum heat load obtained.

3. Results and Discussion

3.1. Proximate analysis and experimental conditions

The composition of raw material was 2.36 wt% extractives (Soxhlet extraction with hexane), 29.4 wt% lignin (determined as acid soluble (1.05 wt%) and insoluble lignin (28.3 wt%)) and 66.8 wt% of carbohydrates. The amount of ash was under the detection limit. The carbohydrates consisted of 24.3 wt% of hemicellulose (determined as xylose, arabinose, galactose, mannose and acetic acid) and 41.3 wt% of cellulose (determined as glucose, fructose and cellobiose). The degradation products were attributed to pyruvaldehyde (0.38 wt.%) and acid formic (0.88 wt.%).

The research was oriented to decipher the influence of three variables: particle size (runs #2,#6 and #4,#8), liquid flowrate (runs #2,#4 and #6,#8) and temperature (runs #1,#5 and #3,#7). Three important magnitudes in autohydrolysis were analysed: pH, Total Organic Carbon and carbohydrate hydrolysis products (mainly sugars and oligomers), as listed in Table 1.

Table 1. Experimental conditions of Holm oak hydrolysis with subcritical water.

Test (Nº)	Mass (gr)	Process time (min)	Temperature (ºC)	Flow ($mL \cdot min^{-1}$)	Particle size (mm)
#1	5,31	94	175	3	3
#2	5,32	94	207	10	3
#3	5,33	94	185	19	3
#4	5,26	94	180	34	3
#5	5,26	94	190	3	6
#6	5,40	94	207	10	6
#7	5,25	94	195	19	6
#8	5,22	94	180	34	6

Experiments using Holm oak biomass were carried out between 175 and 207 ºC at 10 MPa (assuring liquid phase). The solid inside of the reactor after the process time (90

min plus 4-10 min for to heat the system) and a number liquid aliquots were collected, measured and analysed for each test (#1-8).

3.2. Energy savings by heat integration

The use of subcritical water in this process require medium pressures to assure the liquid phase and high temperatures to increase the mass transfer and kinetics. The reaction temperatures were between 175 and 207ºC. The use of a heat exchanger recovering energy makes the process more economically and energetically efficient. In the laboratory plant a heat exchanger (E-01) to heat up the feed flow using the out flow of the reactor was successfully tested. Thus, the heat recovery was between 73.8 and 85.5 % depending on the temperature and flow used in the assays. The second heat exchanger was not used because the temperature of outlet stream was always approximately 30ºC. The overall heat transfer coefficient (U) was between 788 and 619 $W \cdot m^{-2} \cdot ºC^{-1}$.

3.3. Online monitoring: relation among pH, TOC and carbohydrate autohydrolysis

The semicontinuous extraction curve of hydrolysable components in a fixed bed reactor considering the concentration at reactor outlet has a pseudo-Gaussian shape. This means that the concentration exhibited a maximum and decreased afterwards. Figure 2 shows the time required to extract the maximum amount of carbohydrates by HPLC, the maximum total organic carbon measured by TOC equipment and the minimum value of pH for each experiment. An interesting effect was found: in general, the maximum TOC value and the maximum direct concentration of sugars (measured in carbon) appeared at the same time than the minimum pH value (Figure 3). As it will be explained later in detail, this is related to deacetylation of hemicelluloses during the hydrolysis. We have observed this behaviour in all tests and other authors too. Thus, for similar operational conditions than Tests #5 to #8 they found a similar pH and TOC behaviour, although they did not pointed out and analysed it so clearly the maximum-minimum coincidence [18, 22]. In view of all this, the authors propose the pH as the key parameter to follow the process on-line. The main advantage is that the pH is a cheaper parameter than TOC and HPLC analysis. When it is necessary to know the amount of sugars in the sample, a rapid off-line parameter to follow the hydrolysis process is the

use of total organic carbon. Finally, when it is necessary to know about the kind of hydrolysis sugars the authors propose the use of HPLC, or in other cases a methanolysis followed by GC-MS analysis. This technique is more precise but requires more time and it is more expensive than the others discussed before.

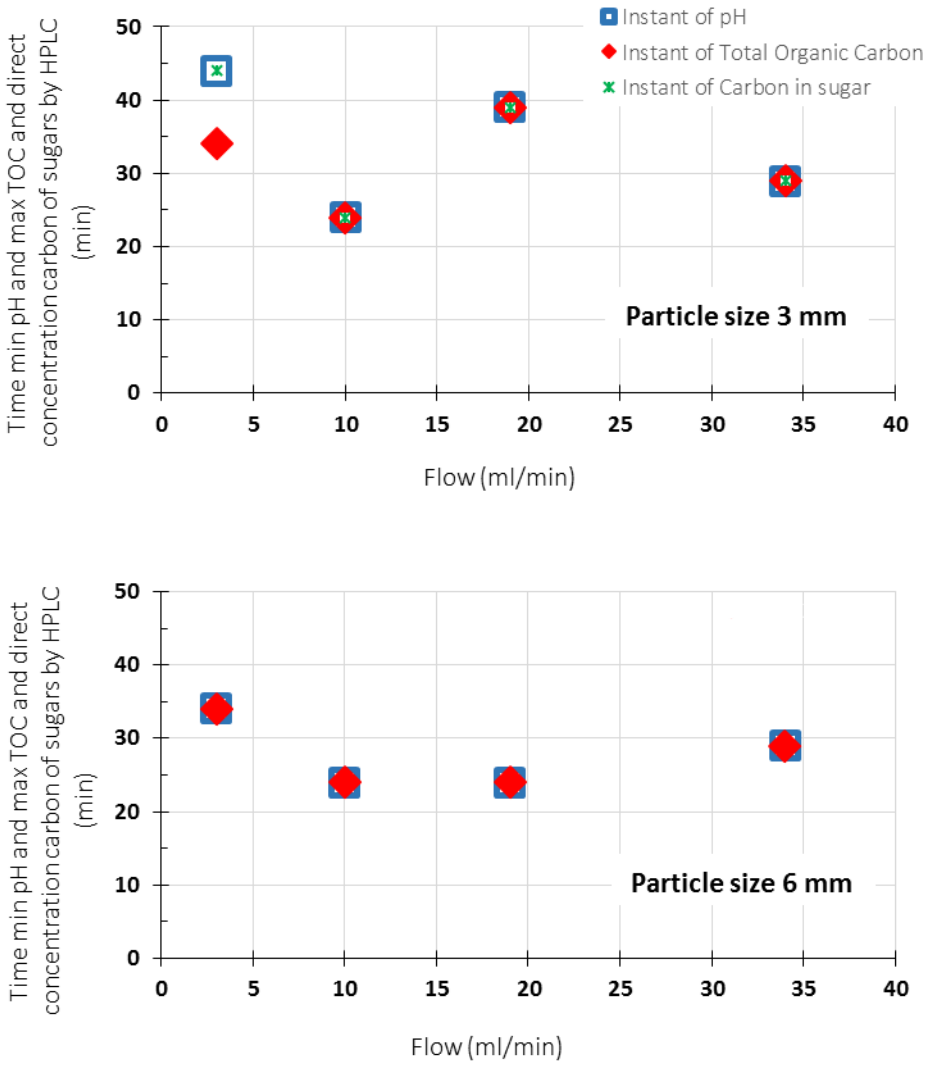

Figure 2. Time required to obtain the minimum pH value and maximum total organic carbon and direct concentration carbon of sugars values in hydrolysis process.

Analysing the pH curve, it is possible to identify the correct time to collect the most representative samples in the process, saving time and money. The authors propose to take five samples, two before the minimum pH, one in the minimum pH and two after

the minimum pH with a comparable time interval to reduce the energy cost and time consumed.

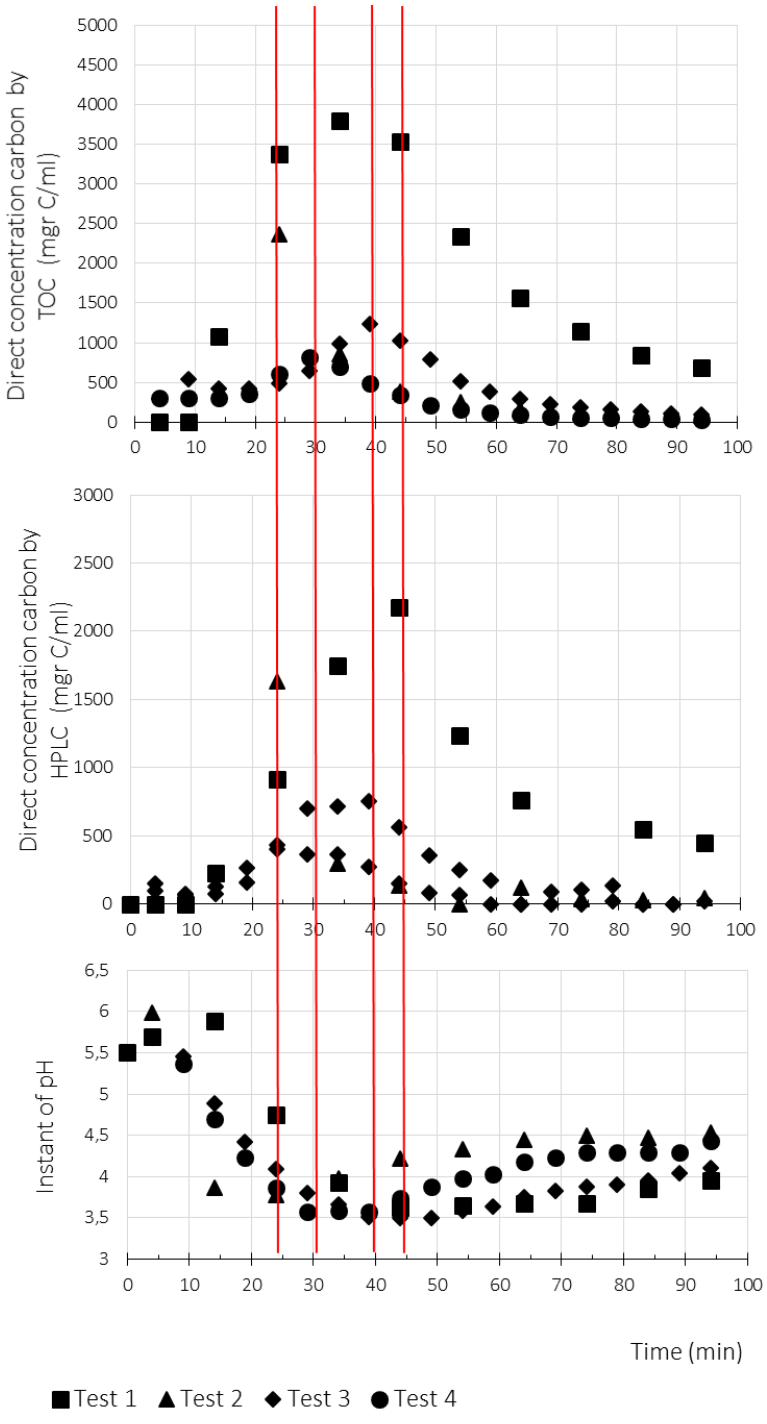

Figure 3. Direct concentration of Total Organic Carbon by TOC and HPLC and pH in function of the time obtained by hydrolysis process.

3.4. The pH variation during autohydrolysis

The behaviour of pH was similar in all experiments, as depicted in Figure 4 and indicated before. The pH value started at about 5.5 corresponding to the distilled water value. The extractives are the first components that can be extracted from biomass, generally in a previous pretreatment. The slight increase in the pH from 5.6 to 6.0 during the first 5 min may suggest the hydrolysis of ash, increasing the basicity of the reaction medium. This behaviour is explained with more detail in the Chapter 5 in the section 3.3.

After that, the pH decreased down to 3.5 to 3.8 during the first 24 to 44 min depending of the operational flow and temperature. The acetyl groups contained in the hemicelluloses are hydrolysed during the hot water extraction, liberating acetic acid that decreases the pH value [24]. The acetic acid act as a catalyst increasing the hydrolysis of carbohydrates and consequently the formation of degradation products [37]. After that, the pH increased up to 3.9 to 4.5 at 94 min suggesting that the reaction time was enough to extract the hemicelluloses, therefore the slight increase could be related with the decrease of concentration of hydrolysed hemicelluloses along time. In addition, this increment in the value of pH can be attributed to the presence of degradation products.

Other researchers have found a similar behaviour, like Sasaki et al. who studied cellulose hydrolysis in sub and supercritical water to recover glucose, fructose and oligomers. At 350ºC and 320ºC, the pH values in the product solution were 3.82 and 3.92 respectively, however at a temperature of 400ºC the pH was 4.72 [38]. The low value of the pH carbohydrates at high temperatures corresponded to the chemical degradation of the carbohydrates extracted, producing organic acids such as formic (pKa=3.75), lactic (pKa=3.86) and acrylic acid (pKa=4.35).

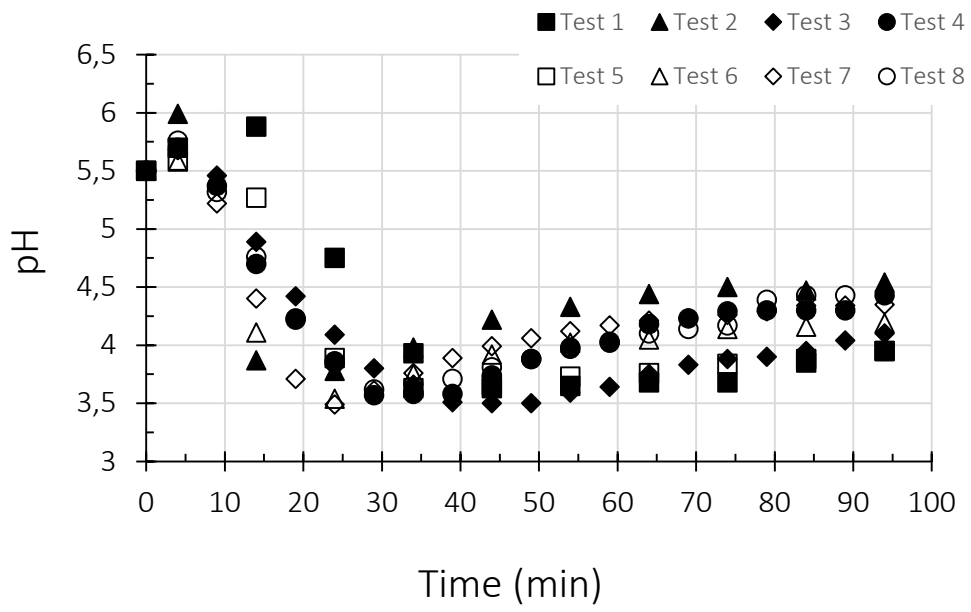

Figure 4. pH of the hydrolysates of Holm oak obtained by subcritical hydrolysis process at different temperatures, flows and particle sizes. Filled symbols correspond to lowest particle size and empty symbols correspond to highest particle size.

The time when the minimum pH value was achieved in each experiment was strongly influenced by temperature as shown in Figure 5. It exhibited a clear trend, the higher the temperature the lower the time when minimum pH appears. This is intimately related to the kinetics of deacetylation. Deacetylation can occur in the solid, directly liberating acetyl groups, or in the liquid, hydrolysing the extracted hemicelluloses and lowering the pH. In addition, the ionic product of water (K_w) increases along with the temperature (up to 220-240ºC), which contributes to a kinetic enhancement. It must be indicated that the protons can also come from sugar degradation into organic acids [38]. On the other hand, the minimum pH value was 3.5-3.8 for all experiments, regardless of experimental conditions of temperature, operational flow and particle size.

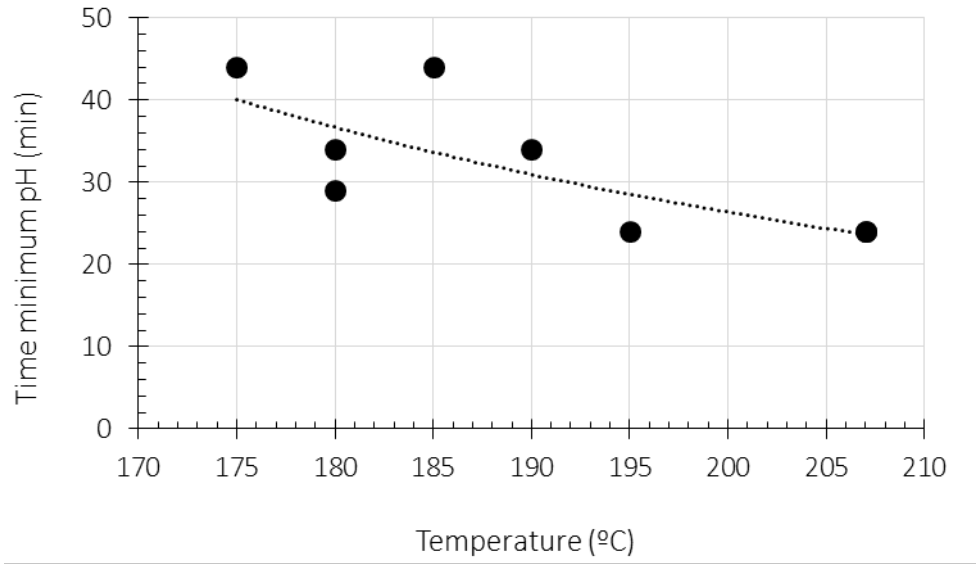

Figure 5. Time for the minimum pH versus temperature.

The influence of particle size in terms of deacetylation was clear too. The cleavage of the acetyl bonds can occur inside or outside the particle. The authors have found that, in general, the time required to obtain the minimum pH value was achieved by increasing the particle size, especially at low flow rates, meaning that deacetylation was eased inside the biomass particles. At higher flow rates the extraction is higher, even some fluidization of the bed may occur and 3 and 6 mm behaved similar. It seems that there is symbiosis between rapid extraction of hemicelluloses (using low particle size) and solubility of the biopolymers extracted. It is well-known that the acetyl groups aid in the solubility of biopolymers in water, so in principle, it is desirable to keep them in place avoiding deacetylation when possible [39]. Summarizing, it seems that spending energy in reducing the particle size benefits lowering the deacetylation rate.

In addition to this, the effect of flowrate is intimately related to mass transfer and residence time of the liquid in the reactor. In this research, it has found that, in general, higher flowrates lead to a faster deacetylation, for both 3 mm and 6 mm particle sizes. Obviously, as the number of acetyl groups inside the reactor is constant (initial batches of biomass were similar), and the key resides in the quantity of bulk liquid moving in the surroundings.

3.5. Total Organic Carbon and severity factor

The severity factor (log R0), that combines two effects: temperature and time, was calculated for each experiment (according to eq. 12).

$$\log R_0 = t \cdot e^{\frac{(T-100)}{14.75}} \quad (12)$$

where t is the process time in min, T is temperature in ºC, '100' is the reference temperature (ºC) indicating that below this temperature there is no reaction and '14.75' is a normalizing parameter used by other authors for similar raw materials [16, 40-42].

The cumulative total organic carbon extracted in the liquid samples varied from 7.70 wt% to 17.3 wt% depending on the severity factor and the flow rate (Figure 6). Thus, the cumulated TOC value was higher when the flow rate was increased. A higher flow rate increases the turbulence inside the system dissolving faster the carbohydrates.

On other hand, when the temperature was increased the severity factor was higher and the cumulative total organic carbon value in the liquid samples increased along, as at higher temperatures kinetics are enhanced.

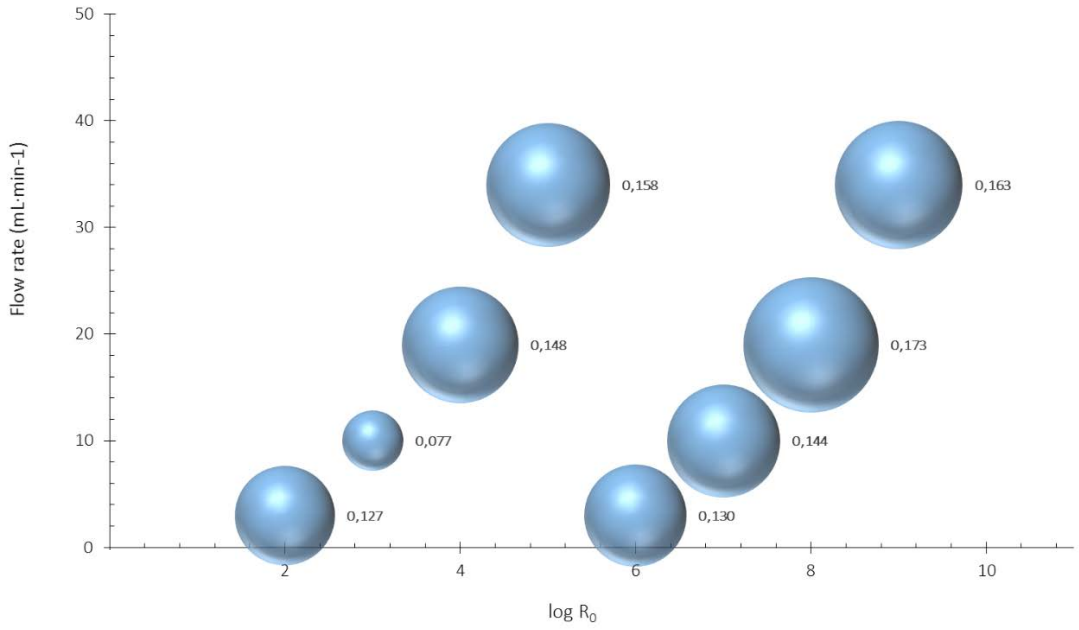

Figure 6. Relation between cumulative total organic carbon, severity factor and flow rate of Holm oak fractionation in subcritical water.

3.6. Solid residue inside of the reactor

The aim of a hydrothermal process can be double-fold: either extract the hemicelluloses usually between 120 and 170ºC, or to co-extract the hemicelluloses and cellulose using temperatures higher than 170ºC, obtaining a remaining solid rich in lignin and cellulose. In both cases degradation of the extracted sugars is observed under these conditions, especially at higher temperatures [43-46]. To avoid the presence of degradation products will be necessary the correct selection of experimental conditions. The influence of temperature and flow on yield of degradation products will be explained in the Section 3.7.

The amount of solid inside of the reactor after the processing time is plotted in Figure 7. The solid residue was between 68-47 wt% for all the experimental conditions studied. These values were lower when the temperature was increased but it was not influenced by the particle size. The lignin content together with the ashes in the raw material accounts for 29.4 wt%; as the solid residue was greater than this value in all cases, this indicated that some sugars were not hydrolysed under such conditions. Cellulose was the main component after fractionation in solid inside of the reactor.

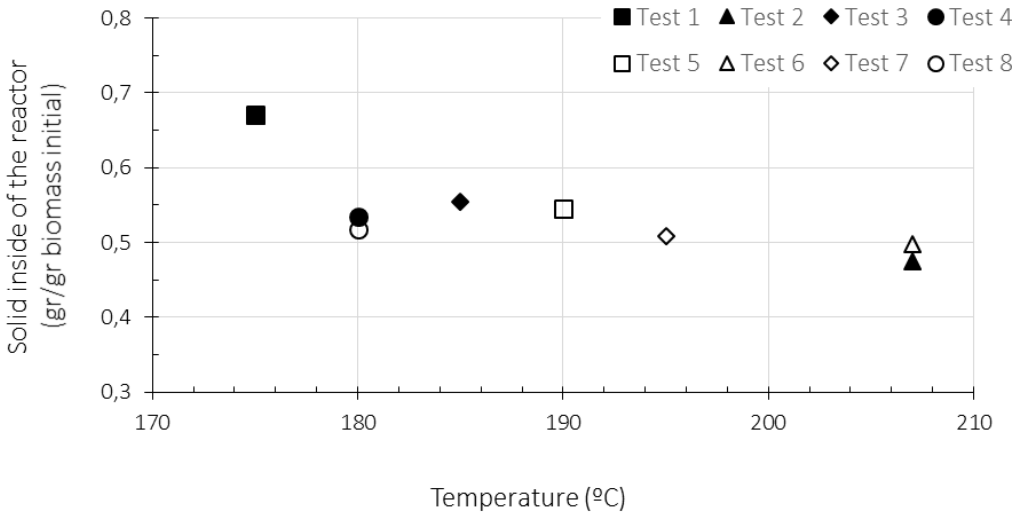

Figure 7. Solid inside of the reactor in function of temperature obtained by subcritical water.

In this work we have focused our attention in the liquid products, more than in the carbonized matter. Nevertheless, SEM and FTIR analyses of the solid samples and raw material have been carried out to evaluate the solid characteristics after the hydrolysis process. Figure 8 shows the SEM images of the raw material and the carbonized solid

after the process time. These images revealed interesting changes in their morphology in relation to the raw biomass. There were major disorganizations in the fibres in all images, indicating the rupturing of the lignocellulosic structure and the extraction of some sugars, as expected from the previous discussed results. The presence of carbon spheres at 207ºC (D and E) was observed, it can be due that the lignin was dissolved and re-precipitated [47]. This behaviour was observed by Nitsos et al. but at lower values of severity factor than observed in this work (log R0=3.5). The existence of porous structure demonstrate a strong dependence with the temperature (to compare B and C). When the temperature increased only 15 ºC, it can be observed to major disorganization in the fibers and the presence of nanostructures (increment of specific surface area) [48]. The influence of particle size was not observed.

