

Article

Pretreatment of Hazelnut Shells as a Key Strategy for the Solubilization and Valorization of Hemicelluloses into Bioactive Compounds

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Abstract: Hazelnut industries generate a large amount of byproducts. Among them, waste hazelnut shells (which account for about 50% of the nut weight), are potential raw materials to produce value added products. Hydrothermal pretreatment enables the solubilization of hemicelluloses, while cellulose and lignin remain in the solid phase almost unaltered, allowing their subsequent processing for an integral valorization of the feedstock. When the reaction was performed at the optimal temperature (210 °C), hemicelluloses were mainly converted into soluble substituted oligosaccharides (OS). Further membrane processing of the liquid phase from hydrothermal pretreatment enabled the refining of the OS, which accounted for up to 90.87 wt% of the nonvolatile solutes (NVC) in the refined solution, which also contained 5 g of natural bound phenolics/100 g NVC. The target products showed a dose-dependent antioxidant activity, conferred by the phenolic components. Substituted OS were made up of xylose backbones with a wide degree of polymerization distribution, and showed structures highly substituted by acetyl and uronic groups. The data included in this study provide the basis for assessing the large-scale manufacture of substituted oligosaccharides with bound phenolics as bioactive components of functional use in foods, cosmetics, or pharmaceuticals.

Keywords: biorefinery; hazelnut shells; hydrothermal pretreatment; hemicelluloses; oligosaccharides; antioxidant activity

1. Introduction

The current model of industrial development, based on an intensive usage of nonrenewable fossil resources, results in a number of negative effects, including health risks and global warming. Therefore, a gradual transition into sustainable alternatives based on cleaner and renewable raw materials is imperative [1]. In this sense, the biorefinery concept, based on the selective separation of the major feedstock components into “fractions” made up of compounds with similar properties, provides a framework for the integral conversion of biomass into a wide scope of fuels, chemicals, and materials. Lignocellulose biorefineries are expected to play a key role in the sustainable development of the industrial sector in the near future, and offer a sustainable path towards a bio-based economy through an efficient conversion of feedstocks from agriculture or forestry, including agroindustrial residues [2]. It can be noted that extracting value from wastes is compatible with the “circular economy” paradigm, which can be complemented with the utilization of green processing technologies.

Hazelnut (*Corylus avellana* L.) is a fruit made up of a shell with a kernel inside, which is used as a food. *Corylus avellana* originated from the Mediterranean region, and is one of the world's major commercial nut crops. Turkey is the main producer of *Corylus avellana*, covering approximately 70 percent of the world's production, followed by Italy, China, Spain, and USA. According to Food and Agriculture Organization (FAO) [3], more than one million tons of hazelnuts (with shell) were produced worldwide in 2017. Hazelnut shells (HS) account for more than 50% of the total nut weight, becoming an important byproduct in the hazelnut industry. HS are currently employed as low-value fuel [4,5]. However, HS is an abundant and low-cost raw material with a large potential as a raw material for biorefineries, where it can yield a number of value added products [6]. Some target products reported are xylooligosaccharides [7], phenolic antioxidants [4,5], bioethanol [8], materials for composites [9], levulinic acid, char [10], and furfural [11].

Nevertheless, the immense complexity of biomass together with the polymeric nature of its structural components makes fractionation almost inevitable. In this sense, decreasing the recalcitrance of the lignocellulosic matrix through cost effective and environmentally friendly technologies is a crucial objective [1]. Pretreatment is an essential processing step that entails the deconstruction of the lignocellulosic biomass by the disarray of lignin and the exposure of polysaccharides (cellulose and hemicelluloses) to hydrolytic reactions [12].

A number of biorefinery pretreatments are proposed in literature [2,13]. Some of them are used for obtaining products with industrial interest from HS as feedstock: hydrothermal [5,7,14], acid [15], alkaline [15,16] or the double step acid/alkaline pretreatments [17], oxidative (i.e., ozonolysis) [18], organosolv [19], or the combined effect of delignification pretreatments before acid hydrolysis [8].

Hydrothermal processing (also called autohydrolysis) is a pretreatment in which biomass is subjected to reaction with compressed hot water, enabling an extensive solubilization of hemicelluloses [20]. The reaction is catalyzed by hydronium ions resulting from water autoionization and in situ generated organic acids (mainly acetic acid coming from the hydrolysis of acetyl groups in hemicelluloses). As a result, hemicelluloses are depolymerized into low molecular weight polysaccharides, oligosaccharides (OS), monosaccharides, and minor amounts of other products. Additionally, the hydrothermal pretreatment increases the surface area and decreases the crystallinity of cellulose, resulting in an improved susceptibility toward the enzymatic hydrolysis. Additionally, lignin can be solubilized in subsequent processing stages.

In HS, hemicelluloses account for 24.6–30 wt% of the raw material. The major hemicellulose constituent is heteroxylan, made up of a backbone of linked xylose units substituted with acetyl and uronic groups, and bound phenolic compounds [5]. Consequently, optimal conditions of hydrothermal processing can result in relevant proportions of hemicellulose-derived xylooligosaccharides (XOS), which can be obtained at a reasonable yield and purity [21].

The implementation of an additional refining stage is necessary when high purity XOS are required, i.e., food-grade OS [22]. This point is important when OS are produced by hydrothermal pretreatment, because a variety of byproducts (monosaccharides, acetic acid, sugar degradation products, extractives, or acid soluble lignin) can be present in the reaction media. Membrane technology is an interesting alternative for XOS purification—the size-dependent separation achieved leads to concentrated solutions of purified XOS, while low molecular weight contaminants are removed in permeate. Some examples of XOS refining by membrane processing were reported for different agricultural residues, i.e., rice husks [23], peanut shells [24], and almond shells [25,26].

XOS are potential prebiotics, defined as nondigestible food ingredients that allow specific changes in both the composition and activity of the gastrointestinal microbiota, as stimulation of the growth of probiotic bifidobacteria and lactic acid bacteria, conferring benefits in the human health [27]. Some beneficial effects described in literature including the maintenance of the human health, the prevention of diseases, and the decreased risk of chronic diseases [22].

XOS containing esterified phenolic compounds are natural antioxidants, with potential applications in food, cosmetic, pharmacy, and nutraceutical industries. This type of XOS is considered as emerging prebiotics [7], and antioxidant activity [28] is derived from the presence of bound phenolics [27]. Therefore, this type of XOS could contribute to satisfying the increasing demand for ingredients of functional foods, whose demand is expected to reach more than 440 billion USD in 2022 [27].

In this work, hydrothermal pretreatment of waste HS was proposed as an initial step of a multistage process allowing the complete utilization of HS. This step aimed at the solubilization of hemicelluloses, leaving a treated solid made up of cellulose and lignin that could be valorized by further processing. HS were processed at different temperatures, and the solid and liquid phases were assayed for composition and yields to allow the formulation of material balances. The liquid phase from hydrothermal processing performed under optimal reaction was refined by membrane processing to yield a final product meeting the purity degree required for commercial food grade OS. The purified OS were assayed for monomeric constituents and structural features. Additionally, the total phenolic content and the *in vitro* antioxidant properties were considered to assess the potential of OS as ingredients for functional foods.

2. Materials and Methods

2.1. Raw Material

Hazelnuts were purchased in a local market (Ourense, Spain). HS were milled, sieved to obtain a particle size between 0.250–1 mm, homogenized, and stored.

2.2. Hydrothermal Pretreatment (Autohydrolysis) of Hazelnut Shells

HS samples were treated in a 600 mL stainless steel reactor (Parr Instrument Company, Moline, IL, USA). The raw material was mixed with distilled water at a liquid to solid ratio of 10 kg water/kg of dry HS, heated under nonisothermal conditions up to reach the target temperature (in the range 190–225 °C), and then cooled by circulating water through an internal loop. Figure 1 shows the heating and cooling profiles that provide the reaction time to reach the temperatures of the experiments performed in this study. At the end of the treatments, liquid and solid phases were separated by vacuum filtration. Solids were washed with distilled water, and air dried. The solids from hydrothermal pretreatment (autohydrolyzed solids, AS) and autohydrolysis liquors (AL) were characterized for moisture, solid yield, and composition, as described in Section 2.4.

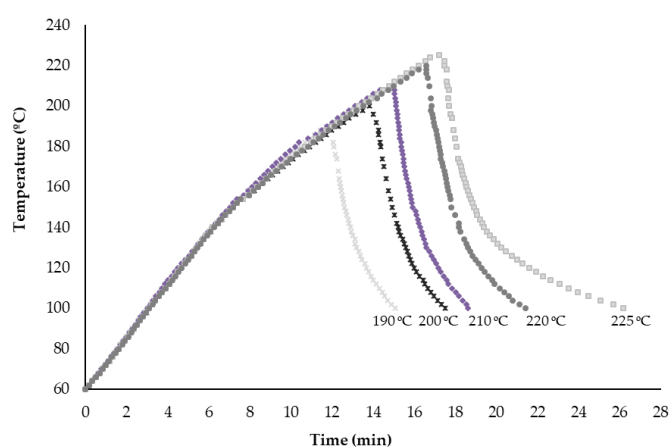


Figure 1. Heating and cooling profiles obtained for the autohydrolysis experiments performed at the assayed temperatures.