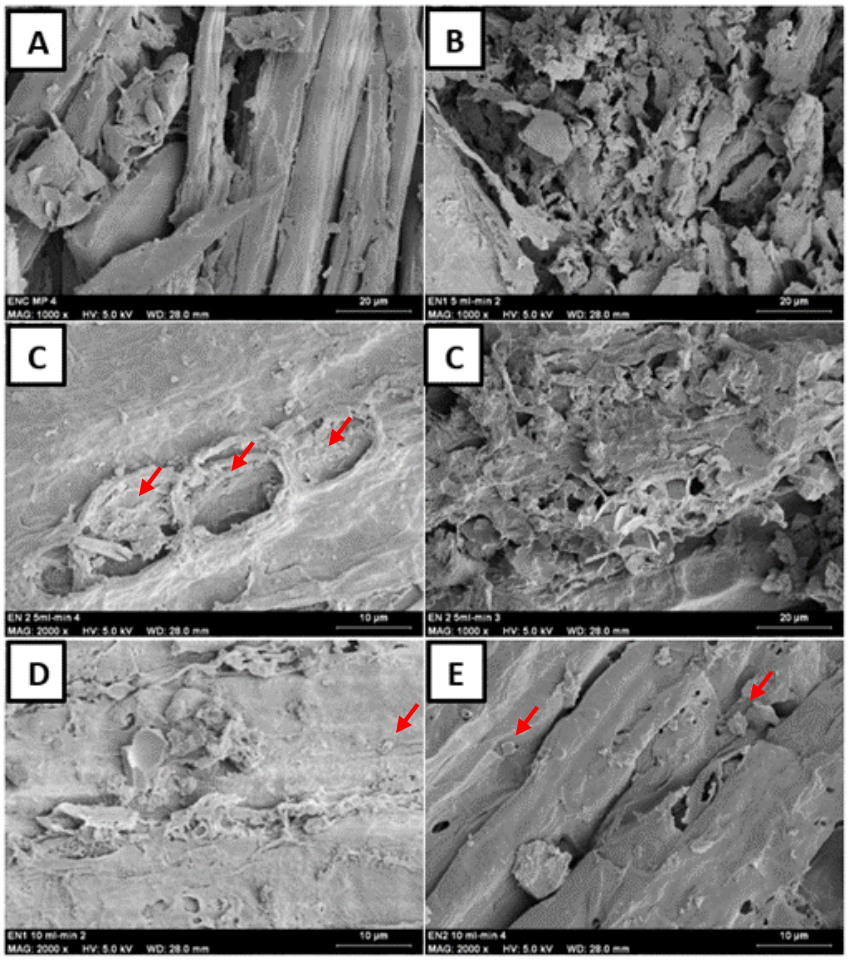

Figure 8. SEM images of raw material (A) and solid residue obtained from hydrolysis process of Holm oak at 175ºC, 3 mL/min and 3mm of particle size (B), 190ºC, 3 mL/min and 6 mm of particle size (C), 207ºC, 10 mL/min and 3 mm of particle size (D), 207ºC, 10 mL/min and 6 mm of particle size (E).

Figure 9 shows FT-IR spectrums of the raw material and the solid products after hydrolysis process (Test 1-8). The band at 1200-1000 cm^{-1} region is a typical region of hemicelluloses by stretching and bending vibrations of C-OH, C-C, C-O and C-O-C bonds [49]. The band at 1165 cm^{-1} represents arabinoxylans structure [49] and the band at 1135 cm^{-1} represents syringyl lignin. These bands were observed in all samples suggesting that lignin and some hemicelluloses were still present in the remaining solid.

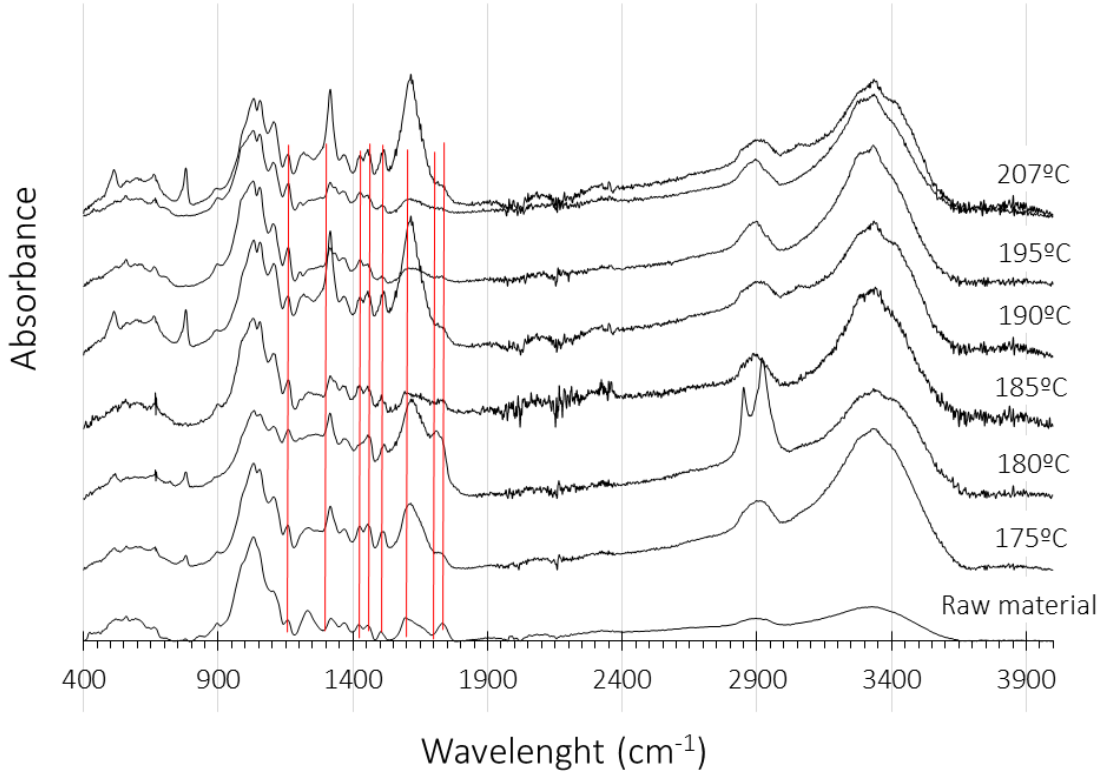

Figure 9. FT-IR spectrums of the raw material and the solid products after hydrolysis process at (Test #1) 175ºC, 3 mL/min and 3mm of particle size, (Test #2) 190ºC, 3 mL/min and 6 mm of particle size, (Test #3) 207ºC, 10 mL/min and 3 mm of particle size, (Test #4) 207ºC, 10 mL/min and 6 mm of particle size, (Test #5) 185ºC, 19 mL/min and 3 mL of particle size, (Test #6) 195ºC, 19 mL/min and 6 mm of particle size, (Test #7) 180ºC, 34 mL/min and 3 mm of particle size and (Test #8) 180ºC, 34 mL/min and 6 mm of particle size.

The band at 1747 cm^{-1} is assigned to ester linked acetyl, feruloyl and p-cuomaryl groups between hemicelluloses and lignin[50]. The absence of the band in all tests suggested that the links between hemicelluloses and lignin were broken during the hydrothermal process. The band at 1717 cm^{-1} represent stretching vibrations of C-O bonds of ketone, ester and carboxyl groups [23]. This band was observed with more intensity in the raw

material and even temperatures of 180ºC suggesting that hydrolysis of hemicellulose was higher when the temperature was increased.

The band at 1500-1610 cm^{-1} region is aromatic skeletal vibrations [50]. The band characteristics of lignin [23] at 1515, 1465, 1424 and 1375 cm^{-1} were observed with more intensity in the solid residue compare to raw material, suggesting more content of lignin in the solid residue according with the results showed in the Figure 10 discussed below.

3.7. Carbohydrate extraction vs Severity factor

Aimed at comparing the extraction of each of the initial fractions in the biomass, Figure 10 depicts the content of hemicellulose, cellulose, lignin and ashes. The amount of ash was under the detection limit in all experiments for this biomass. The percentage of lignin in the solid was between 0.28 and 0.36 gr/gr biomass. The degradation of lignin occurs from 280 up to 365ºC or higher [51] and the cellulose can be hydrolysed at temperatures above 210 up to 260ºC, for this reason the solid residues were rich in cellulose and lignin.

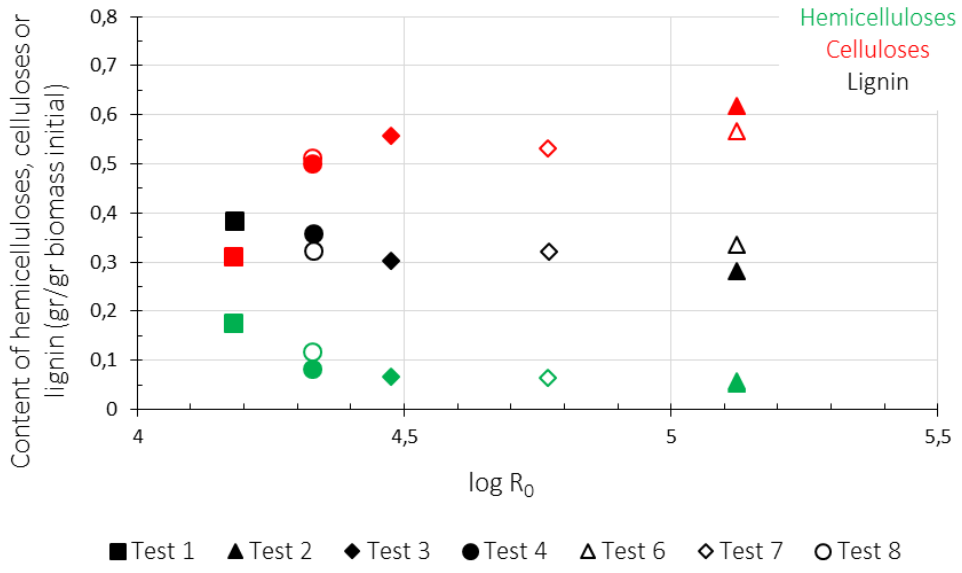

Figure 10. Carbohydrates and lignin composition of the solid residues.

The content of hemicelluloses were expressed as xylose, galactose, mannose and arabinose. The content of cellulose was expressed as glucose, fructose and cellobiose. The presence of derivatized sugars, such as glyceraldehyde, lactic acid, formic acid, acetic acid and levulinic acid, was observed.

The amount of hemicellulose in residue solid decreased by increasing the severity factor. The hemicellulose is the first biomass biopolymer that can be hydrolysed in a hydrothermal process, typically hydrolysis starts at temperatures above 120ºC. For instance, the results obtained by Soledad Mateo et al. showed that the total conversion of hemicelluloses from olive tree pruning was near to log R_0=3.9 using only water and this parameter can be minor using sulphuric acid [52].

In this research, the minimum extraction of hemicelluloses from Holm oak was observed at the lowest temperature (175ºC, log R_0=4.18) with 0.17 gr/gr biomass (0.22 gr/gr hemicelluloses) still remaining in the solid. On the contrary, the maximum extraction of hemicelluloses (0.05 gr/gr biomass; 0.06 gr/gr hemicelluloses) was observed at the highest severity factor (log R_0=5.12) due to that the reaction kinetics increase with the temperature increasing the extraction of carbohydrates.

The particle size also influenced the extraction, the smaller the size the higher the extraction. Thus, using the lower particle size the concentration of hemicelluloses in the solid residue was 0.07 gr/gr biomass (0.08 gr/gr cellulose) compared 0.12 gr/gr (0.14 gr/gr hemicelluloses) biomass for the bigger particle size. This behaviour was in agreement with other studies from Krogell, Korotkova et al. and Qin et al. [53, 54].

On the other hand, the content of cellulose inside of the solid residue was between 0.31 (0.22 gr/gr cellulose) and 0.76 gr/gr biomass (0.55 gr/gr cellulose), as they are more difficult to degrade. The percentage of cellulose inside of the solid residue increased steadily along with the severity factor obtaining a solid rich in lignin and cellulose due to the extraction of hemicelluloses increased along with the severity factor.

As explained in the experimental section, the liquid samples were analysed off-line by hydrolysing them using sulphuric acid, to obtain the amount of monomeric carbohydrates and degradation products as monomers. Total sugars were analysed by HPLC and the results are shown in Table 2.

Table 2. Accumulate total sugars and sugars degradation obtained by hydrolysis process of Holm oak (*Quercus ilex*).

Component (gr accumulate/gr initial biomass)	Test 1	Test 2	Test 3	Test 4
Glucose	0.012	0.008	0.004	0.005
Xylose	0.090	0.177	0.129	0.114
Arabinose	0.019	0.018	0.018	0.013
Pyruvaldehyde	-	0.0004	-	-
Formic acid	0.005	0.007	0.014	0.006
Acetic acid	0.019	0.030	0.028	0.023
Acrylic acid	-	0.004	0.008	0.005
Total sugars and derivates sugars	0.146	0.246	0.203	0.168

The total sugars and sugars derivatized products after acid hydrolysis were between 0.15 and 0.25 gr/gr biomass. The maximum extraction was obtained at 207ºC (Test #2). The maximum recovery of hemicelluloses was 0.80 gr/gr hemicelluloses. The minimum extraction of hemicelluloses was at 175ºC and 3 $mL \cdot min^{-1}$ and the recovery was only 0.45 gr/gr hemicelluloses according with the reaction kinetic is lower at lower temperatures. When the temperature increased 5ºC and the flow was 34 $mL \cdot min^{-1}$, the extraction of hemicelluloses was 0.52 gr/gr hemicelluloses increasing the 7.36% of the extraction of hemicelluloses. When the temperature increased other 5ºC (reaching at 185ºC) and the flow was 19 $mL \cdot min^{-1}$, the extraction of hemicelluloses increased 8.32%, while increasing 22ºC (reaching at 207ºC) the extraction of hemicelluloses increased 19.6%. The hemicelluloses were the main component removed from Holm oak. The xylose was the most abundant sugar in the samples, representing for above of 0.74 gr/gr carbohydrates in the liquid samples. The amount of cellulose was 0.012, 0.008, 0.004 and 0.005 gr/gr biomass in the Test #1, #2, #3 and #4 respectively. These values represented less than 3.0% gr/gr cellulose.

In the hydrolysis process, the extraction of sugars increased along with time and temperature, but some degradation products (from the sugar) appeared too. In this way, the presence of pyruvaldehyde, formic, acetic and acrylic acid was observed. These components are undesirable in some cases, for example in the fermentation process to produce bio-oils [55]. The amount of sugars degradation was 0.02, 0.04, 0.05 and 0.03 gr/gr biomass for the Test #1, #2, #3 and #4 respectively. From our results, it seems that the degradation is also function of the concentration of the sugars extracted, as Prado

and Follegatti-Romero et al. also pointed out [22]. For similar values of hydrolysis of sugars (Test 3 and Test 4) and similar temperatures, the degradation of sugars decreased with the flow because the residence time (see Table 3) inside of the reactor was lower, decreasing the time for the degradation of sugars [22].

The acetic acid was the dominant sugars degradation (0.15-0.18 gr/gr carbohydrates). The amount of acid insoluble lignin in the liquid samples was not observed. The hydrolysis of carbohydrates takes place at temperatures higher than 100ºC, therefore, at temperatures lower than 100ºC, a small hydrolytic reactions can be observed [56]. For this reason, the experimental time was started when the temperature inside the reactor reached 100ºC. The target temperature required for each experiment was reached in around 10 min maximum in general. During the pre-heating time some sugars were extracted and also degraded, but it represented less than 0.01 gr/gr cellulose and 0.01 gr/gr hemicelluloses.

Table 3. Initial and final residence time for each experiment.

Test (Nº)	Initial Residence Time (min)	Final Residence Time (min)
#1	8.3	8.8
#2	3.3	3.6
#3	1.8	1.9
#4	1.0	1.1
#5	12.9	14.2
#6	3.3	3.6
#7	1.6	1.8
#8	0.9	1.0

4. Conclusions

Holm oak hydrolysis in subcritical water was studied at temperatures between 175 and 207ºC and 100 bar employing a semi-continuous reactor during 94 min. In the hydrolysis process, the extraction of sugars increased along with time and temperature, and some degradation products were observed. The maximum yield of carbohydrates and degradation products was obtained at higher temperature. These values were between 0.15 and 0.25 gr/gr biomass. The hemicelluloses were the main component removed from Holm oak being the xylose the most abundant carbohydrate in the liquid sample which value was 0.74 gr/gr carbohydrates. The amount of cellulose represented

less than 3.0% gr/gr cellulose in the liquid sample and consequently a solid rich in lignin and cellulose was obtained. The presence of pyruvaldehyde, formic, acetic and acrylic acid was detected. The amount of sugars degradation was between 0.02 and 0.05 gr/gr biomass and these values depended on the yield of sugars extracted. High yield of hydrolysed carbohydrates was related with high temperatures and it was accompanied with the highest degradation products concentration. The decrease of degradation of sugars with the flow was observed due to lower residence time. The acetic acid was the mainly component of degradation products represented 0.15-0.18 gr/gr carbohydrates.

The behaviour of pH, total organic carbon and yield of carbohydrates was analysed. The pH decreased due to the acetic acid liberation during the cleavage of the hemicelluloses. The acetic acid acts as a catalyst increasing the hydrolysis of hemicelluloses and celluloses and the formation of degradation products. The behaviour of pH showed a minimum value. This point was located in the same time that the maximum TOC value and the maximum carbon instant of sugars were observed suggesting that the maximum yield of hydrolysed carbohydrates was obtained when the cleavage of hemicelluloses is high. The pH is a rapid and cheap parameter. For this reason, the pH can be an excellent parameter to follow on-line the hydrolysis process. Analysing the pH curve, it can possible identify the correct time to collect the samples more representative in this process, saving time and money. This parameter represents a good opportunity to follow to process to industrial scale.

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References

[1] S. Sabiha-Hanim, M.A. Mohd Noor, A. Rosma, Fractionation of oil palm frond hemicelluloses by water or alkaline impregnation and steam explosion, Carbohydrate Polymers 115 (2015) 533-539.

[2] D.A. Cantero, M. Dolores Bermejo, M. José Cocero, Reaction engineering for process intensification of supercritical water biomass refining, The Journal of Supercritical Fluids (2014).

[3] A.M. da Costa Lopes, K.G. João, D.F. Rubik, E. Bogel-Łukasik, L.C. Duarte, J. Andreaus, R. Bogel-Łukasik, Pre-treatment of lignocellulosic biomass using ionic liquids: Wheat straw fractionation, Bioresource Technology 142 (2013) 198-208

[4] N. Labbé, L.M. Kline, L. Moens, K. Kim, P.C. Kim, D.G. Hayes, Activation of lignocellulosic biomass by ionic liquid for biorefinery fractionation, Bioresource Technology 104 (2012) 701-707.

[5] Y.P. Wijaya, R.D.D. Putra, V.T. Widyaya, J.-M. Ha, D.J. Suh, C.S. Kim, Comparative study on two-step concentrated acid hydrolysis for the extraction of sugars from lignocellulosic biomass, Bioresource Technology 164 (2014) 221-231

[6] L. Fu, S.A. McCallum, J. Miao, C. Hart, G.J. Tudryn, F. Zhang, R.J. Linhardt, Rapid and accurate determination of the lignin content of lignocellulosic biomass by solid-state NMR, Fuel 141 (2015) 39-45

[7] T. Cordero, F. Marquez, J. Rodriguez-Mirasol, J.J. Rodriguez, Predicting heating values of lignocellulosics and carbonaceous materials from proximate analysis, Fuel 80 (2001) 1567-1571

[8] X. Lü, S. Saka, Hydrolysis of Japanese beech by batch and semi-flow water under subcritical temperatures and pressures, Biomass and Bioenergy 34 (2010) 1089-1097.

[9] M. Sasaki, T. Adschiri, K. Arai, Fractionation of sugarcane bagasse by hydrothermal treatment, Bioresource Technology 86 (2003) 301-304.

[10] J.M. Prado, T. Forster-Carneiro, M.A. Rostagno, L.A. Follegatti-Romero, F. Maugeri Filho, M.A.A. Meireles, Obtaining sugars from coconut husk, defatted grape seed, and pressed palm fiber by hydrolysis with subcritical water, The Journal of Supercritical Fluids 89 (2014) 89-98.

[11] M.N. Baig, R.C.D. Santos, J. King, D. Pioch, S. Bowra, Evaluation and modelling of continuous flow sub-critical water hydrolysis of biomass derived components; lipids and carbohydrates, Chemical Engineering Research and Design 91 (2013) 2663-2670.

[12] F. Peng, P. Peng, F. Xu, R.-C. Sun, Fractional purification and bioconversion of hemicelluloses, Biotechnology Advances 30 (2012) 879-903

[13] P. Peng, D. She, Isolation, structural characterization, and potential applications of hemicelluloses from bamboo: A review, Carbohydrate Polymers 112 (2014) 701-720

[14] M. Yang, S. Kuittinen, J. Zhang, J. Vepsäläinen, M. Keinänen, A. Pappinen, Co-fermentation of hemicelluloses and starch from barley straw and grain for efficient pentoses utilization in acetone-butanol-ethanol production Bioresource Technology.

[15] C.S. Itziar Egüés, Effect of alkaline and autohydrolysis processes on the purity of obtained hemicelluloses from corn stalks, Bioresource technology 103 (2012) 239-248.

[16] M. Rubio, J.F. Tortosa, J. Quesada, D. Gómez, Fractionation of lignocellulosics. Solubilization of corn stalk hemicelluloses by autohydrolysis in aqueous medium, Biomass and Bioenergy 15 (1998) 483-491

[17] S. Rivas, E. Conde, A. Moure, H. Domínguez, J.C. Parajó, Characterization, refining and antioxidant activity of saccharides derived from hemicelluloses of wood and rice husks, Food Chemistry 141 (2013) 495-502.

[18] J.V. Rissanen, H. Grénman, S. Willför, D.Y. Murzin, T. Salmi, Spruce Hemicellulose for Chemicals Using Aqueous Extraction: Kinetics, Mass Transfer, and Modeling, Industrial & Engineering Chemistry Research 53 (2014) 6341-6350

[19] M.S. Tunc, A.R.P. Van Heiningen, Hemicellulose extraction of mixed southern hardwood with water at 150 °C: Effect of time, Industrial and Engineering Chemistry Research 47 (2008) 7031-7037.

[20] R. Kohn, P. Kovác, Dissociation of D-galacturonic and D-glucuronic acid and their O-methyl derivatives, Chem. zvesti 32 (1978) 478-485.

[21] M.R. Narkhede, C. Nguyen-Trung, D.A. Palmer, Dissociation quotients of D-galacturonic acid in aqueous solution at 0.1 MPa to 1 molal ionic strength and 100°C, Journal of Solution Chemistry 23 (1994) 877-888.

[22] J.M. Prado, L.A. Follegatti-Romero, T. Forster-Carneiro, M.A. Rostagno, F. Maugeri Filho, M.A.A. Meireles, Hydrolysis of sugarcane bagasse in subcritical water, Journal of Supercritical Fluids 86 (2014) 15-22.

[23] F.M. Yedro, J. García-Serna, D.A. Cantero, F. Sobrón, M.J. Cocero, Hydrothermal hydrolysis of grape seeds to produce bio-oil, RSC Advances (2014) (accepted 21 June 2014, DOI: 2010.1039/C2014RA00429A).

[24] F.M. Yedro, J. García-Serna, D.A. Cantero, F. Sobrón, M.J. Cocero, Hydrothermal fractionation of grape seeds in subcritical water to produce oil extract, sugars and lignin, Catalysis Today in press (2014).

[25] G. Garrote, H. Domínguez, J.C. Parajó, Study on the deacetylation of hemicelluloses during the hydrothermal processing of Eucalyptus wood, Holz als Roh- und Werkstoff 59 (2001) 53-59.

[26] T. Forster-Carneiro, J.M. Prado, R. Vardanega, D.L. Perez, M.A.A. Meireles, Subcritical water hydrolysis of sugarcane bagasse and coconut husk assisted by carbon dioxide, Chemical Engineering Transactions, 2014, pp. 391-396.

[27] M.A. Rostagno, J.M. Prado, R. Vardanega, T. Forster-Carneiro, M.A.A. Meireles, Study of recovery of fermentable sugars with subcritical water and carbon dioxide from palm fiber and grape seed, Chemical Engineering Transactions, 2014, pp. 403-408.

[28] S.P.M. Da Silva, A.R.C. Morais, R. Bogel-Łukasik, The CO2-assisted autohydrolysis of wheat straw, Green Chemistry 16 (2014) 238-246.

[29] A.R.C. Morais, A.C. Mata, R. Bogel-Lukasik, Integrated conversion of agroindustrial residue with high pressure CO 2 within the biorefinery concept, Green Chemistry 16 (2014) 4312-4322.

[30] M.D. Carbonero, P. Fernández-Rebollo, Dehesas de encinas. Influencia de la meteorología en la producción de bellotas, Ecosistemas 23 (2014) 55-63.

[31] R. Joffre, S. Rambal, J.P. Ratte, The dehesa system of southern Spain and Portugal as a natural ecosystem mimic, Agroforestry Systems 45 (1999) 57-79.

[32] V. Francescato, E. Antonini, L.Z. Bergomi, Wood fuels handbook, Italian Agriforestry Energy Association, 2008.

[33] N.R.E. Laboratory, Determination of Extractives in Biomass Golden, CO, 2008.

[34] S.-N. Sun, X.-F. Cao, H.-Y. Li, F. Xu, R.-C. Sun, Structural characterization of residual hemicelluloses from hydrothermal pretreated Eucalyptus fiber, International Journal of Biological Macromolecules 69C (2014) 158-164.

[35] N.R.E. Laboratory, Determination of Structural Carbohydrates and Lignin in Biomass, Golden, CO, 2011.

[36] S.-N. Sun, X.-F. Cao, H.-Y. Li, F. Xu, R.-C. Sun, Structural characterization of residual hemicelluloses from hydrothermal pretreated Eucalyptus fiber, International Journal of Biological Macromolecules 69 (2014) 158-164.

[37] S. Liu, Woody biomass: Niche position as a source of sustainable renewable chemicals and energy and kinetics of hot-water extraction/hydrolysis, Biotechnology Advances 28 (2010) 563-582.

[38] M. Sasaki, B. Kabyemela, R. Malaluan, S. Hirose, N. Takeda, T. Adschiri, K. Arai, Cellulose hydrolysis in subcritical and supercritical water, The Journal of Supercritical Fluids 13 (1998) 261-268

[39] H.-Q. Li, W. Jiang, J.-X. Jia, J. Xu, pH pre-corrected liquid hot water pretreatment on corn stover with high hemicellulose recovery and low inhibitors formation, Bioresource Technology 153 (2014) 292-299.

[40] G. Garrote, H. Domínguez, J.C. Parajó, Interpretation of deacetylation and hemicellulose hydrolysis during hydrothermal treatments on the basis of the severity factor, Process Biochemistry 37 (2002) 1067-1073.

[41] A. Banerji, M. Balakrishnan, V.V.N. Kishore, Low severity dilute-acid hydrolysis of sweet sorghum bagasse, Applied Energy 104 (2013) 197-206

[42] J.-W. Lee, T.W. Jeffries, Efficiencies of acid catalysts in the hydrolysis of lignocellulosic biomass over a range of combined severity factors, Bioresource Technology 102 (2011) 5884-5890.

[43] J.Y. Lee, H.J. Ryu, K.K. Oh, Acid-catalyzed hydrothermal severity on the fractionation of agricultural residues for xylose-rich hydrolyzates, Bioresource Technology 132 (2013) 84-90

[44] J.Y. Hong, Y.S. Kim, K.K. Oh, Fractionation and delignification of empty fruit bunches with low reaction severity for high sugar recovery, Bioresource Technology 146 (2013) 176-183.

[45] R. Yáñez, G. Garrote, M.J. Dı´az, Valorisation of a leguminous specie, Sesbania grandiflora, by means of hydrothermal fractionation, Bioresource Technology 100 (2009) 6514-6523.

[46] P. Moniz, H. Pereira, T. Quilhó, F. Carvalheiro, Characterisation and hydrothermal processing of corn straw towards the selective fractionation of hemicelluloses, Industrial Crops and Products 50 (2013) 145-153

[47] C.K. Nitsos, K.A. Matis, K.S. Triantafyllidis, Optimization of hydrothermal pretreatment of lignocellulosic biomass in the bioethanol production process, ChemSusChem 6 (2013) 110-122.

[48] M. Sevilla, A.B. Fuertes, Chemical and structural properties of carbonaceous products obtained by hydrothermal carbonization of saccharides, Chemistry - A European Journal 15 (2009) 4195-4203.

[49] B. Sukhbaatar, E.B. Hassan, M. Kim, P. Steele, L. Ingram, Optimization of hot-compressed water pretreatment of bagasse and characterization of extracted hemicelluloses, Carbohydrate Polymers 101 (2014) 196-202

[50] Y. Xu, B. Chen, Investigation of thermodynamic parameters in the pyrolysis conversion of biomass and manure to biochars using thermogravimetric analysis, Bioresource Technology 146 (2013) 485-493.

[51] J. Hu, D. Shen, S. Wu, H. Zhang, R. Xiao, Effect of temperature on structure evolution in char from hydrothermal degradation of lignin, Journal of Analytical and Applied Pyrolysis 106 (2014) 118-124

[52] S. Mateo, J.G. Puentes, S. Sánchez, A.J. Moya, Oligosaccharides and monomeric carbohydrates production from olive tree pruning biomass, Carbohydrate Polymers 93 (2013) 416-423.

[53] J. Krogell, E. Korotkova, K. Eränen, A. Pranovich, T. Salmi, D. Murzin, S. Willför, Intensification of hemicellulose hot-water extraction from spruce wood in a batch extractor – Effects of wood particle size, Bioresource Technology 143 (2013) 212-220.

[54] Z. Li, M. Qin, C. Xu, X. Chen, Hot Water Extraction of Hemicelluloses from Aspen Wood Chips of Different Sizes, BioResources 8 (2013) 5690-5700

[55] E. Palmqvist, B. Hahn-Hägerdal, Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification, Bioresource Technology 74 (2000) 17-24

[56] S. Kumar, U. Kothari, L. Kong, Y.Y. Lee, R.B. Gupta, Hydrothermal pretreatment of switchgrass and corn stover for production of ethanol and carbon microspheres, Biomass and Bioenergy 35 (2011) 956-968

Chapter 4

Obtaining hemicelluloses from hardwood Holm oak (*Quercus ilex*) using subcritical water in a pilot plant

Abstract

Nowadays, the conversion of fuels and added-value products from lignocellulosic raw materials using water as solvent is an attractive green process. In this study, Holm oak (*Quercus ilex*) was submitted to hydrothermal treatment separating a liquid fraction rich in hemicelluloses. The experiments were carried out using a five reactors connected in series to form a cascade reactor. The temperatures were between 130 and 170ºC. The effects of temperature and reaction time on the conversion and molar mass of hemicelluloses were investigated. The results show that the maximum hydrolysis rate of hemicelluloses depends strongly on the temperature and the biomass used. The maximum conversion (approximately 60%) was obtained at 170ºC during 20 min. After this time, the decreases of conversion can be attributed to the presence of degradation products. On the contrary, the conversion at 130ºC and 140ºC did not exhibit a maximum value indicating that the reaction time was not long enough for complete the hydrolysis. The major component extracted at lower temperatures was glucose (130 and 140ºC) and at higher temperatures (150, 160 and 170ºC) was xylose.

The deacetylation was accompanied by a reduction in the molar mass. The average molar mass of the carbohydrates from hydrolysis of Holm oak decreased with increasing reaction temperature. The average molar mass decreased from 12.9 to 1.75 KDa at 170 ºC during 60 min of hydrolysis. At higher temperatures the hemicelluloses had a

pronounced lower average molar mass after a few minutes of reaction. Compared to Norway spruce (softwood), the average molar mass in Holm oak (hardwood) was lower under the same reaction conditions suggesting that the deacetylation is higher due to a higher content of acetyl groups.

Keywords: Holm oak, glucose, xylose, molar mass, hemicelluloses, extraction, leaching

1. Introduction

The production of fuels, energy and value-added products from renewable resources is of growing interest due to the deflection of fossil resources and the increase of the concentrations of greenhouse gases. In this way, the biorefinery concept has emerged. Lignocellulosic biomass is an alternative as a raw material, the hemicelluloses being one of the main component of biomass. The amount of hemicelluloses (dry weight) in wood material is usually between 20 and 30% [1], but the composition depends on the source. Hemicelluloses are polysaccharides composed mainly of xylose, glucose, arabinose, galactose and mannose. Hardwood contains mainly xylans while softwood mostly consists of galactoglucomannans. The hardwood hydrolysate product contains large amount of xylose and its oligomers [2], and consequently xylose can be used as indicator for following the hydrothermal hemicelluloses process. Dehydration of fructose produces 5-hydroxymethylfurfural and pentoses produce furfural.

During a hydrothermal process, the release of acetyl groups from hemicelluloses [3], catalyses the degradation of hemicelluloses to shorter chains and, consequently, suppresses the molar mass [4]. The knowledge of the chemical composition and molar mass of extracted hemicellulose fractions is crucial for further applications. Targetting long-chain hemi-celluloses, the pH is a key factor during the extraction of carbohydrates from wood. Krogell et al. reported in 2015 that adjusting the pH to 4.8, the molar mass of the extracted hemicelluloses originating from Norway spruce using a batch reactor at 170ºC was higher than the molar mass obtained without pH control [5]. In the same way, Tunc and Heiningen in 2011 observed that the temperature played an important role in the average molar mass obtained in the final biopolymer; it decreases with increasing temperature [6]. The structure of hemicelluloses is mainly amorphous and the molar mass is lower compared with cellulose, consequently it is easier to hydrolyse than the cellulose, being the main component in the hydrolysate product at temperatures below 220ºC [7]. The hydrolysis of cellulose takes place at temperatures higher than 230ºC, therefore, at lower temperatures mainly hemicelluloses are extracted [7]. Sattler et al. reported that the extraction of hemicelluloses from wood flakes begins at 120ºC [5], and correspondingly Leppänen et al. observed that low amounts of hemicelluloses could be

extracted already at 120ºC-160ºC [1], which indicates the practical lower temperature limit for the extraction. Kilpeläinen et al. explained that 70% of xylan can be recovered from ground birch wood at 190ºC in 30 min using a flow-through vessel, but the degree of polymerization of the xylans decreased significantly [8]. Considering the severity factor, pseudo-first order reaction that combines two effects: temperature and residence time allowing the comparison of different treatment conditions, an other important factor is the reaction time [9]. Thus principally, a similar amount of the biopolymer can be extracted or hydrolysed at a higher temperature with less extraction time and vice versa. However, there is a minimum temperature required for the process to work, as explained previously. 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 products [4, 10].

In this study, the extraction of hemicelluloses from Holm oak (*Quercus ilex*) using subcritical water was investigated, focusing on the effect of temperature and reaction time on the yield of carbohydrates and molar mass of the product. The presence of degradation products was not desirable. 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 select the best reaction conditions to obtain the high yield of hemicelluloses with low yield of degradation products and with an specific value of molar mass.

2. Materials and methods

2.1. Materials

The Holm oak (*Quercus ilex*) 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 according to Section 2.3. 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)

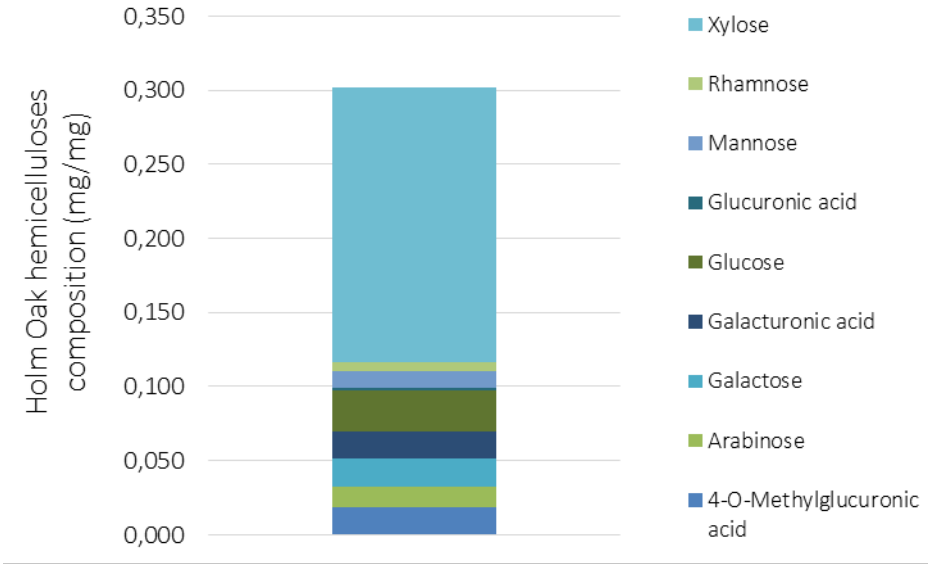

Figure 1. Hemicelluloses composition in the raw material.

The compounds identified in the gas chromatographic analysis were: xylose (Xyl), rhamnose (Rha), mannose (Man), glucuronic acid (GlcA), glucose (Glu), galacturonic acid (GalA), galactose (Gal), arabinose (Ara), 4-O-methylglucuronic acid (4-O-MeGlcA), and sorbitol and resorcinol standards. For the determination of the hemicelluloses content the following reagents were used: pyridine, hydrochloric acid (HCl), methanol, hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS).

2.2. Experimental device

The experiments were carried out in a cascade reactor comprising five reactors connected in series, as explained elsewhere [11]. 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 raw material. The 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 and the temperature was measured continuously inside and outside of the reactor to 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 170ºC. Each reactor was charged with 5 g of

hardwood (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 34 in the reactor. The liquid inside in the by-pass part of the system was rapidly heated to desired temperature. After that, the by-pass section was opened allowing the circulation of the hot water through the reactors. This moment was set to time zero (i.e. reaction started). From a macroscopic point of view the reaction system behaved like a perfectly agitated reactor, as the flowrate was very high and it was operating in recirculation mode. Figure 2 shows a simplified scheme of the experimental device.

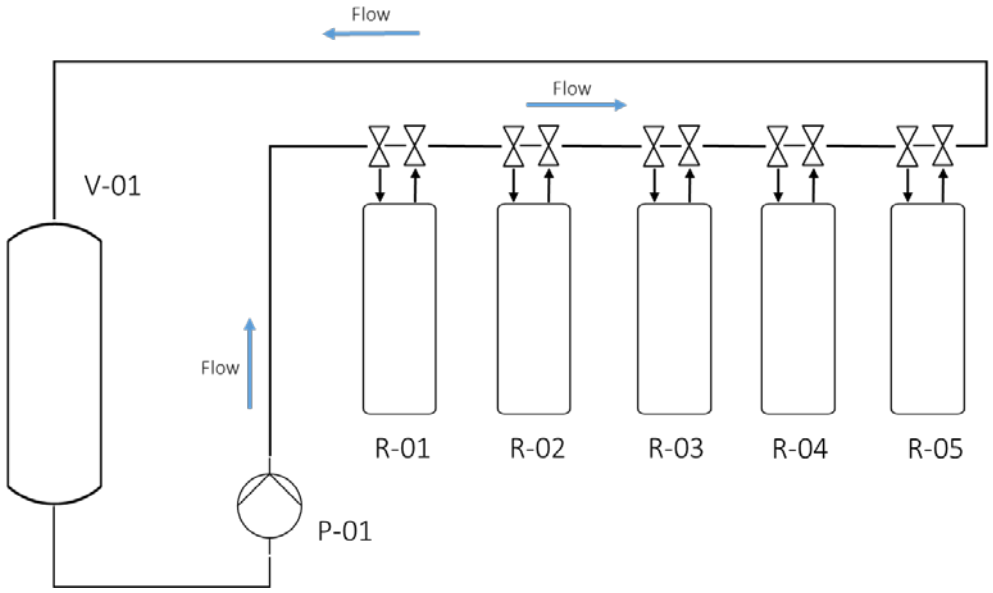

Figure 2. Schematic flow diagram of the experimental system. Equipment: V-01 Collector vessel, P-01 Pump, R-01/R-05 Reactors.

As the pre-established sampling time was reached (see Table 1), one of the reactors was again by passed and cooled down rapidly. The cooled down performed was carried out introducing directly the reactor into a cold water vessel. Five liquid and solid samples were obtained from a single experiment. The liquid and solid samples were collected and then analysed further as explained in Section 2.3.

Table 1. Sampling time for each experimental temperature

	130ºC	140ºC	150ºC	160ºC	170ºC
Reactor (Nº)	Sampling time (min)	Sampling time (min)	Sampling time (min)	Sampling time (min)	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

2.3. Analytical methods

2.3.1. Analysis

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

2.3.2. Hemicelluloses content

Liquid phase samples were subjected to acid methanolysis [12] using resorcinol and sorbitol as internal standards to analyse the hemicelluloses content. The total content of carbohydrates was determined by measuring the mass of a sample after the water was removed by evaporation using an oven. After that, a certain amount of liquid or solid sample which content of carbohydrates was about 0.1 mg was freeze-dried in vacuum. The calibration samples were prepared using a carbohydrate calibration solution. 2 mL of 2M HCl/MeOH anhydrous was added to samples and the calibration samples and heated at 100 ºC for 3 h. The excess of acid was neutralized with 170 µL of pyridine. After that, 1 mL of sorbitol (0.1 mg/mL in MeOH) and 1 mL resorcinol (0.1 mg/mL in MeOH) were added. Then, the solution was evaporated under nitrogen gas at 50ºC 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.

2.3.3. Monomer content

The monomer content was analysed using a similar method applied for the total hemicelluloses content. The derivatised samples were analysed by gas chromatography.

2.3.4. Gas chromatographic method

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, 2 ºC/min, 8 min – 170 ºC, 12ºC/min, 300 ºC, 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.

2.3.5. Molar mass

The weight-average and number-average molar weights of hemicelluloses were determined by high-performance size-exclusion chromatography (HPSEC) equipped with multiangle laser-light scattering (MALLS) and 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 $NaNO_3$ at a flowrate of 0.5 mL/min at 40ºC. 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 solvent. In order to optimize the hydrolysis of hemicelluloses from biomass, the influence of temperature and extraction/hydrolysis time on the yield and molar mass were studied. After the hydrothermal process, two main fractions were obtained: liquid and solid. The liquid phase was analysed to determine the enrichment in hemicelluloses. The most accessible polymers from biomass are hemicelluloses, consequently the selection of reaction temperature was closely related with that `accessibility´. The exposure time is a key factor, too. Oligosaccharides and monosaccharides were the final products after the biomass was treated hydrothermally. At longer treatment times, the oligomers and monomers began to decompose generating degradation products. The temperature was

another key factor in the hydrolysis of hemicelluloses. Rissanen et al. [13] observed that the conversion was only 10% at 120ºC using the same pilot plant, consequently the temperatures selected were higher than 120ºC.

3.1. Evolution of pH

The pH values of the liquid samples as a function of the time for the Holm oak at different temperatures are shown in Figure 3. The pH was strongly influenced by the temperature and the behaviour was similar in all cases. The pH started at about 5.5 corresponding to the pH of distilled water. Then, the pH decreased during the experiment time from 5.5 to about 4-4.3, depending on the reaction temperature. The decrease of pH was due to the hydrolysis of acetyl groups from hemicelluloses producing acetic acid, which increased the hydronium ion concentration in the reaction medium, leading to significantly faster hydrolysis. The acetic acid catalyses the hydrolysis of carbohydrates. The pentoses and hexoses hydrolysed can be converted to degradation products. The xylose and arabinose can be transformed into furfural through a dehydration [14]. The glucose, in a hydrothermal medium, can be converted into carbohydrate and degradation products. The glucose can be transformed into 1,6-anhydroglucose through a dehydration and glycolaldehyde by retro-aldol condensation. By isomerization reaction, the glucose can be converted into fructose. The fructose can be transformed into 5-hydroxymethylfurfural (5-HMF) by dehydration reactions and glyceraldehyde by retro-aldol condensation [15]. The 5-HMF can be degraded into levulinic and formic acid [16] while the furfural can be transformed into formic acid. It can be observed a simplified reaction pathway for pentoses and hexoses and subsequent reactions in Chapter 2, Figure 6. The low value of the pH can be attributed to the presence of degradation products as well [17].

At 130ºC, the temperature was not enough to depolymerize the hemicelluloses and the behaviour was almost linear with time. Lower temperatures led to lower reaction kinetics, generating low carbohydrates hydrolysis and consequently more basic pH values, decreasing the production of degradation products when the working pH range was between 4 and 7 [13].

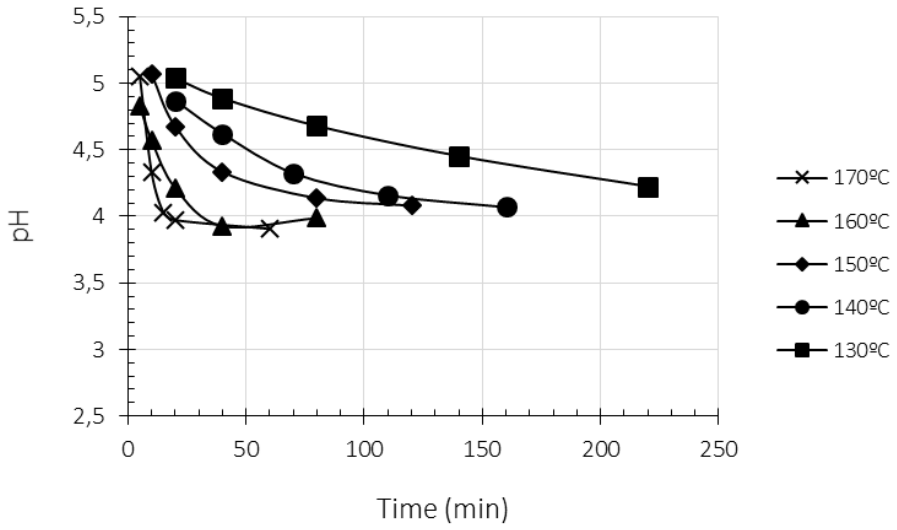

Figure 3. The pH behaviour in function of time at different temperatures.

The largest difference was observed when the temperature was increased from 130 to 140ºC suggesting that the structure of biomass was broken and the hemicelluloses were more available for the extraction process. Increasing the temperature up to 160-170ºC exhibited a faster deacetylation, and the pH exhibited a plateau at about 20-30 min. This plateau value is an indicator that the hemicelluloses depolymerisation was stopped. At 160ºC can be observed an increment of the value of pH after 40 min. This increment can be attributed to the presence of degradation products which increased during the reaction time. The degradation products are obtained from the hydrolysis of pentoses and hexoses extracted and 5-hydroxymethylfurfural, furfural, levulinic acid, etc can be observed [16]. These components will maintain the pH low. The rapid removal of hydrolysed products (not time to subsequent reactions) and the addition of sodium carbonate might have advantages, it could help in minimizing the formation of degradation products [1, 18]. The adittion of sodium carbonate increases the value of pH, decreasing the `effect´ of acetyl groups that acts as catalyst and consequently reducing the formation of degradation products.

The minimum pH was observed at the same time as the conversion of hemicelluloses was at a maximum. This effect was explained with more details in the Chapter 3 of this document. This behaviour was observed at temperatures exceeding 150ºC. The pH can

be used as an indicator for following the hydrothermal process and identifying the reaction time necessary to reach the maximum conversion [19].

3.2. Hemicelluloses extraction kinetics

Hemicelluloses hydrolysates were affected by temperature (see Figure 4). At higher temperatures, a higher amount of hemicelluloses was obtained. The behaviour at 130ºC and 140ºC was approximately linear, indicating that the reaction time or the temperature was not high enough to remove all of the hemicelluloses (the minimum pH was not reached). At temperatures exceeding 150ºC two different stages in Figure 3 can be observed.

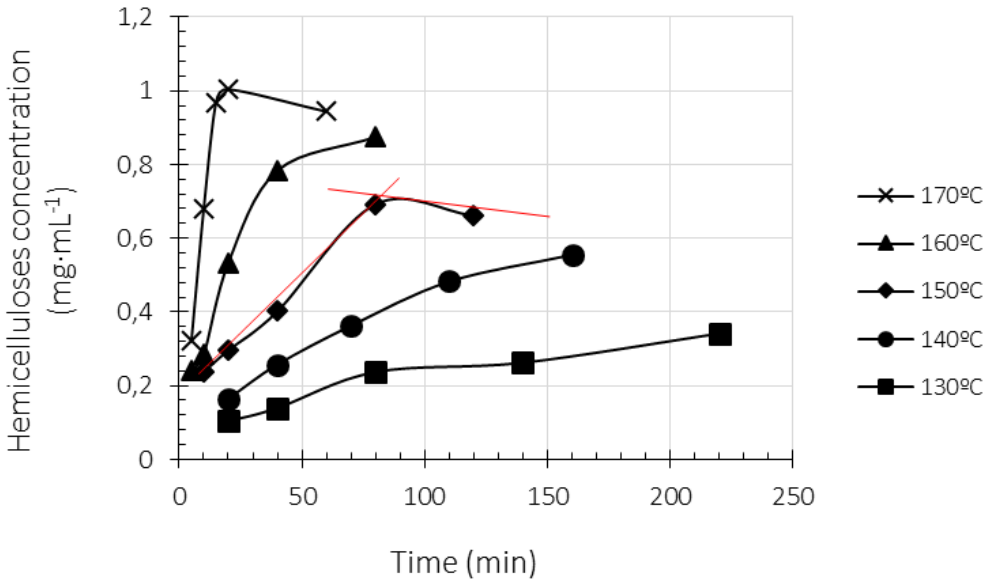

Figure 4. Concentration of hydrolysed hemicelluloses as a function of time at different temperatures.

The first stage corresponds to the extraction and hydrolysis of hemicelluloses. The second stage (negative) indicates the presence of degradation products according to the behaviour of pH discussed previously as well as the decrease in carbohydrate concentration with time. The higher acidity could also indicate that a more severe deacetylation took place during extraction resulting in major extraction/hydrolysis of hemicelluloses as well as the formation of degradation products. The time required to hydrolyse the maximum amount of hemicelluloses depended on the reaction temperature. The time necessary to reach the maximum concentration of hemicelluloses was 80 min at 150ºC but only 20 min at 170ºC indicating that the reaction kinetics were

faster at higher temperatures. At the highest temperature studied, after 20 min, the concentration of hemicelluloses decreased indicating that the reaction time should be shorter to avoid the formation of undesirable products. The errors in the data was lower than 10% in mass, indicating a good reproducibility in the experiments.

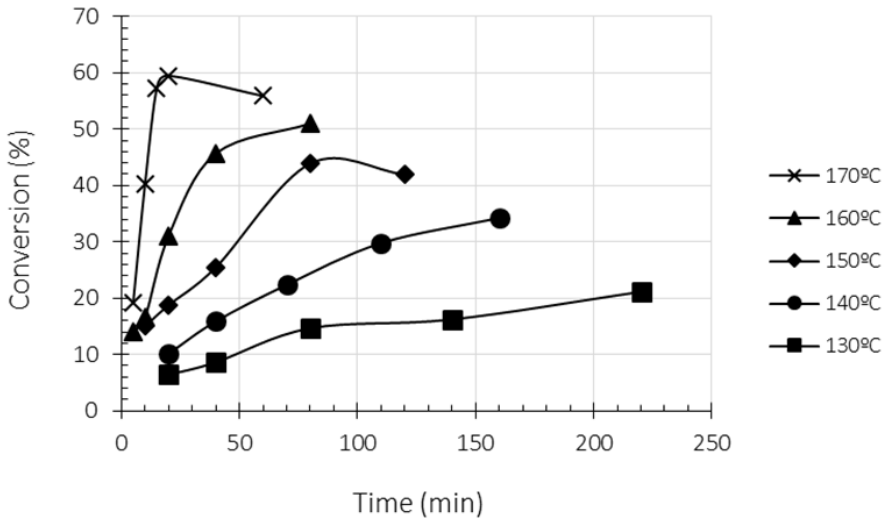

Figure 5. Conversion of hemicelluloses as a function of time at different temperatures.

The conversion of hemicelluloses depended strongly on the reaction time and temperature (Figure 5) but the maximum hydrolysis of hemicelluloses depends on the type of biomass treated (Figure 6). The time required to obtain a 30% hemicellulose conversion was 110 min at 140ºC while it was 50 min at 150ºC and only 8 min at 170ºC. 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 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 was related to the reaction temperature. The ionic product of water increases with temperature (until 374ºC) [20] increasing the reaction kinetics and consequently the hydrolysis of hemicelluloses is faster.

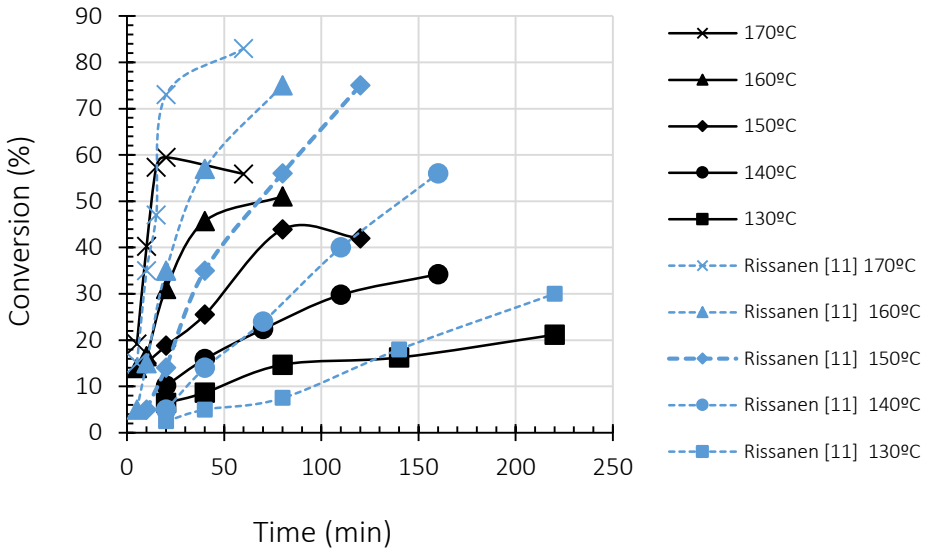

Figure 6. Conversion of hemicelluloses as a function of the time at different temperatures for Holm oak and Norway spruce.

The conversion obtained at different temperatures was compared to the data of Rissanen et al. who studied the hydrolysis of Norway spruce (softwood) hemicelluloses using subcritical water extraction [11]. As shown in Figure 6, in all cases, the conversion obtained for the softwood species was higher than for our hardwood species, and larger differences in the extraction of hemicelluloses at the same temperature were observed at higher temperatures. Hardwood has a higher content of acetyl groups than the softwood [21] but hardwood has a lower content of lignin compared to softwood [22]. Two reasons may explain the higher extraction of softwood hemicelluloses under similar experimental conditions: a) less deacetylation generates lower ion hydronium concentration in the liquid decreasing the formation of degradation products and increasing the amount of hemicelluloses in the liquid phase and b) the low content of lignin in the raw material increases the accessibility of hemicelluloses increasing the extraction of hemicelluloses, too.

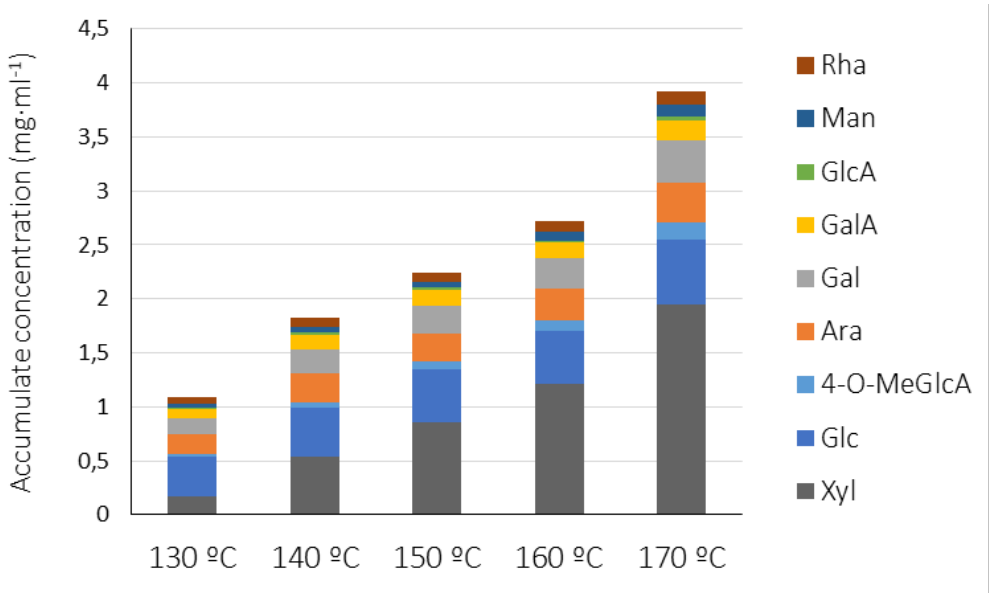

Figure 7. Accumulate concentration of hemicelluloses and composition in hydrothermal process.

As shown in Figure 7, the final chemical composition of the hydrolysed liquid was influenced by temperature and time. The major component extracted from hemicelluloses was glucose at 130 and 140ºC and xylose at 150, 160 and 170ºC [6]. The xylose concentration was 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 160ºC as did glucose (R^2 between 0.97 and 0.95) at less than 150ºC indicating that the reaction time was not enough to extract the maximum amounts of the hemicelluloses at these temperatures. The xylose exhibited a slope at 170ºC after 60 min and the glucose at 160ºC after 80 min suggesting the presence of dehydration and retro-aldol condensation reactions [20]. Xylose and glucose represented between 49.7 and 65.0% wt. of the hemicelluloses extracted.

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 cases: the largest hemicelluloses were extracted at the shortest time and it decreased as the temperature was increased. Figure 8 depicts the evolution of molar mass during the autohydrolysis process at different temperatures.

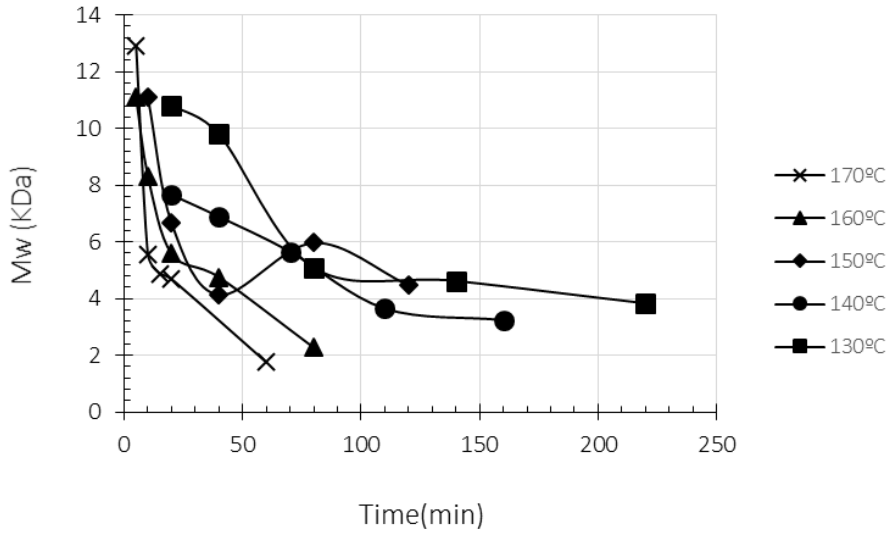

Figure 8. Change in the molar mass during autohydrolysis at different temperatures.

A diversified mix of lower molar mass hemicelluloses was produced during the hydrolysis process. The highest molar mass obtained was 12.9 KDa (72 DP) working at 170ºC for 5 min reaching the lowest molar mass after an hour of extraction according to the pH value. At 160 and 170ºC, the hemicelluloses had a significantly lower molar mass 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. [11] for softwood extraction (Norway spruce). The molar mass of carbohydrates depends on the wood species. As shown in Figure 9, 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. Probably this hydrolysis 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 [11]. The results indicate that if high molar mass is targeted it is better to use softwood.

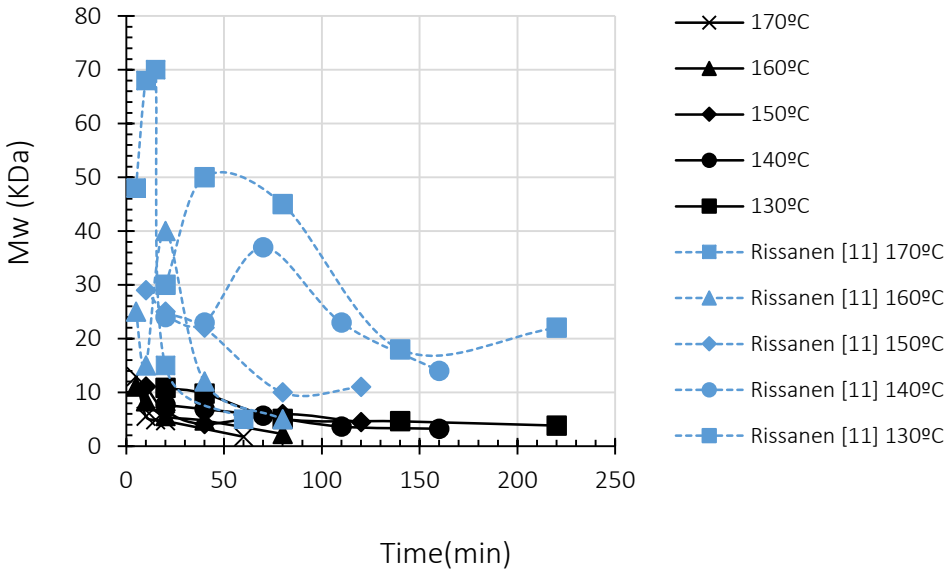

Figure 9. Molar mass along time for softwood and hardwood species.

4. Conclusions

The hydrothermal process can be used to recover carbohydrates and lignin from lignocellulosic biomass. Holm oak hydrolysis in subcritical water was studied at temperatures between 130 and 170ºC employing five reactors in series to form a cascade during 60-220 min using a particle size of 1.25-2 mm. The conversion was influenced with the reaction temperature. The final conversion varied from 21.1% at 130ºC to 55.9 % at 170ºC, mostly constituted by glucose and xylose. The behaviour at lower temperatures was linear indicating that the time was not high enough to hydrolyse the hemicelluloses. Increasing the temperature up 150ºC, two stages were observed. The first stage (positive slope) corresponds to the hydrolysis of hemicelluloses and the second stage (negative slope) suggested the presence of degradation products. The conversion of hemicelluloses from Norway spruce (softwood) was higher than Holm oak (hardwood). This difference can be attributed to two effects: lower content of acetyl groups in softwood specie generating lower ion hydronium concentration and reducing the presence of degradation products and lower content of lignin in hardwood increasing the `accessibility´ to hemicelluloses and consequently the formation of degradation products.

The pH was strongly influenced by temperature, reaching levels of about 4-4.3. The largest difference was observed from 130ºC to 140ºC suggesting that the structure of

hardwood specie was broken taking more `accessibility´ to hemicelluloses. At higher temperatures, a faster deacetylation was observed together with a faster decreased of molar mass. The yield and molar mass obtained after the hydrolysis process is 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, being the highest molar mass extracted of 12.9 KDa at 170ºC after 5 min and the lowest molar mass was of 1.75 KDa after 60 min. Contrary, if high molar mass is targeted it is better to use softwood. The results showed that a diversified mix of lower molar mass of hemicelluloses can be obtained indicating that the selection of experimental conditions can be used to achieve a desirable molar mass. The `versatility and flexibility´ of the system changing only the experimental conditions is an important point for further industrial application.

In the next work, the hydrothermal treatment of several types of lignocellulosic biomass with a heat recovery system will be tested. In addition, the combination of semi continuous hydrothermal process with a continuous ultrafast hydrolysis in supercritical water will be analysed.

Acknowledgements

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References

[1] K. Leppänen, P. Spetz, A. Pranovich, K. Hartonen, V. Kitunen, H. Ilvesniemi, Pressurized hot water extraction of Norway spruce hemicelluloses using a flow-through system, Wood Science and Technology 45 (2010) 223-236

[2] H.A. Ruiz, R.M. Rodríguez-Jasso, B.D. Fernandes, A.A. Vicente, J.A. Teixeira, Hydrothermal processing, as an alternative for upgrading agriculture residues and marine biomass according to the biorefinery concept: A review, Renewable and Sustainable Energy Reviews 21 (2013) 35-51

[3] X. Chen, M. Lawoko, A.V. Heiningen, Kinetics and mechanism of autohydrolysis of hardwoods, Bioresource Technology 101 (2010) 7812-7819.

[4] X.J. Ma, X.F. Yang, X. Zheng, L. Lin, L.H. Chen, L.L. Huang, S.L. Cao, Degradation and dissolution of hemicelluloses during bamboo hydrothermal pretreatment, Bioresource Technology 161 (2014) 215-220.

[5] J. Krogell, K. Eränen, A. Pranovich, S. Willför, In-line high-temperature pH control during hot-water extraction of wood, Industrial Crops and Products 67 (2015) 114-120.

[6] M.S. Tunc, A.R.P. van Heiningen, Characterization and molecular weight distribution of carbohydrates isolated from the autohydrolysis extract of mixed southern hardwoods, Carbohydrate Polymers 83 (2011) 8-13

[7] H. Ando, T. Sakaki, T. Kokusho, M. Shibata, Y. Uemura, Y. Hatate, Decomposition Behavior of Plant Biomass in Hot-Compressed Water, Industrial & Engineering Chemistry Research 39 (2000) 3688-3693.

[8] N.S. Thompson, Hemicellulose, Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, Inc. %* Copyright © 2001 by John Wiley & Sons, Inc. %@ 9780471238966, 2000.

[9] Y. Kim, T. Kreke, N.S. Mosier, M.R. Ladisch, Severity factor coefficients for subcritical liquid hot water pretreatment of hardwood chips, Biotechnology and Bioengineering 111 (2014) 254-263.

[10] E.L. Springer, Prehydrolysis of hardwoods with dilute sulfuric acid, Industrial & Engineering Chemistry Product Research and Development 24 (2002).

[11] J.V. Rissanen, H. Grénman, S. Willför, D.Y. Murzin, T. Salmi, Spruce Hemicellulose for Chemicals Using Aqueous Extraction: Kinetics, Mass Transfer, and Modeling, Industrial & Engineering Chemistry Research 53 (2014) 6341-6350

[12] A. Sundberg, K. Sundberg, C. Lillandt, B. Holmbom, Determination of hemicelluloses and pectins in wood and pulp fibres by acid methanolysis and gas chromatography Nordic Pulp and Paper Research Journal (1996) 11 (1996) 216-219

[13] J.V. Rissanen, H. Grénman, C. Xu, S. Willför, D.Y. Murzin, T. Salmi, Obtaining spruce hemicelluloses of desired molar mass by using pressurized hot water extraction, ChemSusChem 7 (2014) 2947-2953.

[14] B. Danon, W. Hongsiri, L. van der Aa, W. de Jong, Kinetic study on homogeneously catalyzed xylose dehydration to furfural in the presence of arabinose and glucose, Biomass and Bioenergy 66 (2014) 364-370

[15] D.A. Cantero, M.D. Bermejo, M.J. Cocero, Kinetic analysis of cellulose depolymerization reactions in near critical water, Journal of Supercritical Fluids 75 (2013) 48-57.

[16] F. Peng, P. Peng, F. Xu, R.-C. Sun, Fractional purification and bioconversion of hemicelluloses, Biotechnology Advances 30 (2012) 879-903

[17] M. Sasaki, B. Kabyemela, R. Malaluan, S. Hirose, N. Takeda, T. Adschiri, K. Arai, Cellulose hydrolysis in subcritical and supercritical water, The Journal of Supercritical Fluids 13 (1998) 261-268

[18] A.D. Espinoza, R.O. Morawicki, Effect of Additives on Subcritical Water Hydrolysis of Whey Protein Isolate, Journal of Agricultural and Food Chemistry 60 (2012) 5250-5256.

[19] F.M. Yedro, J. García-Serna, M.J. Cocero, Monitoring alternatives and main sugar products for the autohydrolysis of Holm oak hemicelluloses using pressurized hot water, XXXX ((under review)).

[20] D.A. Cantero, A. Álvarez, M.D. Bermejo, M.J. Cocero, Transformation of glucose into added value compounds in a hydrothermal reaction media, The Journal of Supercritical Fluids 98 (2015) 204-210.

[21] G. Garrote, H. Domínguez, J.C. Parajó, Hydrothermal processing of lignocellulosic materials, Holz als Roh - und Werkstoff 57 (1999) 191-202.

[22] W.-S. Lim, J.-W. Lee, Influence of pretreatment condition on the fermentable sugar production and enzymatic hydrolysis of dilute acid-pretreated mixed softwood, Bioresource Technology 140 (2013) 306-311

Chapter 5

Hydrothermal fractionation of woody biomass: lignin effect over sugars recovery

Abstract

Subcritical water was employed to fractionate woody biomass into carbohydrates and lignin. Nine urban trees species (hardwood and softwood) from Spain were studied. The experiments were carried out in a semi-continuous reactor at 250ºC for 64 min. The hemicellulose and cellulose recovery yields were between 30% wt. and 80% wt. while the lignin content in the solid product ranged between 32% wt. and 92% wt. It was observed that an increment of solubilized lignin disfavored the hydrolysis of hemicelluloses. It was determined that the maximum extraction of hemicellulose was achieved at 20 min of solid reaction time while the extraction of celluloses not exhibited a maximum value. The hydrolysis of hemicellulose and cellulose would be governed by the hydrolysis kinetic and the polymers accessibility. In addition, the extraction of hemicellulose was negatively affected by the lignin content in the raw material while cellulose hydrolysis was not affected by this parameter.

Keywords: Biorefinery, glucose, process flexibility, xylose

1. Introduction

Lignocellulosic biomass has emerged as a potential renewable resource for the production of fuels [1], energy and added value chemical products [2]. To achieve this, different treatments should be applied to the raw material in a concept industry called: Biorefinery [3]. Lignocellulosic biomass is a complex material composed mainly of three biopolymers: hemicelluloses, celluloses and lignin. The nature of these polymers is quite different, hemicellulose is composed of C-5 molecules like xylose, arabinose, mannose and galactose while cellulose is only composed of glucose. On the other hand lignin is the most complex polymer of biomass composed of phenolic units linked in a three dimensional network [4]. These polymers interact between them by covalent, hydrogen and Van deer Walls bonds. So, prior applying the reaction engineering to produce chemicals or fuels in a selective way, it is needed to break the linkages between the three main polymers of biomass, which will allow the separation of them. Once cellulose, hemicellulose and lignin is obtained, several products can be produced like: ethanol [5], hydrogen [6], glycolaldehyde [7-10], pyruvaldehyde [11, 12], 5-hydroxymethylfurfural (5-HMF) [13] and lactic acid [14] among others.

Sub and supercritical water (SCW) processes have gained attention as a promising solvent for performing the reactions of fractionation and hydrolysis of biomass.

One of the main advantages of SCW is the solvent: water, particularly in the field of 'green chemistry'. Water is environmentally friendly and represents an alternative to corrosive and toxic solvents being an attractive reaction media for a large number of applications. The variations in the properties of water near to its critical point (374ºC and 22.1 MPa) only by changing pressure and temperature make it a promising reaction medium to set reaction conditions depending on the desired product. The main properties that can be modified by pressure and temperature and will define the identity of the medium are: dielectric constant, ionic product, density, miscibility and transport properties [15, 16] . Water can adopt different roles in the reaction medium: as solvent, reactant or catalyst [17]. The ionic reactions are favoured using high density and high ionic product. Contrary, the radical reactions are favoured using supercritical water conditions [18]. These properties can be adjusted by manipulating temperature and pressure being the process more flexible to obtain a specific product stream. The

challenge is to find the experimental conditions which can be adaptable to all kind of biomass contributing to the decentralization and versatility of the process to industrial scale [19].

Several studies consider woody biomass like potential feedstock for the production of chemicals and fuels. Numerous pretreatments, including dilute acid [20], steam explosion [21], ammonia fibre expansion [22], ozonolysis [23], liquid hot water [24-27], supercritical water[28] have been studied to determine the optimum process conditions to obtain high yield of carbohydrates recovered using low reaction time from plant biomass [2]. The pretreatment is necessary to alter the structure of biomass and to increase the hydrolysis of hemicelluloses and celluloses to the enzymes for monosaccharides production [29] and increase the porosity of the materials. The fractionation of vegetal biomass using liquid hot water has been investigated obtaining high recovery of the biomass component in relative low treatment times. The hydrolysis enables the depolymerisation of hemicelluloses [30, 31] and the extraction of lignin [32] and cellulose. These hydrolyses reaction are usually followed by the formation of byproducts such as furfural, 5-HMF, acetic acid and lactic acid [33, 34]. The hydrothermal fractionation of biomass is usually carried out between 150ºC and 250ºC, at pressures lower than 10 MPa [35-37]. The recovery yields of hemicellulose usually range between 60% wt. and 100% wt. while cellulose recovery is lower than 60% wt. However, the fractionation yields of plant biomass can be affected by the nature of the biomass: structural and chemical properties. So, the selection of the experimental conditions play an important role in the obtaining of high yield of hydrolysis of biopolymer. For instance, hardwood hemicelluloses can be removed/hydrolysed at lower temperatures than the softwood hemicelluloses [37]. The main compound in hemicellulose is xylose for hardwood and mannose for softwood. The content of lignin for hardwood is generally lower than softwood [38]. The hardwood has higher content of acetyl groups than softwood, which increase the deacetylation of hemicelluloses being this specie more favourable in the hydrolysis of carbohydrates [39].

In this work, nine species of urban trees (hardwood and softwood) were hydrolysed at one temperature using water in subcritical conditions as solvent. The influence of the composition and nature of the biomass on the yield of hemicelluloses and celluloses as well as a solid rich in lignin were studied. The main objective was studied the system flexibility on the yield of hemicelluloses and celluloses from different raw materials trying to find a general trend relating these yields with the initial lignin content in biomass.

2. Materials and methods

2.1. Materials

The raw materials used in this work to conduct the extraction/hydrolysis process were 9 species of urban trees from Valladolid region, in Spain. These species were: Linden (*Large-leaved linden*), Plane (*Platanus x acerifolia*), Eucalyptus (*Eucalyptus globulus*), Catalpa (*Catalpa bignonioides*), Holm oak (*Quercus ilex*), Maple (*Acer saccharum*), Almond (*Prunus dulcis*), Pine (*Pinus pinea*) and Cedar (*Juniperus oxycedrus*).

The standards used in High Performance Liquid Chromatography (HPLC) analysis were: cellobiose (+98%), glucose (+99%), fructose (+99%), glyceraldehyde (95%), pyruvaldehyde (40%), arabinose (+99%), 5-hydroxymethylfurfural (99%), lactic acid (85%), formic acid (98%), acrylic acid (99%), mannose (+99%), xylose (+99%) and galactose (+99%) purchased from Sigma and used without further modification.

For the determination of extractives, n-hexane (96%) supplied by Sigma was used. For the determination of carbohydrates, lignin and ash, sulphuric acid (96%) and calcium carbonate (≥ 99.0%) were purchased from Panreac and used as reagents without further modification. Distilled water was used as reaction medium in the experiment and Milli-Q water was used as mobile phase in the HPLC analysis.

2.2. Methods

Two products were obtained after the hydrothermal treatment (explained in section 2.3) of the raw material: solid and liquid. The solid sample was the remaining amount of biomass in the fixed bed reactor after the treatment. On the other hand, the liquid sample was produced due to the extraction/hydrolysis of the hemicellulose and cellulose fractions of the raw material during the treatment. The raw materials and the

liquid and solid samples taken during hydrothermal fractionation were analysed to determine the carbohydrates and lignin content. The content of hemicelluloses, celluloses, lignin and ash were determined according to the National Renewable Energy Laboratory (NREL) – Determination of Structural Carbohydrates and Lignin in Biomass [40-42]. The content of extractives was determined according to the Determination of Extractives in Biomass [42, 43]. The solid samples were dried at 105ºC to constant weight to obtain chemical composition in dry weight basis.

2.2.1. Liquid chemical composition

The liquid samples were subjected to different analysis. The pH was measured and the total organic carbon was also determined directly to each sample. The composition in sugars from cellulose and hemicellulose was determined by hydrolysing the extracted oligo- and polysaccharides to their simple monomer: glucose, xylose, arabinose, mannose and galactose. To do so, 10 ml of the liquid sample taken after the hydrothermal process was set in a hydrolysis bottle (100 mL) together with 4 ml of sulphuric acid. The bottle was incubated at 30ºC for 30 min and then, 84 ml of distilled water was added. After this, the hydrolysis bottle was incubated at 121ºC for 1 h. After that, the bottle was cooled to room temperature and calcium carbonate was added to neutralize the medium obtaining a final pH between 6 and 7. Then, the sample was filtrated with nylon filters membranes (0.20 µm) and analysed by HPLC. The HPLC chromatograms were analyzed using Fast Fourier Transform and band-adjustment by Gaussian functions. The yield of hemicellulose products was determined in terms of initial mass and initial hemicellulose as shown in equation 1 and 2 respectively. In the same way, cellulose yield was determined in function of initial mass and initial cellulose as shown in equations 3 and 4 respectively. The hydrolysis product yield was determined by equation 5.

$$Hemicelluloses\ (g/g_{biomass}) = [xylose(g)+arabinose\ (g)+galactose(g)+mannose(g)]*m_{biomass}(g)^{-1} \quad (1)$$

$$Hemicelluloses\ (g/g_{hemicellbiomass}) = [xylose(g)+arabinose\ (g)+galactose(g)+mannose(g)]*[m_{biomass}(g)*X_{hemi}]^{-1} \quad (2)$$

$$Celluloses\ (g/g_{biomass}) = [glucose(g)+fructose\ (g)+cellobiose(g)]*m_{biomass}(g)^{-1} \quad (3)$$

$$Celluloses\ (g/g_{cellulosebiomass}) = [glucose(g)+fructose\ (g)+cellobiose(g)]*[m_{biomass}(g)*X_{cellulosebiomass}]^{-1} \quad (4)$$

Derived-products ($g/g_{biomass}$)= [glyceraldehyde(g)+pyruvaldehyde(g)+lactic-acid(g)+formic-acid(g)+acrylic-acid(g)+5-hydroxymethylfurfural(g)]$m_{biomass}(g)^{-1}$* (5)

The amount of extraction for each component was calculated as it is shown in equation 6.

$$dX_y = C_y . f . dt \quad (6)$$

Where $m_{biomass}$ is the initial content of biomass in the reactor (gr); $X_{hemibiomass}$ is the initial content of hemicelluloses in the biomass (% wt.); $X_{cellulosebiomass}$ is the initial content of celluloses in the biomass (% wt.); X_y is concentration of component y in gr; C is concentration of component y in ppm; f is the flow in ml/min and t is the time in min.

2.2.2. Solid chemical composition

The raw material and the solid samples taken from the reactor after the hydrothermal treatment were analysed in the same way for carbohydrate and lignin determination. However, the raw materials were subjected to an extraction process using n-hexane as solvent prior the acid hydrolysis. For carbohydrates and lignin content determination, 300 mg (W_1) of the solid sample was set in a hydrolysis bottle together with 3 mL (72%) of sulphuric acid and it was incubated at 30ºC for 30 min. Then, 84 mL of distilled water was added to the bottle and it was incubated at 121ºC for 1 h. Finally, the bottle was cooled to room temperature and the hydrolysates were filtrated under vacuum obtaining two products: a solid composed of insoluble lignin and ash, and a liquid composed of carbohydrates and soluble lignin. The solid was dried at 105ºC for 24 h (W_2) and then it was heated at 550ºC for 24 h in a muffle and weighted (W_3). The content of acid insoluble lignin (AIL) and ash (A) were calculate using the equations (7) and (8) respectively.

$$AIL(g) = \frac{W_2(g) - W_3(g)}{W_1(g)} 100 \quad (7)$$

$$A(g) = \frac{W_3(g)}{W_1(g)} 100 \quad (8)$$

On the other hand, two aliquots of 50 ml were collected per sample. Calcium carbonate was added to one of these sample to neutralize the medium obtaining a pH between 6

and 7 and then was analysed by HPLC analysis. The other sample was analysed using a UV-visible spectrophotometer to determine the soluble lignin content. The wavelength was set at 320 nm and the absorptivity value was 30 $L \cdot g^{-1} \cdot cm^{-1}$. The soluble lignin content was determined by equation 9

$$SL(g) = \frac{UV_{abs} * volume_{filtrate} * Dilution}{\varepsilon * ODW * Pathlength} \quad (9)$$

Where SL is the soluble lignin (g/gbiomass); UV_{abs} is the average UV-Visible absorbance; volume filtrate is the volume hydrolysis liquor (ml); Dilution is the dilution factor (adim); ε is Absorptivity of biomass at specific wavelength; ODW is the weight of sample (milligrams) and Pathlength is pathlength of UV-Vis cell (cm).

2.2.3. Analysis

The content of total organic carbon (TOC) and pH were analysed in the liquid samples. TOC was measured using a Shimadzu TOC-VCSH analyser. The pH was measured by Nahita model 903 pH-meter using an electrode Glass- Body ElectroJelly PH5101-3B.

The sugars content was determined by HPLC. A SH-1011 column from Shodex was used to perform the separation of the compounds with 0.01 N of sulphuric acid as mobile phase with a flow of 0.8 ml/min and at 50ºC. The HPLC equipment is equipped with two detectors: Waters IR detector 2414 (210 nm) to identify the carbohydrates and their derived products and Waters dual λ absorbance detector 2487 at 254 nm to identify the 5-hydroxymethylfurfural.

The raw materials and the solid obtained after the fractionation hydrolysis were analysed by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The FTIR analysis were carried out in a Bruker Tensor 27 spectrometer. The analysed region was between 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans was recorded. The surface morphology of these samples was determined by SEM JSM-820 (Joel). A gold evaporator Balzers SCD003 with a gold thickness 25-30 nm was used. The accelerating voltage was 20 kV. The samples were placed under high vacuum conditions.

2.2.4. Residence times

In a semicontinuous process can be observed two residence times: a solid and a liquid residence time. The solid residence time was always defined as 64 min. This is the time that the insolubilized fraction of the raw material remains inside the reactor. The liquid residence time depends on the flowrate and the mass inside of the reactor (variable value). The liquid reaction time was calculated as the average between the initial and final reaction time determined by equation 10 and 11 respectively.

$$LRT_i = \left[VR - (m_i / density)\right] / f \quad (10)$$

$$LRT_f = \left[VR - (m_f / density)\right] / f \quad (11)$$

Where LRT_i is initial liquid residence time in min; VR is the volume of the reactor in m^3; m_i is the initial solid inside of the reactor in Kg; density is the density of the material inside of the reactor in $Kg \cdot m^3$; f is the flow in $ml \cdot min^{-1}$; LRT_f is final liquid residence time in min; m_f is the solid inside of the reactor after the solid residence time in Kg.

2.3. Experimental setup and operation

The process configuration is shown in the Figure 1. The hydrothermal process was carried out in a semi-continuous reactor. The system essentially consists of a pump feeding system (PU-2080 model), a preheater (E-01, 200 cm of 1/8" AISI 316 piping), a reactor (R-01, 38 cm length, 1/2" O.D. SS316 piping), an oven (HP5680 model) and two heat exchangers (E-02, 15 cm of concentric tube heat exchanger 1/4"-3/8" countercurrent operation and E-03, 70 cm of concentric tube heat exchanger 1/4"-3/8" countercurrent operation). The pressure of the system was controlled using a go-back pressure valve (BPV-01).

The reactor was charged approximately with 5 gr of dried trees sawdust. Two filters were placed at the top and the bottom of the reactor to avoid the loss of raw material during the assays. The heat exchanger E-01 was used to pre-heat the flow and the heater E-02 was used to get the water stream at the desired temperature. The working temperature was 250ºC, the process time was 64 min, the pressure was fixed to 10 MPa and the flowrate was 10 ml/min. The time required to obtain the minimum pH was achieved faster (it can be observed in the Chapter 3) using 10 ml/min. As mentioned in

the Chapter 3, one correlation between pH and total organic carbon was observed. The maximum total organic carbon was located at the same time that the minimum pH value suggesting a high hydrolysis of carbohydrates. Liquid samples were taken every 10 min. After the process time, the pump was set to zero flow and the system was cooled down back to room temperature and depressurized. The solid inside the reactor was analysed as well as the liquid samples.

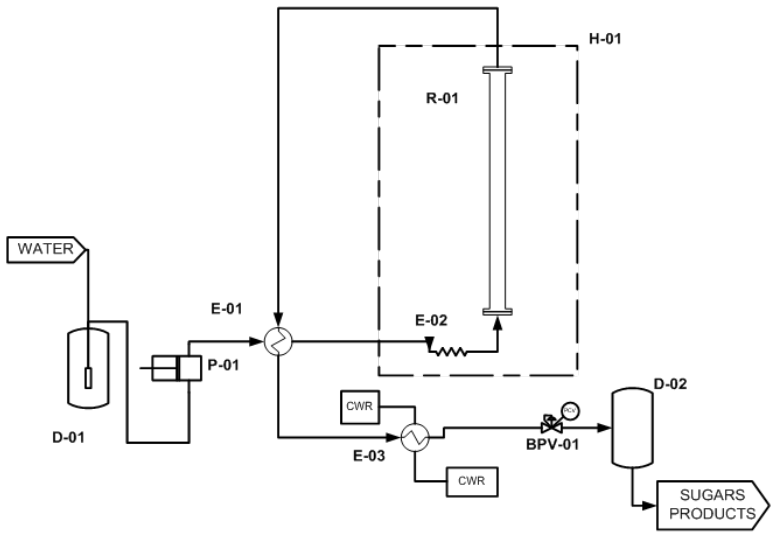

Figure 1. Schema of the hydrolysis process. Equipment: D-01 Feeder, P-01 Pump, E-01 Heat exchanger, E-02 Preheater, R-01 Reactor, H-01 oven, E-03 Heat exchanger, BPV-01 Go-backpressure valve, D-02 Liquid sampling vessel.

3. Results and Discussion

Different species of urban trees were hydrothermally treated in a fixed bed reactor using water as solvent. The goal of these experiments was the extraction and hydrolysis of the cellulosic and hemicellulosic sugars of biomass. The used solvent, hot pressurized water, was able to extract and hydrolyze hemicellulose and cellulose while most of the lignin fraction remained insolubilized. So, after hydrothermal treatment of plant biomass two main products were obtained: a liquid and a solid product. These two products can be obtained from hydrothermal treatment at temperatures between 150ºC and 400ºC and pressures between 10 and 25 MPa independently of the operation way (batch, semi-batch or continue) [4]. The reaction time is closely related with the treatment temperature. Generally, the reaction times for hydrothermal treatment at mild conditions (250ºC) range between 30 min and 90 min. However, the reaction time is drastically decreased when the hydrolysis temperature is close to the

critical point of water. It those situations, the use of continuous reactors is mandatory to set reaction times lower than minutes, which will avoid degradation reactions [4]. In this work, the fractionation of different plant biomass was carried out at 250ºC in a semi-continuous reactor. This temperature was selected from previous studies because it was the optimum for extracting the maximum amount of carbohydrates [44]. The pressure was fixed to 10 MPa for all the experiments in order to ensure a liquid phase extraction and hydrolysis. The liquid flowing through the reactor has a residence time that is a function of the reactor volume and the flow (reaction time liquid). Although high solid reaction times are needed in order to extract most of the hemicellulose and cellulose fraction, the liquid reaction time should be as low as possible to avoid sugars degradation. Depending on the reaction time of these sugars in the reactor, considerable amount of degradation products can be produced. The addition of small amount of NaOH can be prevented the formation of undesirable degradation products recovering a major amount of hemicelluloses [34]. In the experimental system used in this work, the liquid reaction time was between 3 and 3.6 min considering the variations in the porosity of the reactor.

3.1. Raw materials characterization

The content of lignin, cellulose, hemicellulose, ash and extractives for each urban tree were analyzed in duplicate samples following the protocol described in section 2.2.2. The content of lignin was calculated as the sum of acid soluble and insoluble lignin. After the hydrolysis, the content of hemicellulose was calculated as the sum of xylose, arabinose, galactose, mannose and acetic acid in the liquid hydrolysate. In the same way, the concentration of cellulose was determined as the sum of glucose, fructose and cellobiose. In Figure 2 it is shown the composition of the raw materials used in this study. The lignin content was between 21.6 and 39.1 %wt. The maximum amount of acid soluble lignin was 2.14% wt. indicating that the lignin content is due mainly to the presence of acid insoluble lignin. It can be seen that Linden contained the lowest amount of lignin while Cedar the highest amount of lignin. The amount of hemicellulose was similar for all urban trees analyzed. These values were between 20.0% wt. (Almond) and 25.5% wt. (Pine). On the other hand, the content of cellulose was variable depending on the raw material. The content of cellulose was between 21.7% wt.

(Almond) and 45.2 % wt. (Eucalyptus). A low content of ash and extractives was observed for all the analyzed raw materials. The ash content was lower than 1% wt. for all the studied raw materials and the amount of extractives varied from 0.6 % wt. (Plane) to 5.1% wt. (Linden). The mass balance ranged from 90.0 to 100 % wt. These value is acceptable is acceptable considering the small raw material used for the analysis.

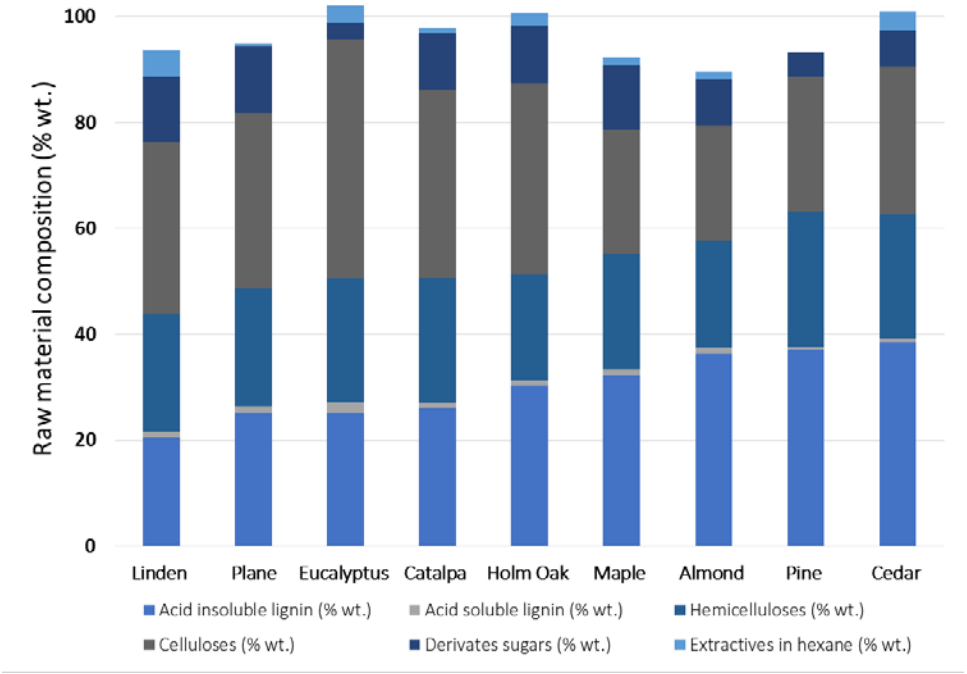

Figure 2. Chemical composition of the raw materials.

3.2. Product distribution

In Figure 3 it is shown the removal percentage of hemicellulose, cellulose, derived products and lignin from raw material as well as the solid residue obtained after the hydrothermal treatment. The hemicellulose and cellulose yields were calculated as it was explained in section 2.2.1. The main compound produced from sugars hydrolysis were glyceraldehyde, glycolaldehyde, 5-HMF, formic acid, levulinic acid and acetic acid. Also, lactic acid was observed in the liquid sample from the hydrolysis of Almond wood. The solubilized lignin was calculated as the difference between the content of lignin in raw material and the content of lignin in the solid residue.

The mass balance ranged from 0.75 to 1 g/g, however, most of the experiments gave a mass balance higher than 0.8 g/g. These values were acceptable considering the small amount of solid used as raw material. The main loss of material can be attributed to: filling and emptying of the reactor and the difficulty to identify hemicellulose and cellulose derived products by HPLC. Also, it should be taken into account that 8 samples were taken in each experiment to represent a 64 min experiment. So, an uncertainty should be added to the calculation of the accumulative yield of cellulose and hemicellulose.

The sum of hemicelluloses and solubilized lignin was similar for all the experiments, representing about 0.3 g/g initial biomass. The studied urban trees exhibited a similar tendency: the presence of high amount of hydrolyzed lignin together with a low amount of hydrolyzed hemicellulose [45]. The cellulosic sugars collected after the hydrothermal treatment ranged from 0.11 to 0.28 g/g initial biomass. The amount of derived products was between 0.10 and 0.27 g/g initial biomass. The content of acetic acid was higher in hardwood than softwood species as it was also observed in literature [33].

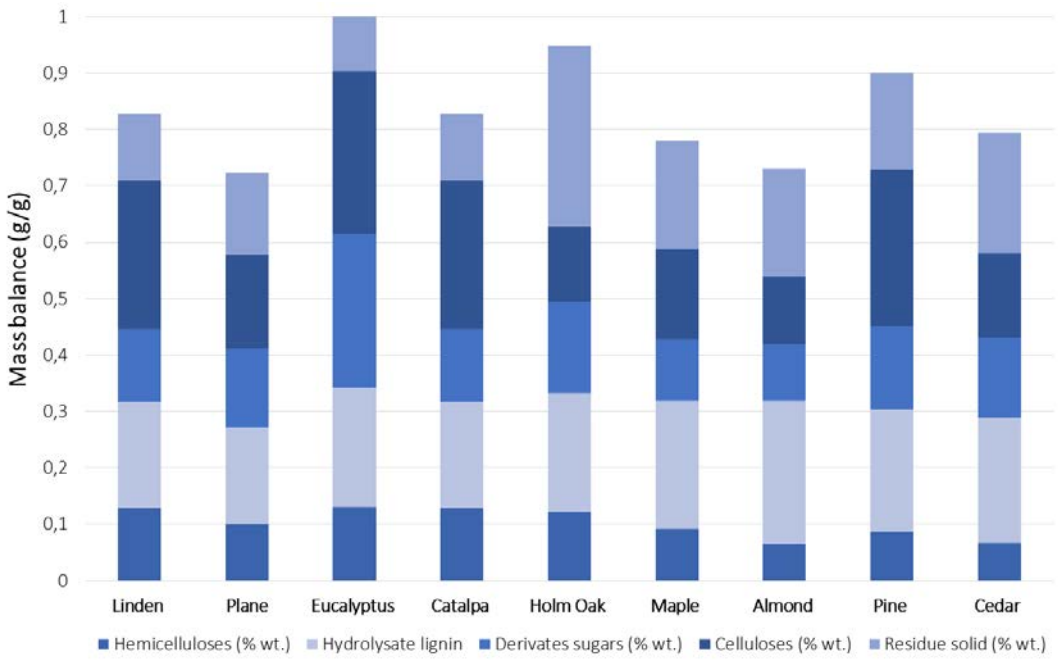

Figure 3. Material balance for hydrothermally urban trees.

3.3. Liquid Products

The sampling of the liquids products started just before the pumping was started. The samples taken from the reactor outlet were subjected to many analysis as explained in section 2.2.1. One of the easiest ways to follow the fractionation of plant biomass is the measurement of the pH. In Figure 4 it can be seen the pH values of the liquid samples in function of the solid reaction time for the 9 analyzed urban trees. First, the pH values for Eucalyptus globulus were on-line measured every 1 min. From that measurements, it can be observed that the pH shows 3 different behaviors along solid reaction time. First the pH was increased, then it was reduced drastically and finally the pH is increased softly. The values of pH for Eucalyptus fractionation were used to decide the sampling time. The first sample was taken at zero time, then the second sample was taken around the pH peak (4 min). The third sample was taken after the minimum pH is achieved (14 min). Finally, five samples were taken every 10 min in the last part of the experiment. In this way, the analytical and time expenditures were reduced. The pH started at about 5.5 corresponding to the distilled water value. The increase in the pH (0 min < t < 4 min) from 5.5 (distilled water value) to almost 6 can be attributed to the extraction of ash, which will increase the basicity of the medium. In order to test it, an experiment was run in which the pumping and heating were stopped after 4 min of treatment. The purpose of this experiment was to determine the composition of the solid inside the reactor. It was observed that 40% wt. of the biomass ashes were extracted in the first 4 min suggesting that the pH increment in the first minutes of the treatment takes place due to the ash solubility. The decrease in pH from t=4 min to t=10 min is due to the release of acetyl groups linked to hemicellulose during the hydrolysis. The release of acetic acid generate a decrease of the pH [46] and acts as a catalyst in the polysaccharide hydrolysis and degradation of carbohydrates because of the presence of hydronium ions [47]. The third behaviour of pH was detected from t= 10 min to t= 64 min. In that stage of the hydrolysis, the pH value was slightly increase from 3.3 to 3.7. This effect can be attributed to a dual effect: (1) an increase because the hydrolysis product concentration decrease along time, so the pH should increase to reach the distilled water value and (2), production of degradation products [48] such as 5-hydroxymetylfurfural (5-HMF), formic [49], furfural, uronic acid [34], which will

maintain the pH low. This behaviour was observed in all the experimented cases. As shown in Figure 3, the minimum pH value for the nine studied species was between 3.2 and 3.6 at around 10 min of hydrothermal treatment. The variability in the minimum pH value and the extraction time which the pH was minimum can be related with the biomass structure as well as the content of solubilized lignin in the liquid samples [45]. The final pH value was between 3.7 and 3.9. These values were lower compared to the range 4-7 recommended for other researchers to prevent the formation of derived products [47].

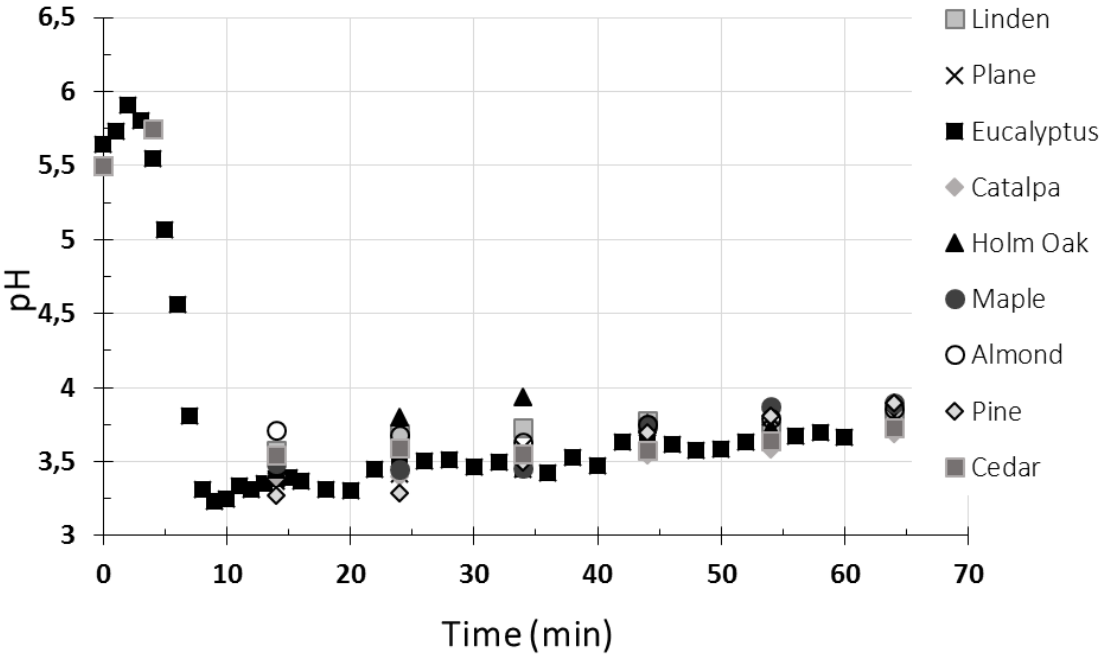

Figure 4. Behaviour of pH during wood autohydrolysis process.

The liquid products were composed of oligosaccharides and saccharides from hemicellulose and cellulose and also their derived products. The concentration of these components after the hydrothermal process depends strongly on two parameters: the liquid reaction time and temperature. At 180ºC the hemicellulose is hydrolyzed and some lignin is removed at about 200ºC [50]. At high temperatures, the kinetics of hydrolysis of hemicelluloses and cellulose are fast, which is desirable to reduce the reactors volume. Morever, if the reaction time is high a big amount of derived products will be produced [4]. The choice of operational conditions is important to avoid the formation of undesirable products.

The hemicellulose recovery was plotted along solid reaction time in Figure 5-A. It can be observed that the hemicellulose recovery was quite different depending on the starting biomass. The recovery yield varied from 0.28 to 0.79 g/g hemicellulose in raw material. The main component of hemicellulose was xylose and it can be used as indicator to follow the hydrolysis of this fraction. The maximum extraction of hemicellulose was observed around 20 min of solid reaction time. An increase of the process time favored the formation of undesired products. The cellulose recovery was plotted along solid reaction time in Figure 5-B. As it was observed for hemicellulose, the cellulose recovery was highly dependent of the initial raw material, ranging the yield from 0.36 to 1.00 g/g cellulose in raw material. The process time was not enough to get the maximum extraction value of cellulose indicating that the complete hydrolysis of celluloses was not achieved for 64 min in most of the experiments.

The hemicellulose and cellulose have chemical structures and composition very different. Hemicellulose polymerization of its compositional saccharides takes place in a branch way. For cellulose, the polymerization of glucose take place in a linear way allowing the fibers of cellulose to interact between them forming crystals [4]. This difference makes hemicellulose a more accessible polymer for the hydrolysis process. So, it was expected a different behavior under the hydrothermal treatment. This can be one of the main reasons because of cellulose needs more time/temperature than hemicellulose to be completely hydrolyzed. In fact, the hemicellulose recovery yield (Figure 5-A) and the cellulose recovery yield (Figure 5-B) follow different behaviors.

The hydrolysis of hemicellulose and cellulose seems to be governed by two parameters: the kinetic of hydrolysis and the accessibility of the polymer. The maximum yield of hemicellulose for each specie was achieved at the same solid reaction time, around 20 min. This suggests that the kinetic of hemicellulose hydrolysis was the same for the different studied species. However, the maximum recovery yield varied a lot depending in the treated biomass. This suggests that the availability of the hemicellulose fraction in the raw material was different for each biomass. As it was analyzed for hemicellulose, the maximum values of cellulose recovery yield would be affected by the accessibility of cellulose in the raw material. Therefore, the hydrolysis of hemicellulose and cellulose

will be affect by the composition of the raw material and the distribution of the cellulose and hemicellulose fractions in the biomass.

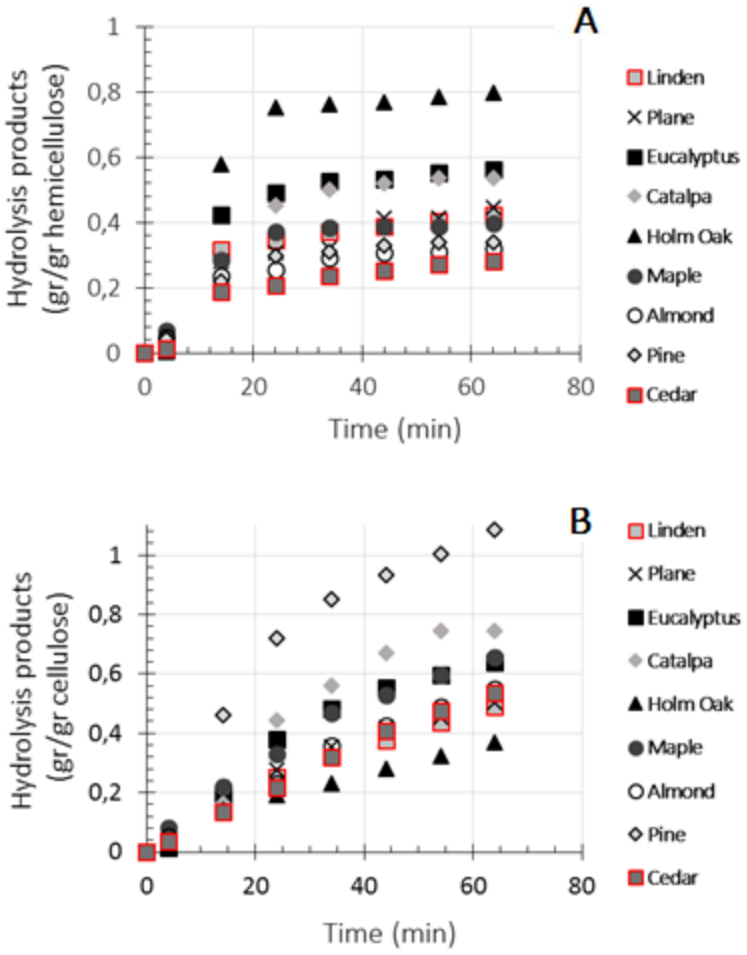

Figure 5. Yield of hemicelluloses (A) and celluloses (B) recovered after hydrolysis process at 250ºC and 64 min.

As it can be seen in Figure 5, the hydrolysis of hemicellulose and cellulose depends on the treated raw material. In order to evaluate the interaction of the biomass polymers (hemicellulose, cellulose and lignin), the extraction yield of hemicellulose and cellulose was analysed in function of the lignin content in biomass. So, the final amount of hemicellulose and cellulose extracted against the lignin content in the raw material was plotted in Figure 6-A and 6-B respectively. The extraction of hemicellulose showed to be improved when the lignin content of the biomass was reduced. In spite of one experimental point (Holm Oak), which did not follow the trend, a general tendency was found for the extraction of hemicellulose (Figure 6-A). A low lignin content may

suggests a big hemicellulose accessibility. However, the extraction of cellulose was not influenced by the lignin content of the raw material. In Figure 6-B, it can be seen that the recovery of cellulose yielded 60 ±10 % wt (except for Pine) independently of the used biomass. Following the analysis of these results, it can be concluded that the low content of lignin makes more accessible the hemicellulose fraction. Even, it can be though that hemicellulose is mainly placed around/between three dimensional lignin structures [51]. On the other hand, the cellulose fractions seems to be embedded inside the lignin matrix making cellulose hydrolysis independent of lignin content.

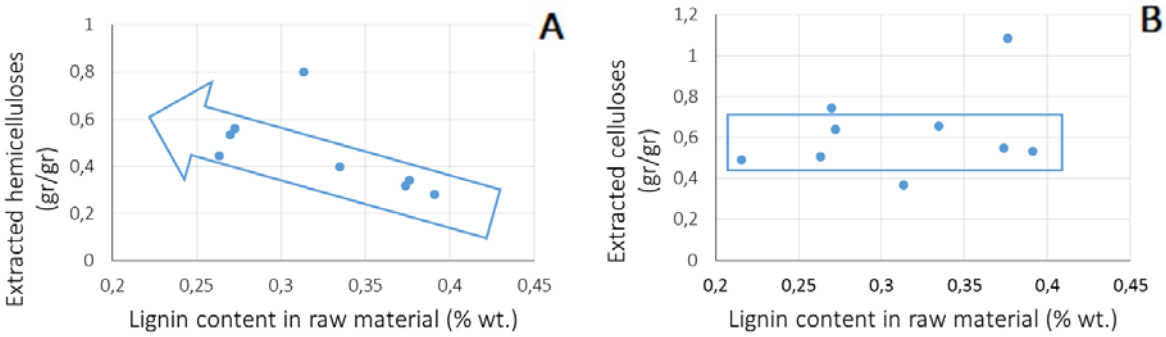

Figure 6. Yield of hemicelluloses (A) and celluloses extracted (B) after the hydrolysis process using nine species of urban trees at 250ºC.

The carbohydrates produced from cellulose and hemicellulose hydrolysis can follow mainly two different reaction pathways: dehydration or retro-aldol condensation. The formation of 5-HMF is due to a dehydration reaction while that the formation of glycolaldehyde is due to a retro-aldol condensation reaction [52]. The 5-HMF is a degradation product from hexose sugars that could act as inhibitor during the fermentation of sugars to produce ethanol [47]. In those cases, it is necessary a previous detoxification step, which increase the fermentation cost at industrial scale to obtain this alcohol [34]. After the hydrothermal treatment of the biomass, 5-HMF and glycolaldehyde were observed in the liquid samples (see Figure 7). The yield of 5-HMF was lower than 0.05 gr/gr biomass and it was below the threshold limit of fermentation inhibitory level [29]. The content of glycolaldehyde at 64 min was lower than 0.06 gr/gr biomass.

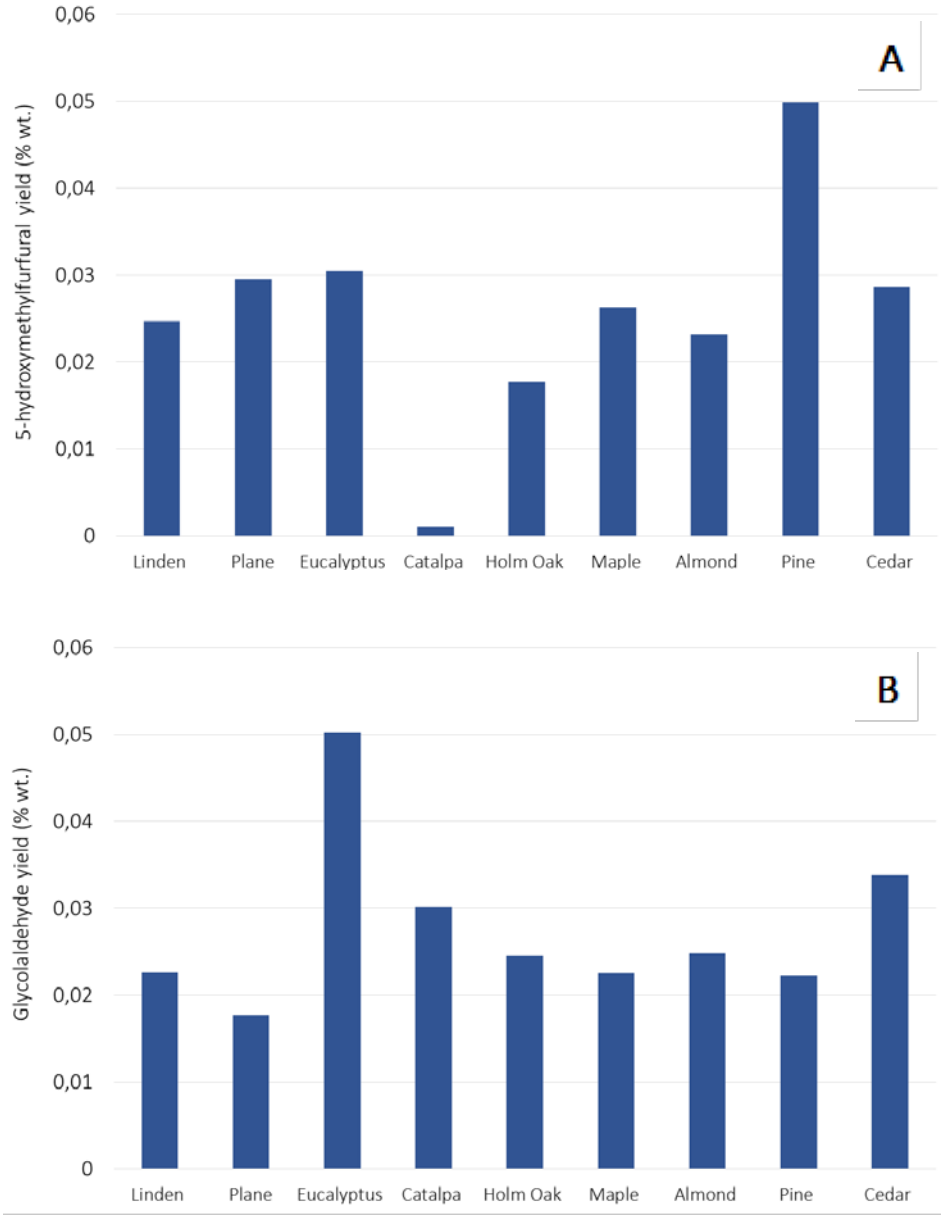

Figure 7. Content of 5-hydroxymetylfurfural (A) and glycolaldehyde (B) after the hydrothermal process in the liquid samples.

3.4. Solid Products

The content of solid residue after hydrolysis process varied from 0.10 to 0.32 gr/gr initial biomass (see Table 1) and the color of these solids was completely black. These values were lower than the initial content of lignin in the raw material indicating that some lignin was hydrolyzed. The structure of lignin could be composed of an amorphous and crystalline regions; the amorphous region would be easily hydrolysable while that the crystalline region would be more resistant to hydrolysis [53]. The solid residue was analyzed to know the carbohydrates, lignin and ash content. The ash

content was not detected. The carbohydrates were the most common `contaminant´ in the solid [54]. The lignin content was between 0.56 and 0.92 gr/gr biomass (except to Linden and Holm oak) being the main component in the solid product. These values corresponded to Linden and Pine respectively. The content of lignin in raw material was higher in Pine than in Linden. The difference in the quality of solid (measured as lignin recovery content) can be due to the lignin component is more resistant to thermal degradation.

Table 1. Amount and characterization of solid obtained after the hydrothermal process.

Biomass	Solid after hydrolysis (gr/gr biomass)	Lignin recovery content (gr/gr biomass)
Linden	0,25	0,41
Plane	0,14	0,63
Eucalyptus	0,11	0,56
Catalpa	0,10	0,69
Holm Oak	0,32	0,32
Maple	0,15	0,56
Almond	0,19	0,63
Pine	0,17	0,92
Cedar	0,11	0,78

In the Figure 8 it can be seen the structural characterization by FTIR of Cedar and Linden before and after hydrothermal process. The aromatic skeleton vibration can be seen at 1610 and 1460 cm^{-1} while the vibrations at 1135 cm^{-1} represents the aromatic C-H for syringyl type. These bands were observed in the raw material and the solid product suggesting that the aromaticity properties remained after the hydrolysis process [55]. At 1735 cm^{-1} it can be observed the linkage between hemicellulose and lignin [1]. This band was observed in the two raw materials as well as in the Linden solid product but not in the Cedar solid product suggesting that the linkage was broken. This agree with the results presented before, where the presence of low amount of hydrolysed lignin increases the hydrolysis of hemicelluloses. The results proved that the solid residue was composed of the aromatics groups as was discussed earlier (see Table 1). The peak at 765 cm^{-1} corresponds to presence of polysaccharide [1] and it was observed in all

samples indicating the presence of carbohydrates in the raw material as well as in the solid residue. This agree with the results showed in the Table 1.

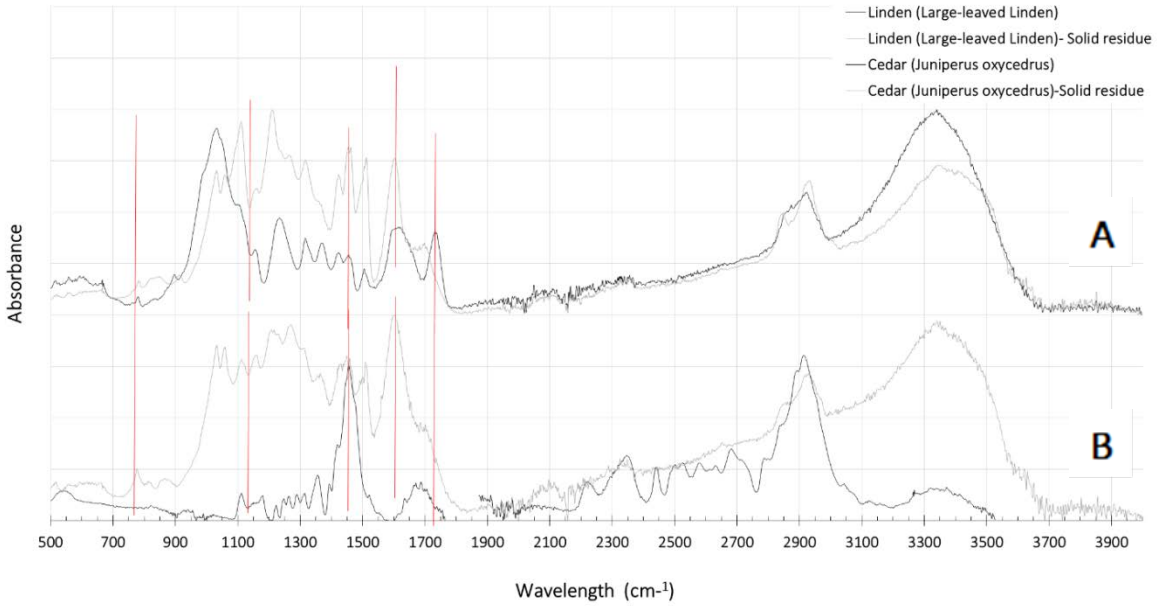

Figure 8. Characterization by FT-IR of two raw materials (Linden(A) and Cedar(B)) and two solid residue (Linden(A) and Cedar(B))

4. Conclusions

The hydrothermal process can be used to recover sugars, added value products and lignin. The extraction/hydrolysis of carbohydrates using a hydrothermal medium at 250ºC for 64 min using a flowrate of 10 mL/min was studied. The hemicelluloses hydrolysed varied from 0.28 to 0.79 gr/gr hemicellulose, mostly constituted by xylose. The cellulose hydrolysed ranged from 0.36 to 1 g/gr cellulose being the glucose the main component. The hydrolysis of hemicellulose and cellulose was affected by the composition of the raw material and the distribution of hemicelluloses and celluloses inside of matrix-biomass. The maximum yield of hemicelluloses for each specie was achieved at about 20 min but the amount of hemicelluloses hydrolysed depended on raw material used, suggesting that the hydrolysis kinetic of hemicelluloses was independent of the raw material used and the accessibility of hemicelluloses was strongly dependent of the biomass treated, respectively. Contrary, the maximum yield of cellulose was not observed indicating that the process time was not enough to obtain

the maximum extraction of cellulose. In the same way that the yield of hemicelluloses, the amount of cellulose depended on biomass used suggesting that the distribution of cellulose inside of matrix-biomass was dependent of the raw material treated.

Finally, the hydrolysis of hemicelluloses can be improved if the lignin content in the raw material is reduced suggesting that the accessibility of hemicelluloses depended on the low lignin content. Contrary, the hydrolysis of celluloses was not affected by this parameter.

In the next work, the combination of semicontinuous hydrothermal process in subcritical water with a continuous ultrafast hydrolysis in supercritical water will be analysed.

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References

[1] S.K. Thangavelu, A.S. Ahmed, F.N. Ani, Bioethanol production from sago pith waste using microwave hydrothermal hydrolysis accelerated by carbon dioxide, Applied Energy 128 (2014) 277-283

[2] Y.P. Wijaya, R.D.D. Putra, V.T. Widyaya, J.-M. Ha, D.J. Suh, C.S. Kim, Comparative study on two-step concentrated acid hydrolysis for the extraction of sugars from lignocellulosic biomass, Bioresource Technology 164 (2014) 221-231

[3] J.J. Bozell, Feedstocks for the future - Biorefinery production of chemicals from renewable carbon, Clean - Soil, Air, Water 36 (2008) 641-647.

[4] D.A. Cantero, M. Dolores Bermejo, M. José Cocero, Reaction engineering for process intensification of supercritical water biomass refining, The Journal of Supercritical Fluids 96 (2015) 21-35

[5] P. Binod, R. Sindhu, R.R. Singhania, S. Vikram, L. Devi, S. Nagalakshmi, N. Kurien, R.K. Sukumaran, A. Pandey, Bioethanol production from rice straw: An overview, Bioresource Technology 101 (2010) 4767-4774.

[6] R.D. Cortright, R.R. Davda, J.A. Dumesic, Hydrogen from catalytic reforming of biomass-derived hydrocarbons in liquid water, Nature 418 (2002) 964-967.

[7] H. Kishida, F. Jin, X. Yan, T. Moriya, H. Enomoto, Formation of lactic acid from glycolaldehyde by alkaline hydrothermal reaction, Carbohydrate Research 341 (2006) 2619-2623

[8] M. Sasaki, K. Goto, K. Tajima, T. Adschiri, K. Arai, Rapid and selective retro-aldol condensation of glucose to glycolaldehyde in supercritical water, Green Chemistry 4 (2002) 285-287.

[9] A. Wang, T. Zhang, One-Pot Conversion of Cellulose to Ethylene Glycol with Multifunctional Tungsten-Based Catalysts, Accounts of Chemical Research 46 (2013) 1377-1386.

[10] D.A. Cantero, A. Álvarez, M.D. Bermejo, M.J. Cocero, Transformation of glucose in high added value compounds in a hydrothermal reaction media, in: Prosciba, (Ed.), III Iberoamerican Conference on Supercritical Fluids, Prosciba, Cartagena de Indias (Colombia), 2013, pp. 1-7.

[11] T.M. Aida, K. Tajima, M. Watanabe, Y. Saito, K. Kuroda, T. Nonaka, H. Hattori, R.L. Smith Jr, K. Arai, Reactions of D-fructose in water at temperatures up to 400 °C and pressures up to 100 MPa, The Journal of Supercritical Fluids 42 (2007) 110-119.

[12] B.M. Kabyemela, T. Adschiri, R. Malaluan, K. Arai, Degradation Kinetics of Dihydroxyacetone and Glyceraldehyde in Subcritical and Supercritical Water, Industrial & Engineering Chemistry Research 36 (1997) 2025-2030.

[13] Y. Román-Leshkov, J.N. Chheda, J.A. Dumesic, Phase Modifiers Promote Efficient Production of Hydroxymethylfurfural from Fructose, Science 312 (2006) 1933-1937.

[14] K.L. Wasewar, A.A. Yawalkar, J.A. Moulijn, V.G. Pangarkar, Fermentation of Glucose to Lactic Acid Coupled with Reactive Extraction: A Review, Industrial & Engineering Chemistry Research 43 (2004) 5969-5982.

[15] S.S. Toor, L. Rosendahl, A. Rudolf, Hydrothermal liquefaction of biomass: A review of subcritical water technologies, Energy 36 (2011) 2328-2342.

[16] I. Pavlovič, Ž. Knez, M. Škerget, Hydrothermal reactions of agricultural and food processing wastes in sub- and supercritical water: a review of fundamentals, mechanisms, and state of research, Journal of Agricultural and Food Chemistry 61 (2013) 8003-8025.

[17] Ž. Knez, E. Markočič, M.K. Hrnčič, M. Ravber, M. Škerget, High pressure water reforming of biomass for energy and chemicals: A short review, The Journal of Supercritical Fluids 96 (2015) 46-52

[18] A.A. Peterson, F. Vogel, R.P. Lachance, M. Fröling, M.J. Antal Jr, J.W. Tester, Thermochemical biofuel production in hydrothermal media: A review of sub- and supercritical water technologies, Energy and Environmental Science 1 (2008) 32-65.

[19] K. Arai, R.L. Smith Jr, T.M. Aida, Decentralized chemical processes with supercritical fluid technology for sustainable society, The Journal of Supercritical Fluids 47 (2009) 628-636.

[20] C. Rong, X. Ding, Y. Zhu, Y. Li, L. Wang, Y. Qu, X. Ma, Z. Wang, Production of furfural from xylose at atmospheric pressure by dilute sulfuric acid and inorganic salts, Carbohydr Res 350 (2012) 77-80.

[21] S. Sabiha-Hanim, M.A. Mohd Noor, A. Rosma, Fractionation of oil palm frond hemicelluloses by water or alkaline impregnation and steam explosion, Carbohydrate Polymers 115 (2015) 533-539.

[22] A.N. Hoover, J.S. Tumuluru, F. Teymouri, J. Moore, G. Gresham, Effect of pelleting process variables on physical properties and sugar yields of ammonia fiber expansion pretreated corn stover, Bioresource Technology 164 (2014) 128-135.

[23] R. Travaini, M.D.M. Otero, M. Coca, R. Da-Silva, S. Bolado, Sugarcane bagasse ozonolysis pretreatment: Effect on enzymatic digestibility and inhibitory compound formation, Bioresource Technology 133 (2013) 332-339.

[24] J.M. Prado, L.A. Follegatti-Romero, T. Forster-Carneiro, M.A. Rostagno, F. Maugeri Filho, M.A.A. Meireles, Hydrolysis of sugarcane bagasse in subcritical water, Journal of Supercritical Fluids 86 (2014) 15-22.

[25] G. Garrote, H. Domínguez, J.C. Parajó, Autohydrolysis of corncob: Study of non-isothermal operation for xylooligosaccharide production, Journal of Food Engineering 52 (2002) 211-218.

[26] J.V. Rissanen, H. Grénman, S. Willför, D.Y. Murzin, T. Salmi, Spruce Hemicellulose for Chemicals Using Aqueous Extraction: Kinetics, Mass Transfer, and Modeling, Industrial & Engineering Chemistry Research 53 (2014) 6341-6350

[27] J.M. Prado, T. Forster-Carneiro, M.A. Rostagno, L.A. Follegatti-Romero, F. Maugeri Filho, M.A.A. Meireles, Obtaining sugars from coconut husk, defatted grape seed, and pressed palm fiber by hydrolysis with subcritical water, The Journal of Supercritical Fluids 89 (2014) 89-98.

[28] P.C. Veggi, R.N. Cavalcanti, M.A.A. Meireles, Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction: Experimental data and economic evaluation, Journal of Food Engineering 131 (2014) 96-109.

[29] C.S. Gong, N.J. Cao, J. Du, G.T. Tsao, Ethanol Production from Renewable Resources, in: P.D.G.T. Tsao, A.P. Brainard, H.R. Bungay, N.J. Cao, P. Cen, Z. Chen, J. Du, B. Foody, C.S. Gong, P. Hall, N.W.Y. Ho, D.C. Irwin, P. Iyer, T.W. Jeffries, C.M. Ladisch, M.R. Ladisch, Y.Y. Lee, N.S. Mosier, H.M. Mühlemann, M. Sedlak, N.Q. Shi, G.T. Tsao, J.S. Tolan, R.W. Torget, D.B. Wilson, L. Xia, (Eds.), Recent Progress in Bioconversion of Lignocellulosics, Springer Berlin Heidelberg, 1999, pp. 207-241 %* ©1999 Springer-Verlag Berlin Heidelberg

[30] H. Grenman, K. Eranen, J. Krogell, S. Willfor, T. Salmi, D.Y. Murzin, Kinetics of aqueous extraction of hemicelluloses from spruce in an intensified reactor system, Industrial and Engineering Chemistry Research 50 (2011) 3818-3828.

[31] M.S. Tunc, A.R.P. Van Heiningen, Hemicellulose extraction of mixed southern hardwood with water at 150 °C: Effect of time, Industrial and Engineering Chemistry Research 47 (2008) 7031-7037.

[32] J. Hu, D. Shen, S. Wu, H. Zhang, R. Xiao, Effect of temperature on structure evolution in char from hydrothermal degradation of lignin, Journal of Analytical and Applied Pyrolysis 106 (2014) 118-124

[33] B. Du, L.N. Sharma, C. Becker, S.-F. Chen, R.A. Mowery, G.P. van Walsum, C.K. Chambliss, Effect of varying feedstock-pretreatment chemistry combinations on the formation and accumulation of potentially inhibitory degradation products in biomass hydrolysates, Biotechnology and Bioengineering 107 (2010) 430-440.

[34] H.-Q. Li, W. Jiang, J.-X. Jia, J. Xu, pH pre-corrected liquid hot water pretreatment on corn stover with high hemicellulose recovery and low inhibitors formation, Bioresource Technology 153 (2014) 292-299.

[35] W.S.L. Mok, M.J. Antal Jr, Uncatalyzed solvolysis of whole biomass hemicellulose by hot compressed liquid water, Industrial and Engineering Chemistry Research 31 (1992) 1157-1161.

[36] S.-N. Sun, X.-F. Cao, H.-Y. Li, F. Xu, R.-C. Sun, Structural characterization of residual hemicelluloses from hydrothermal pretreated Eucalyptus fiber, International Journal of Biological Macromolecules 69C (2014) 158-164.

[37] L. Wei, A. Shrestha, M. Tu, S. Adhikari, Effects of surfactant on biochemical and hydrothermal conversion of softwood hemicellulose to ethanol and furan derivatives, Process Biochemistry 46 (2011) 1785-1792.

[38] W.-S. Lim, J.-W. Lee, Influence of pretreatment condition on the fermentable sugar production and enzymatic hydrolysis of dilute acid-pretreated mixed softwood, Bioresource Technology 140 (2013) 306-311

[39] G. Garrote, H. Domínguez, J.C. Parajó, Hydrothermal processing of lignocellulosic materials, Holz als Roh - und Werkstoff 57 (1999) 191-202.

[40] S.-N. Sun, X.-F. Cao, H.-Y. Li, F. Xu, R.-C. Sun, Structural characterization of residual hemicelluloses from hydrothermal pretreated Eucalyptus fiber, International Journal of Biological Macromolecules 69 (2014) 158-164.

[41] N.R.E. Laboratory, Determination of Structural Carbohydrates and Lignin in Biomass, Golden, CO, 2011.

[42] J.B. Sluiter, R.O. Ruiz, C.J. Scarlata, A.D. Sluiter, D.W. Templeton, Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods, J. Agric. Food Chem. 58 (2010) 9043-9053.

[43] R.R. A. Sluiter, C. Scarlata, J. Sluiter, and D. Templeton Determination of Extractives in Biomass, NREL Laboratory Analytical Procedures, National Renewable Energy Laboratory, Golden, Colorado, 2008.

[44] F.M. Yedro, J. García-Serna, D.A. Cantero, F. Sobrón, M.J. Cocero, Hydrothermal fractionation of grape seeds in subcritical water to produce oil extract, sugars and lignin, Catalysis Today in press (2014).

[45] W.S.L. Mok, M.J. Antal, Uncatalyzed solvolysis of whole biomass hemicellulose by hot compressed liquid water, Industrial & Engineering Chemistry Research 31 (1992) 1157-1161

[46] S. Liu, Woody biomass: Niche position as a source of sustainable renewable chemicals and energy and kinetics of hot-water extraction/hydrolysis, Biotechnology Advances 28 (2010) 563-582.

[47] S. Kumar, U. Kothari, L. Kong, Y.Y. Lee, R.B. Gupta, Hydrothermal pretreatment of switchgrass and corn stover for production of ethanol and carbon microspheres, Biomass and Bioenergy 35 (2011) 956-968

[48] M. Sasaki, B. Kabyemela, R. Malaluan, S. Hirose, N. Takeda, T. Adschiri, K. Arai, Cellulose hydrolysis in subcritical and supercritical water, The Journal of Supercritical Fluids 13 (1998) 261-268

[49] A.S. da Silva, R.S. Sobral Teixeira, R.d. Oliveira, V. Santana, R.d.R.O. de Barros, M. Antonieta, E.P. da Silva Bo, Sugarcane and Woody Biomass Pretreatments for Ethanol Production, in: A. Chandel, (Ed.), Sustainable Degradation of Lignocellulosic Biomass - Techniques, Applications and Commercialization, InTech %@ 978-953-51-1119-1 2013.

[50] O. Bobleter, Hydrothermal degradation of polymers derived from plants, Progress in Polymer Science 19 (1994) 797-841

[51] S. Wang, Y. Zhou, T. Liang, X. Guo, Catalytic pyrolysis of mannose as a model compound of hemicellulose over zeolites, Biomass and Bioenergy 57 (2013) 106-112.

[52] D.A. Cantero, A. Álvarez, M.D. Bermejo, M.J. Cocero, Transformation of glucose into added value compounds in a hydrothermal reaction media The Journal of Supercritical Fluids.

[53] S. Kumar, R.B. Gupta, Biocrude Production from Switchgrass Using Subcritical Water, Energy & Fuels 23 (2009) 5151-5159

[54] D. Fengel, G. Wegener, Wood: chemistry, ultrastructure, reactions, Walter de Gruyter 1983.

[55] J. Zhang, H. Deng, Y. Sun, C. Pan, S. Liu, Isolation and characterization of wheat straw lignin with a formic acid process, Bioresource Technology 101 (2010) 2311-2316.

Conclusions

Fractionation process of surplus biomass by autohydrolysis in subcritical water obtaining added value products

The fractionation/hydrolysis of biomass was intensively studied using water as solvent in subcritical conditions. The experimental setup was designed for this thesis and it was able to operate up to 400ºC and 25 MPa, operating in a semicontinuous reaction mode to develop the experiments included in this PhD thesis (Chapters 1,2,3 and 5). In this kind of reactor two residence times were defined: one related to residence time of solid and one associated to residence time of liquid. The semicontinuous operation mode of the reactor showed to be adequate for the fractionation of biomass obtaining the following hydrolysis products: carbohydrates, lignin and components with low content of oxygen.

The hydrothermal process was studied at temperatures between 150 and 340ºC and pressures between 10 MPa and 16 MPa. It was observed that the temperature played an important role in the fractionation/hydrolysis process on hydrolysis products obtained. The variations of temperature allowed for the extraction/hydrolysis of the main carbohydrates and lignin. The major extraction of hemicelluloses was observed at about 170-200ºC being the content of celluloses lower than 10% in the final liquid product. In the same way, the extraction of hemicellulose showed to be improved when the lignin content of the biomass was reduced. It can be concluded that the low content of lignin makes more accessible the hemicellulose fraction. The maximum hydrolysis of celluloses was observed at higher temperatures, at about 250-265ºC. The step to extract the remaining carbohydrates from the solid inside of the reactor, after a previous fractionation process, using higher temperatures (at about 320ºC) is not necessary. These results were expected as the polymerization of hemicelluloses and celluloses take place in a different way: the hemicelluloses take place in a branch way while the cellulose takes place in a linear way. This difference makes hemicellulose a more accessible polymer for the hydrolysis process. Thus, controlling temperature the process behaves different and fractions rich in hemicelluloses, cellulose and lignin can be obtained possible.

Moreover, the behaviour of pH was studied thoroughly. The same performance was observed independently of the temperature, flowrate, particle size and raw material used. Considering pH profile with time, three different behaviours were observed. First the pH increased, then it dropped drastically and finally the pH increased softly. The first increase in the pH can be attributed to the extraction of basic ashes, which will increase the basicity of the medium. The decreased of pH was due to the hydrolysis of acetyl

groups from hemicelluloses producing free acetic acid, which increased the hydronium ions concentration in the reaction medium. The acetic acid acted as a catalyst in the polysaccharide autohydrolysis and degradation of carbohydrates. The second increase was attributed to two factors: presence of degradation products and decrease o hydrolysis product concentration along time. The most important result of our research was to demonstrate in a number of experiments, that the minimum pH was located at the same time as the content of carbon in the liquid samples was maximum. This point is important due to that the pH can be used as indicator for following the hydrothermal process identifying the reaction time necessary to reach the maximum hydrolysis of hemicelluloses. Consequently, the number of samples taken can be reduced reducing the analytical and time expenditures and at the same time understanding the behaviour of the system. The performance of pH can help to follow a hydrothermal process to industrial scale.

Some of the experiments were carried out in a 5-reactor recirculation pilot plant, specifically designed to extract hemicelluloses. These experiments were carried out thanks to the collaboration of "Reaction Engineering and Industrial Chemistry Laboratory" at Åbo Akademi (Turku, Finland) with Dr. Henrik Grenmán and Ak. Prof. Tapio Salmi. Here, we found that the deacetylation was accompanied by a reduction in the molecular weight (M_w) of hemicelluloses. The target was to obtain high molecular weight hemicelluloses, but from hardwood (i.e. Holm oak) only 12.9 kDa was obtained as the maximum. The longest hemicelluloses were extracted at the beginning and the molecular weight decreased along the increasing both temperature and time.

Future work

From the studies developed in this PhD, it can be concluded that the fractionation of biomass using a semicontinuos reactor in a hydrothermal process is a good option to obtain high yields of carbohydrates and lignin.

It was observed in all studies that the amount of degradation products is lower. If the purpose of the carbohydrates hydrolysed is further conversions into added value products such as lactic acid, 5-Hydroxymhetilfurfural, glycolaldehyde, etc., the use of this semicontinuos reactor will not be adequate. The higher amount of degradation products using this reactor can be obtained using higher residence time of the liquid phase,

manipulating two variables: increasing the reactor volume or reducing the flow. These options are unviable to industrial scale.

The installation of a continuous reactor in the outlet liquid stream of a semicontinuous reactor could be a good option, to reduce the amount of oligomers increasing the reduced sugars content. This new design, which in the first reactor can be obtained a high yield of carbohydrates and in the second reactor can be obtained high selectivity and yield of added value products, could reduce the equipment cost changing the residence time from minutes to milliseconds in the second reactor.

Resumen

Proceso de fraccionamiento de biomasa excedentaria por autohidrólisis en agua subcrítica obteniendo productos de valor añadido

Los problemas que se generan a partir de las fuentes de combustibles fósiles como son los elevados precios del petróleo y del gas, el calentamiento global causado por las emisiones de gases de efecto invernadero y el progresivo agotamiento de las fuentes fósiles han incrementado en los últimos años la demanda y el uso de recursos renovables para la obtención de productos de valor añadido, biocombustibles y de energía. En la actualidad, uno de los grandes desafíos es conseguir una sociedad basada en el concepto de bioeconomía. La bioeconomía se refiere a la producción sostenible y conversión de biomasa en una amplia gama de alimentos, salud, productos industriales y energía.

La biomasa lignocelulósica es una materia prima potencial para la producción de bioproductos, biocombustibles y energía. El primer reto para la conversión de biomasa en productos de valor añadido es la de fraccionar su estructura en sus tres principales componentes: hemicelulosas, celulosa y lignina. Uno de los métodos más prometedores es el proceso hidrotermal, en el que se utiliza agua presurizada a alta temperatura como disolvente y medio de reacción; lo que permite tener unas adecuadas condiciones para realizar la hidrolisis y es un disolvente limpio, seguro y "amigable" medioambientalmente.

Objetivos

El objetivo principal de este trabajo es desarrollar un proceso capaz de obtener productos de valor añadido a partir del fraccionamiento de biomasa lignocelulósica utilizando el agua en condiciones subcríticas como disolvente y medio de reacción.

Con el fin de lograr el propósito de esta tesis, se definen los siguientes objetivos:

- Diseño, construcción y optimización de una planta piloto con el fin de estudiar el proceso de fraccionamiento hidrotermal utilizando agua como disolvente y medio de reacción en condiciones subcríticas. La temperatura máxima y la presión requeridas fueron de 400ºC y 25 MPa.
- Estudio de la hidrólisis de semillas de uva
    - Análisis del efecto de la temperatura sobre los rendimientos de heavy bio oil, light bio oil, azúcares y sólidos.
    - Análisis del efecto de la temperatura en el rendimiento de azúcares hidrolizados y sólido.

- Análisis de la extracción solvotermal (utilizando como solvente una mezcla de etanol/agua) sobre el rendimiento de polifenoles y aceite.

- Estudio de la hidrólisis de encina
  - Análisis de la temperatura, flujo y tamaño de partícula sobre el rendimiento de los carbohidratos hidrolizados y del sólido obtenido.
  - Alternativas para seleccionar un parámetro que simplifique el seguimiento del comportamiento del proceso hidrotermal.
  - Análisis de la temperatura y del tiempo de reacción en la hidrólisis de hemicelulosas y del peso molecular promedio obtenido.

- Estudio de la hidrólisis en especies de madera dura y blanda
  - Análisis de la influencia de la composición de la materia prima en el rendimiento de azúcares extraídos y solido obtenido.

Discusión de los resultados

Esta Tesis Doctoral ha sido estructurada en cinco capítulos. En cada uno de los mismos se han presentado los objetivos así como una pequeña revisión bibliográfica relacionada con el tema tratado. A continuación se detallan los principales resultados como así también las conclusiones más relevantes de cada capítulo.

Para poder realizar cada uno de los estudios, la planta piloto fue diseñada y construida. En todos los casos las condiciones de operación de presión y temperatura, fueron condiciones subcríticas, por lo tanto la temperatura máxima de operación fue de 340ºC con presiones de hasta 165 bares. Las principales ventajas del sistema experimental son:

- El reactor puede considerarse isotérmico debido a los cortos tiempos de calentamiento y enfriamiento.
- Versatilidad del sistema, principalmente debido a la posibilidad de trabajar con distintos tipos de biomasa.

En el Capítulo 1 titulado como “Hydrothermal hydrolysis of grape seeds to produce bio-oil” se presenta un estudio sobre el efecto de la temperatura en la producción de heavy bio-oil y light bio-oil como así también del sólido residual y azúcares obtenidos durante 60 minutos de hidrólisis de semillas de uva. Las temperaturas seleccionadas fueron: 250, 300 y 340ºC. El flujo empleado fue de 5 ml/min. Los rendimientos obtenidos para light bio-oil variaron entre 8.1-15.7% wt. y para heavy bio-oil entre el 10.6-16.2% wt. Estos resultados fueron obtenidos para la temperatura más alta de operación: 340ºC. La hidrólisis de celulosa y hemicelulosas genera azúcares que pueden convertirse en otros compuestos tales como cetonas y aldehídos (vía condensación retro-aldólica y reacción de deshidratación) dependiendo del tiempo y la temperatura empleada. Estas reacciones son favorecidas a altas temperaturas incrementando el heavy bio-oil y por lo tanto la cantidad total de bio-oil. El sólido residual varió desde el 35.8 %wt. hasta el 25.6 % wt. La máxima cantidad de sólido residual fue obtenido a la más baja temperatura indicando que la cinética de la hidrólisis de azúcares es más rápida a más altas temperaturas de operación. El espectro FT-IR mostró que los principales grupos aromáticos estaban presentes en el sólido residual, mientras que la intensidad de las bandas de los grupos funcionales de hemicelulosas y celulosa no se observaban o su intensidad era menor que antes de ser sometidas al proceso hidrotermal. El balance de masa final fue de 80.2-86.3 % wt. (ver Figura 1) siendo un valor aceptable si se considera que únicamente se han utilizado para la experimentación 4 gramos de materia prima.

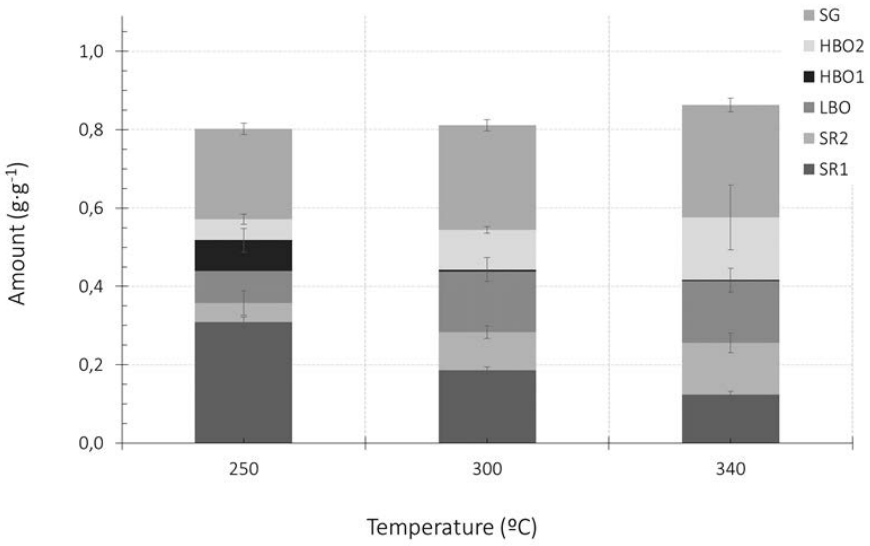

Figura 1. Variación de los productos de hidrólisis con la temperatura

En el Capítulo 2 titulado "Hydrothermal fractionation of grape seeds in subcritical water to produce oil extract, sugars and lignin" se presenta un estudio de la combinación de dos procesos: una extracción solvotermal seguida de un fraccionamiento hidrotermal. Primeramente, se sometió a la materia prima a una extracción con etanol/agua (70/30% wt.) durante 60 min a 90ºC. Se obtuvo un 13.0% wt. de aceite y extractables cuyo contenido de polifenoles fue de 4.46% wt en el producto líquido. A continuación, el proceso de fraccionamiento hidrotermal consistió en un perfil de temperaturas (desde 150ºC hasta 340ºC) con el objetivo de hidrolizar inicialmente las hemicelulosas, luego la celulosa y por último aquellos azúcares que no habían sido hidrolizados en las etapas anteriores con el fin de obtener un sólido rico en lignina.

Como puede observarse en la Figura 2, la cantidad total de azúcares hidrolizados fueron: 0.20 g/g para el siguiente perfil de temperaturas: 150/250/320ºC y aproximadamente 0.23 g/g para 165/265/330ºC y 180/280/340ºC. Estos valores representaron una hidrólisis de entre el 54% wt. y el 63% wt. referidos a la cantidad de hemicelulosas y celulosa contenida en la materia prima (36.8% wt.). La máxima cantidad de C5, C6 y oligosacáridos para el primer rango de temperaturas fue obtenido a los 180ºC. Esta corriente fue rica en hemicelulosas indicando que las mismas se extraen a bajas temperaturas. En el segundo rango de temperaturas pudo observarse que la máxima concentración de azúcares en el producto líquido se obtuvo entre los 250 y 265ºC. Como se puede observar en la Figura 2, la curva presenta dos pendientes, correspondientes a

los siguientes tiempos de operación: 105-120 min y 120-150 min. La primera pendiente se considera que fue debida al calentamiento del reactor para alcanzar el segundo rango de temperaturas de trabajo. La segunda pendiente fue menor, indicando que la mayor extracción de azúcares se realizó durante el calentamiento. La corriente obtenida en esta etapa fue rica en celulosa. En el tercer rango de temperaturas se pudo observar un ligero aumento en la hidrólisis de azúcares, indicando que la mayoría de los azúcares fueron extraídos en las etapas previas.

La mejor combinación de temperaturas para hidrolizar azúcares fue: 180ºC - (250-265ºC) – (330-340ºC).

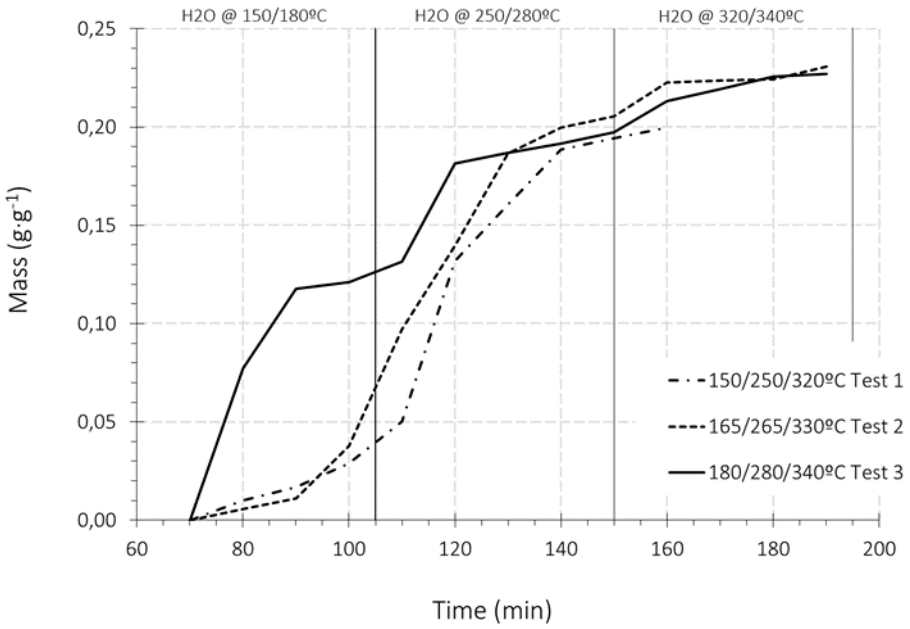

Figura 2. Masa total extraída ($g \cdot g^{-1}$) durante el proceso de fraccionamiento hidrotermal.

En el Capítulo 3 titulado como "Monitoring alternatives and main sugar products for the autohydrolysis of Holm oak hemicelluloses using pressurized hot water" se presenta un estudio basado en encontrar un parámetro que permita el seguimiento del tratamiento hidrotermal. En este capítulo también se analiza el rendimiento de azúcares recuperados bajo la influencia de distintas condiciones de operación tales como flujo, temperatura y tamaño de partícula. La eficiencia del proceso, desde el punto de vista energético, también es estudiada. La biomasa utilizada fue Encina (*Quercus ilex*) la cual fue sometida a diferentes condiciones de temperatura (entre 175 y 207 ºC), de flujo (3, 10, 19 y 34 ml/min) y de tamaños de partícula: 3 y 6 mm. La recuperación de calor fue entre el 73.8

y el 85.5% dependiendo del flujo y la temperatura de operación. El coeficiente global de transferencia de calor (U) fue entre 788 y 619 Wm^{-2} $ºC^{-1}$. Se encontró un interesante efecto entre el pH, el Carbono Orgánico Total (TOC) y la cantidad de carbohidratos medidos por HPLC (High-Pressure Liquid Chromatography). Los máximos valores de TOC y carbohidratos medidos por HPLC se obtuvieron al mismo tiempo en el que el valor del pH fue mínimo, indicando que la máxima hidrólisis de carbohidratos se da cuando la mayoría de las hemicelulosas son extraídas. En la Figura 3 puede observarse este comportamiento.

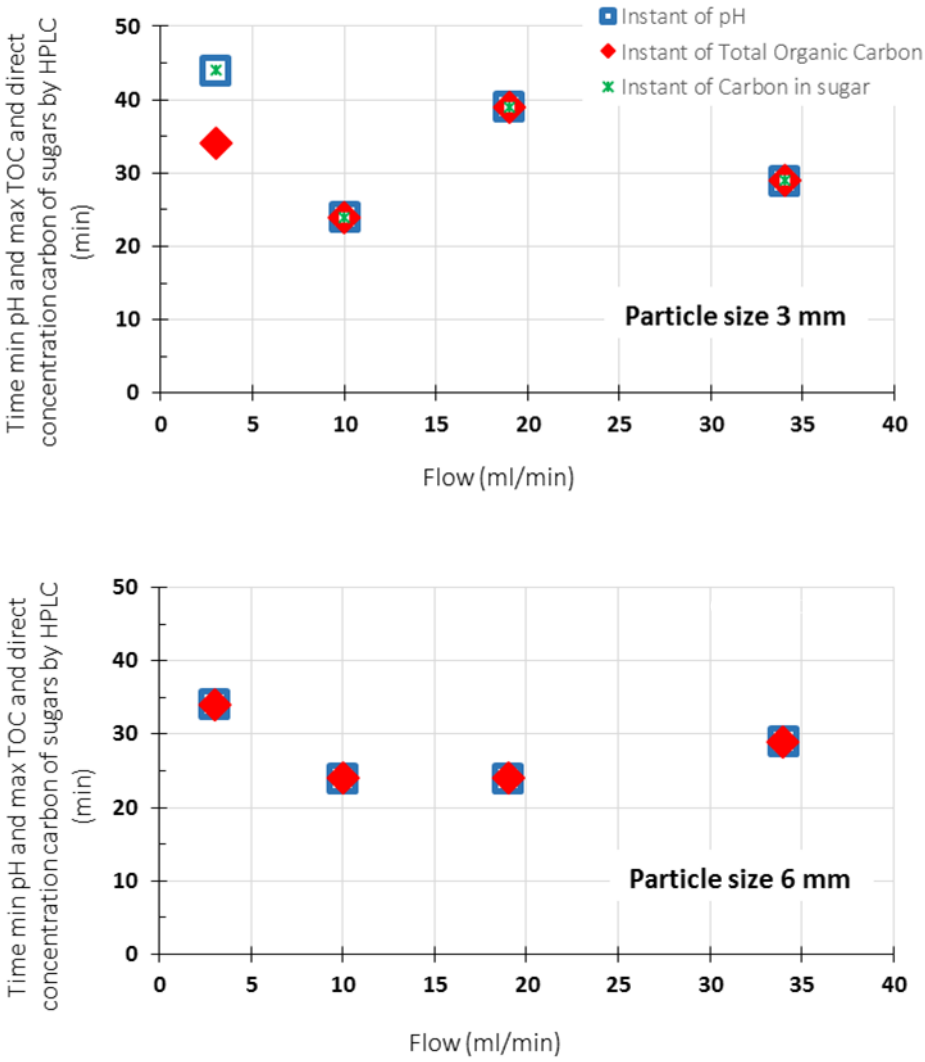

Figura 3. Tiempos en los que se obtiene las máximas concentraciones de carbono orgánico total y carbohidratos y el mínimo pH en función de los flujos.

El pH mostró un comportamiento similar en todos los casos como se puede apreciar en la Figura 4. El pH comenzó en 5.5 (valor de pH del agua destilada) y luego decreció alcanzando valores entre 3.5-3.8 durante los primeros 24-44 minutos dependiendo de

las condiciones operativas empleadas. Las hemicelulosas contienen grupos acetilos que al ser hidrolizados liberan ácido acético generando una disminución del pH. Tras esto se produce un crecimiento del pH hasta alcanzar valores entre 3.9-4.5. El pH final puede estar relacionado con valores de productos de degradación tales como ácido láctico (pKa=3.86), ácido fórmico (pKa=3.75) y ácido acrílico (pKa=4.35).

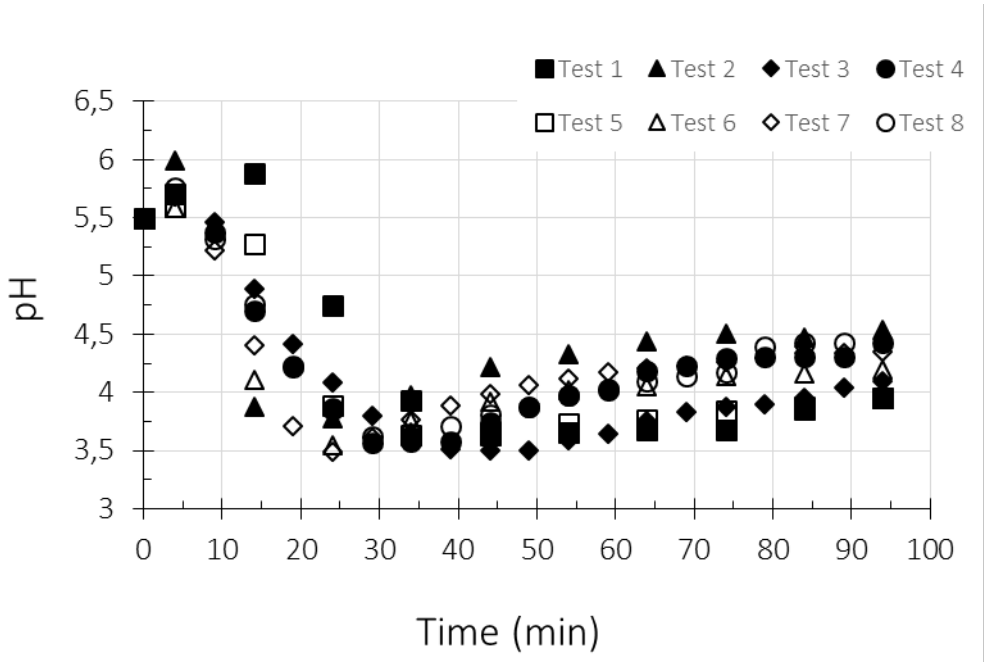

Figura 4. Comportamiento del pH durante la hidrólisis de Encina a diferentes condiciones de temperatura, flujo y tamaño de partícula.

Se propone el pH como parámetro clave para seguir el proceso en línea, ya que se trata de un parámetro que es muy fácil de medir y económico. Si es necesario conocer la cantidad de carbohidratos extraídos se propone utilizar el análisis de TOC fuera de línea. Finalmente, si se quiere conocer el tipo de azúcares hidrolizadas se propone utilizar el análisis por HPLC. Los autores proponen el análisis de cinco muestras para poder entender el comportamiento del sistema, disminuyendo el costo de análisis y consecuentemente el tiempo: la primera que coincida con el valor mínimo de pH, luego dos antes de este tiempo y dos después de este tiempo, a intervalos de tiempo comparables.

En el Capítulo 4 titulado como "Obtaining hemicelluloses from hardwood Holm oak (*Quercus ilex*) using subcritical water in a pilot plant" se presenta un estudio basado en el efecto de la temperatura y el tiempo de reacción sobre el rendimiento y el peso molecular

de hemicelulosas extraídas usando cinco reactores conectados en serie formando una "cascada de reactores" donde el flujo fue recirculado durante todo el análisis. Las temperaturas de operación fueron entre 130 y 170ºC. Los resultados mostraron que la disminución del pH durante la hidrólisis de la hemicelulosas fue fuertemente dependiente de la temperatura y estuvo acompañada de una reducción en el peso molecular de las hemicelulosas extraídas. A altas temperaturas las hemicelulosas mostraron una pronunciada disminución del peso molecular luego de pocos minutos de comenzado el proceso hidrotermal. El peso molecular de las hemicelulosas extraídas a 170ºC durante 60 min varió entre 12930 a 1752 $g \cdot mol^{-1}$. El peso molecular final fue entre 3833 y 1752 $g \cdot mol^{-1}$ dependiendo de la temperatura y del tiempo de reacción empleado. Comparando los resultados obtenidos en este estudio con una especie softwood, Picea, el peso molecular obtenido a partir de la hidrólisis de Encina fue inferior, probablemente debido a una desacetilación mayor producida por un mayor contenido de grupos acetilos en las especies hardwood.

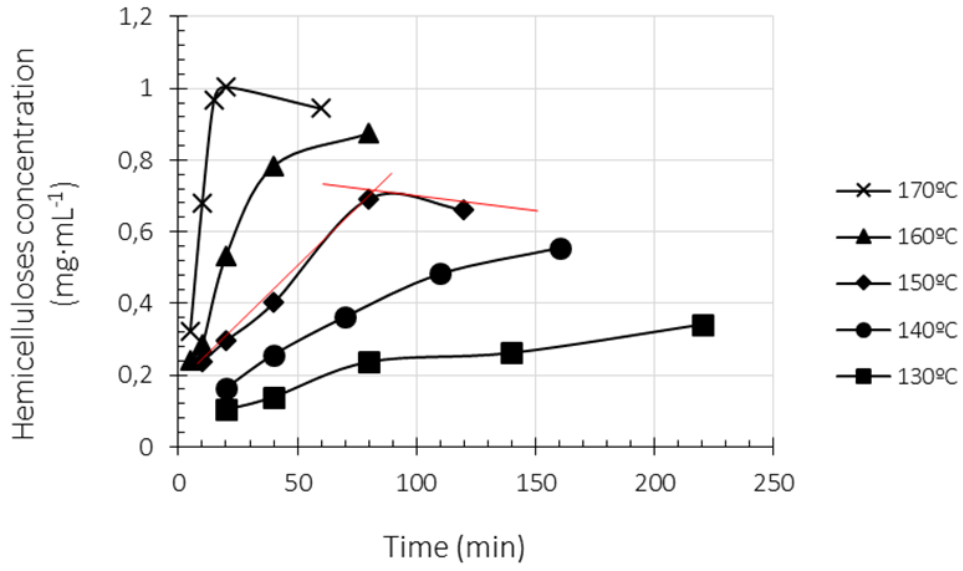

Figura 7. Concentración de hemicelulosas hidrolizadas en función del tiempo a diferentes temperaturas.

Como se puede observar en la Figura 7, la mayor concentración de hemicelulosas extraídas fue obtenida a las más altas temperaturas. El tiempo necesario para alcanzar la máxima concentración de hemicelulosas fue de 80 min a 150ºC mientras que de solo 20 min a 170ºC indicando que las cinéticas de reacción son mayores a mayor temperatura.

La máxima conversión de hemicelulosas hidrolizadas fue un 60% trabajando a 170ºC durante 20 min.

El comportamiento a 130 y 140ºC fue aproximadamente lineal indicando de esta forma que el tiempo de reacción o la temperatura no fueron suficientes para hidrolizar las hemicelulosas. Para temperaturas por encima de los 150ºC, se observaron dos pendientes en la concentración de hemicelulosas hidrolizadas. La primera pendiente de la curva fue causada por la hidrólisis de hemicelulosas. La segunda pendiente fue menos pronunciada que la primera sugiriendo la presencia de productos de degradación.

En el Capítulo 5 titulado como "Hydrothermal fractionation of woody biomass: lignin effect over sugars recovery" se presenta un estudio basado en la influencia de la composición de la materia prima sobre el rendimiento obtenido en la hidrólisis de hemicelulosas y celulosa y en la obtención de un sólido rico en lignina. Se trata de un análisis en la versatilidad del sistema. Nueve especies de árboles urbanos fueron tratados hidrotermalmente usando un reactor semicontinuo a 250ºC usando un flujo de 10 ml/min. La presión fue fijada a 10 MPa para asegurar que el solvente estuviera en fase líquida. Dos productos fueron obtenidos: un producto sólido y un producto líquido. En este trabajo se definieron dos tiempos: el tiempo relacionado con el sólido que se encuentra en el interior del reactor el cual fue de 64 minutos y un tiempo relacionado con el líquido, el cual es función de la porosidad del lecho y del flujo empleado que varió entre 3 y 3.6 min.

En la Figura 5A y 5B se muestra la cantidad de hemicelulosas y celulosa recuperadas en función del tiempo de reacción del sólido respectivamente. Como se puede observar, la cantidad de hemicelulosas recuperadas (Figura 5A) depende de la biomasa utilizada y varia entre 0.28 y 0.79 g/g hemicelulosas. En el caso del rendimiento de la celulosa recuperada, éste varió entre el 0.36 y 1 g/g celulosa. Las gráficas muestran que el tiempo no fue suficiente para alcanzar la máxima extracción de celulosa.

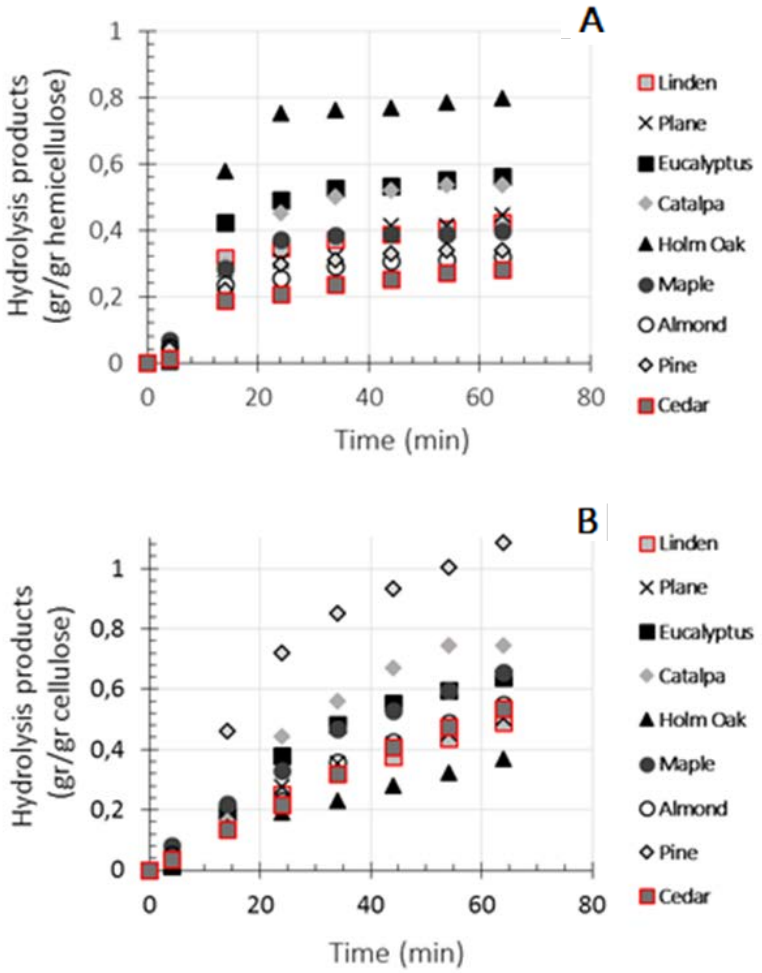

Figura 5. Rendimiento de hemicelulosas (A) y celulosa (B) recuperadas luego del proceso hidrotermal a 250ºC durante 64 min.

Los autores proponen que la hidrólisis de hemicelulosas y celulosa está gobernada por dos parámetros: la cinética de hidrólisis y la accesibilidad al polímero. La máxima extracción de hemicelulosas fue observada a los 20 minutos, y fue el mismo tiempo (referido al tiempo de reacción del sólido) para todas las especies de árboles estudiadas, sugiriendo que la cinética de hidrólisis de hemicelulosas fue la misma para todas las materias primas empleadas. Sin embargo, el máximo rendimiento de extracción/hidrólisis de hemicelulosas dependió de la biomasa tratada sugiriendo que la accesibilidad y disposición de hemicelulosas depende de la materia prima empleada. Del mismo modo, los máximos valores de celulosa recuperada podrían estar afectados por la accesibilidad a este componente en la materia prima utilizada.

Para evaluar la interacción de los principales componentes de la biomasa, en la Figura 6 se muestra la extracción de las hemicelulosas y celulosa en función del contenido de lignina en la materia prima.

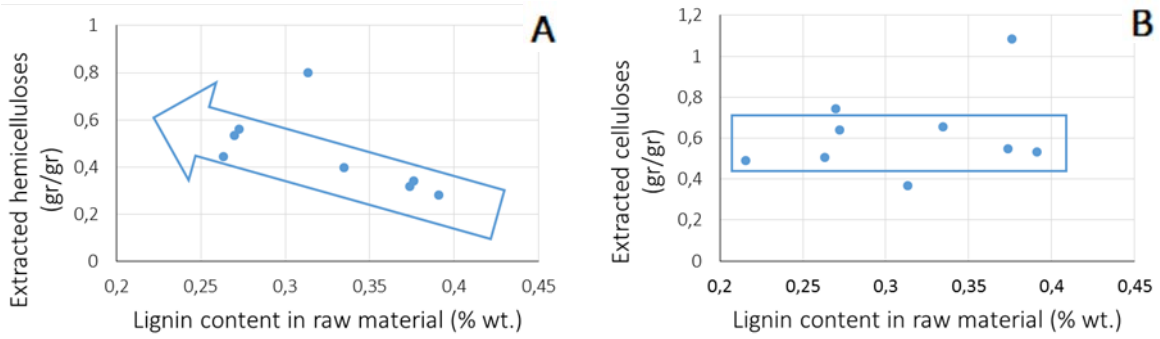

Figura 6. Rendimiento de hemicelulosa (A) y celulosa (B) extraídas luego de un proceso hidrotermal utilizando nueve especies de árboles urbanos a 250ºC.

Una tendencia general fue encontrada en la extracción de hemicelulosas (Figura 6A): un bajo contenido de lignina sugirió una mayor accesibilidad en las hemicelulosas recuperadas. En el caso de la celulosa, el rendimiento de la celulosa recuperada fue de 60±10% gr/gr celulosa independientemente de la biomasa utilizada.

Los autores sugieren que la hidrólisis de hemicelulosas y celulosa podría estar gobernada por dos efectos: la cinética de hidrólisis y la accesibilidad al polímero en la materia prima. En el primer caso, la cinética de hidrólisis de celulosa y hemicelulosa es independiente de la biomasa estudiada. Sin embargo, la accesibilidad del agua a éstas fracciones podría estar influenciada por la estructura del material afectando la producción final de carbohidratos.

Conclusiones

Además de las conclusiones aportadas en cada estudio específico realizado en cada capítulo, se dan unas conclusiones generales la tesis que se concretan a continuación:

El proceso de hidrólisis-fraccionamiento de biomasa se estudió en un medio hidrotermal. El dispositivo experimental consiste en un reactor semicontinuo capaz de trabajar con temperaturas de hasta 400ºC y presiones de hasta 25 MPa. En este tipo de reactores dos tiempos son definidos: uno relacionado con el tiempo de residencia del sólido y uno relacionado con el tiempo de residencia del líquido. El modo de operación semicontinuo

mostró ser adecuado para el fraccionamiento de biomasa en la obtención de los siguientes productos de hidrólisis: azúcares, lignina y productos de bajo contenido de oxígeno.

El proceso hidrotermal fue estudiado a temperaturas entre 150 y 340ºC y presiones entre 10 MPa y 16 MPa. Se observó que la temperatura juega un papel importante en el proceso de hidrólisis/fraccionamiento de biomasa sobre la obtención de productos hidrolizados. Las variaciones en temperatura condujeron a la extracción/hidrólisis de los principales azúcares y lignina.

La mayor extracción de hemicelulosas fue observada entre 170-200ºC siendo el contenido de celulosa en el producto líquido final menor que el 10%. Del mismo modo, la extracción de hemicelulosas mostró que puede ser mejorada cuando el contenido inicial de lignina en la materia prima sometida al proceso hidrotermal es menor. Se puede concluir que el bajo contenido de lignina hace más accesible la fracción de hemicelulosa. La máxima hidrólisis de celulosa fue observada a más altas temperaturas, entre los 250 y 265ºC. Los resultados mostraron que someter a la biomasa a una tercera etapa de extracción/hidrolisis a alta temperatura (alrededor de 320ºC) no es necesario ya que dos etapas son suficientes para hidrolizar la mayor cantidad de azúcares. Los resultados obtenidos en esta tesis doctoral son los esperados debido a que la polimerización de las hemicelulosas y las celulosa tienen lugar de diferentes modos: las hemicelulosas tienen lugar en forma de “rama” mientras que la celulosa en forma lineal. Éstas diferencias en la estructura del polímero hacen que la hemicelulosa sea de más fácil hidrólisis. Esta diferencia en el comportamiento de ambos polímeros sometidos a un proceso hidrotermal puede ser beneficioso si un adecuado control de la temperatura es aplicado, obteniendo de esta forma fracciones ricas en cada uno de los polímeros (hemicelulosa, celulosa y lignina).

El comportamiento del pH fue intensivamente estudiado. El mismo comportamiento fue observado independientemente de la temperatura, flujo, tamaño de partícula y material utilizado. Tres diferentes comportamientos fueron observados. Primero el pH incrementó, luego disminuyó drásticamente y posteriormente se incrementó suavemente. El primer incremento puede ser atribuido a la presencia de cenizas, los cuales incrementan la basicidad del medio. El decrecimiento drástico del pH fue debido

a la hidrólisis de los grupos acetilos provenientes de las hemicelulosas produciendo ácido acético en el medio de reacción. El ácido acético actúa como catalizador en la hidrólisis de polisacáridos y en la degradación de azúcares. El segundo incremento, más suave, fue atribuido a dos factores: presencia de productos de degradación y disminución en la concentración de los productos de hidrólisis a lo largo del tiempo. Adicionalmente, el valor de pH mínimo fue localizado al mismo tiempo en el que el contenido de carbono en las muestras líquidas fue máximo. Este punto es importante debido a que el pH puede ser usado como indicador para seguir el comportamiento en un proceso hidrotermal identificando el tiempo de reacción necesario para alcanzar la máxima hidrólisis de hemicelulosas. Consecuentemente, el número de muestras necesarias para seguir el proceso pueden ser disminuidas reduciendo el gasto en tiempo y dinero y al mismo tiempo entender el comportamiento del sistema. El comportamiento del pH en un proceso hidrotermal puede ser beneficioso para aplicaciones a escala industrial.

La desacetilación fue acompañada por una reducción en el peso molecular de hemicelulosas. El peso molecular promedio exhibió un comportamiento similar para todas las temperaturas estudiadas: hemicelulosas con más alto peso molecular fueron extraídas a más bajos tiempos de reacción y éstos decrecieron a medida que la temperatura incrementó.

Trabajo futuro

De los estudios desarrollados en esta tesis doctoral, se puede concluir que el fraccionamiento hidrotermal de biomasa usando reactores semicontinuos es una buena opción para obtener buenos rendimientos de azúcares hidrolizados y lignina.

En todos los estudios realizados, la cantidad de productos de degradación fue baja. Si el fin de los azúcares hidrolizados es la conversión a productos de valor añadido tales como ácido láctico, 5-hidroximetilfurfural, glicolaldehído, etc, el uso de reactores semicontinuos no será adecuado ya que serían necesarios mayores tiempos de residencia de la fase líquida o mayores temperaturas. Los mayores tiempos de residencia pueden obtenerse manipulando dos variables: un aumento en el volumen del reactor o una disminución en el flujo. Estas opciones son inviables a escala industrial.

La instalación de un reactor continuo a la salida de la corriente del líquido del reactor semicontinuo puede ser una buena opción. Cantero et al. Estudió la intensificación de la hidrólisis de celulosa y biomasa usando agua en condiciones supercríticas como medio de reacción. Sus investigaciones resultaron en alta selectividad y alto rendimiento de los productos obtenidos. Este nuevo diseño de dos reactores en serie, donde en el primer reactor pueden ser obtenidos altos rendimientos de azúcares y en el segundo alta selectividad y altos rendimientos de productos de valor añadido, podría reducir el costo de equipamiento cambiando el tiempo de residencia en el segundo reactor de minutos a milisegundos.

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About the author

Florencia M. Yedro was born in Córdoba, Argentina on September 21, 1984. She graduated from high school in 2002 from San Buenaventura Institute obtaining the degree of Economy and Management of Organizations. She started the studies of Chemical Engineer at the National University of Río Cuarto in 2003 in Argentina. In March 2012, she started a M.S. in Thermodynamic Engineering of fluids at the University of Valladolid in Spain and continued with the Ph.D. Thesis in the High Pressure Processes Group of the Department of Environmental and Chemical Engineering at the University of Valladolid. In September 2014, she was in the Åbo Akademi University doing a doctoral stay during three months in the Reaction Engineering and Industrial Chemistry Department in Turku, Finland. Her Ph.D. was focused in the fractionation of biomass using subcritical water.

List of Publication

Álvaro Cabeza, Francisco Sobrón, **Florencia M. Yedro**, Juan García Serna. Modelling of biomass isothermal fractionation with subcritical water in a packed bed reactor. Fuel. 148 (2015), pp. 212–225.

Florencia M. Yedro, Juan García Serna, Danilo A. Cantero, Francisco Sobrón, M. José Cocero. Hydrothermal fractionation of grape seeds with subcritical water to produce polyphenols, sugars and lignin. Catalysis Today. doi:10.1016/j.cattod.2014.07.053

Florencia M. Yedro, Juan García Serna, Danilo A. Cantero, Francisco Sobrón, M. José Cocero. Hydrothermal extraction and hydrolysis of grape seeds to produce bio-oil. RSC Advances. 4, 30332-30339.

Submitted/ In preparation

Obtaining hemicelluloses from hardwood Holm oak (*Quercus ilex*) using subcritical water in a pilot plant.

Two-phase modelling and simulation of the hydrothermal fractionation of Holm oak in a packed bed reactor with hot pressurized water. *Chemical Engineering Science*

Hydrothermal fractionation of woody biomass: lignin effect over sugars recovery. *Bioresources Technology.*

Monitoring alternatives and main sugar products for the autohydrolysis of Holm oak hemicelluloses using pressurized hot water. *Sustainable Chemistry & Engineering*

Conference contributions

Oral presentations

Florencia M. Yedro, Juan García Serna, M. José Cocero. Monitoring the Holm oak autohydrolysis process using online parameter. 11th International Conference on Renewable Resources and Biorefineries. June 2015.

Álvaro Cabeza, **Florencia M. Yedro**, Juan García Serna. Autocatalytic kinetic model for thermogravimetric analysis of biomass and biomass fractions. 10th International Conference on Renewable Resources and Biorefineries. June 2014.

Florencia M. Yedro, Juan García Serna, M. José Cocero. Fractionation of biomass from urban trees using subcritical water. 10th International Conference on Renewable Resources and Biorefineries. June 2014.

Florencia M. Yedro, Juan García Serna, M. José Cocero. Hydrothermal treatment with subcritical water to produce bio-oils and to fractionate biomass. The example of grape seeds. 5th International Seminar on Engineering Thermodynamic of Fluids. July 2013.

Juan García Serna, **Florencia M. Yedro**, Danilo A. Cantero, María Pinilla, Francisco Sobrón, M. José Cocero. Semicontinuous fractioning of grape seed biomass in subcritical water. ANQUE´s International Congress of Chemical Engineering. June 2013.

Poster presentation

Yedro F.M.; García Serna J.; Sobrón F.; Cocero M.José. "Rationalization of analyses for the monitoring of the hydrothermal fractionation of biomass". 4th International Congress on Green Process Engineering. April 2014.

Sobrón F.; **Yedro F.M.**; García Serna J.; Cocero M.José. "Modelling the fractionation process of biomass by hydrothermal extraction". 9th International Conference on Renewable Resources & Biorefineries. July 2013.

Yedro F.M.; García Serna J.; Cocero M.José. "Fractionation of grape seeds using hot pressurised water-ethanol extraction and subcritical water hydrolysis". 9th International Conference on Renewable Resources & Biorefineries. July 2013.

Sobrón F.; **Yedro F.M.**; Cabeza A.; García Serna J. "Modeling TGA for the prediction of biomass components during conversion processes". ANQUE´s International Congress of Chemical Engineering. June 2013.

García Serna J.; **Yedro F.M.**; Sobrón F.; Cocero M.José. "Semicontinuous fractioning of grape seed biomass in subcritical water for lignin production". 8th edition of the International Conference on Renewable Resources & Biorefineries. June 2012.