2.3. Refining of Oligosaccharides by Membrane Processing

AL obtained under optimal conditions for OS production was refined by discontinuous diafiltration (DD). Assays were performed in a stirred Amicon cell (Millipore) operating at a transmembrane pressure of 3.5 bar and room temperature. Water was added to AL at 2:1 volume ratio, diafiltered through a 0.3 kDa cutoff membrane (GE Osmonics Inc., Minnetonka, MN, USA) up to achieve a retentate volume equal to one of the feed. Samples from retentate and permeate were assayed for composition (using the same methodology as described in Section 2.4) and structural features.

2.4. Analytical Procedures

The moisture contents of HS and AS were assayed according to the T-264-cm-97 standard method [29]. The T-249-cm-85 method [30] was employed for measuring the contents of structural carbohydrates and Klason lignin. This method consisted of a two step quantitative acid hydrolysis performed with 72% and 4% H_2SO_4 , respectively. The method led to an insoluble lignin residue (Klason lignin) and to a liquid phase. Samples from the liquid phase were filtered through 0.45 μm cellulose acetate membranes and analyzed by High Performance Liquid Chromatography (HPLC) using an Agilent 1200 series instrument (Agilent Technologies, Santa Clara, CA, USA), fitted with a refraction index detector (RID). Samples were assayed for monosaccharides (glucose, xylose, and arabinose), organic acids (acetic acid), and furans (furfural and hydroxymethylfurfural) using a 300 $\times$ 7.8 Aminex HPX-87H column (BioRad Life Science Group, Hercules, CA, USA) kept at 50 $^\circ\text{C}$ and eluted with 0.003 N H_2SO_4 at a 0.6 $\text{mL}\cdot\text{min}^{-1}$ flow rate. The ash content was assayed according to the T-211-om-02 method [31].

Samples of AL were filtered through 0.45 μm cellulose acetate membranes and assayed by HPLC as described above. Aliquots of AL were subjected to quantitative posthydrolysis (4% of H_2SO_4 at 121 $^\circ\text{C}$ for 20 min). The increases in the concentrations of monosaccharides and acetic acid caused by posthydrolysis provided the measure of oligomers concentration and their degree of substitution with acetyl groups. The content of total nonvolatile compounds (NVC) was measured by oven-drying samples at 105 $^\circ\text{C}$ until constant weight. All the analytical determinations were performed in triplicate.

Uronic acids were determined spectrophotometrically at 520 nm, using galacturonic acid as a standard [32].

High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAED–PAD) was used to assess the types of oligomers present in the reaction media. The analyses were performed using an ICS300 instrument (Dionex, Sunnyvale, CA, USA) equipped with a 250 $\times$ 2 mm CarboPac PA-1 column in combination with a 25 $\times$ 2 mm CarboPac PA guard column [33]. Matrix-Assisted Laser Desorption and Ionization Time-of-Flight Mass Spectrometry (MALDI TOF–MS) analyses were employed to allow a detailed structural characterization of the oligomers contained in AL. Assays were performed using an Autoflex III smartbeam instrument (Bruker Daltonics, Bremen, Germany) operating in linear positive ion mode. Spectra were acquired and treated using the Flex Control 3.0 and Flex-Analysis 3.0 software (Bruker Daltonics), respectively [34]. Commercial XOS (degree of polymerization, DP 2–6) from Megazyme (Wicklow, Ireland) were used as standards for HPAED–PAD and MALDI TOF–MS analyses.

The total phenolic content (TPC) was determined using the Folin–Ciocalteu assay [35], and the results are expressed as Gallic Acid Equivalents (GAE). The determination of antioxidant activities was carried out using the 2,2-diphenyl-1-picrylhydrazyl assay ((DPPH) [36], Trolox-Equivalent Antioxidant Capacity assay (TEAC) [37], and Ferric Reducing Ability of Plasma assay (FRAP) [38] methods. Identification of phenolic compounds was carried out by High Performance Liquid Chromatography with Diode Array Detection (HPLC–DAD) [39].

3. Results and Discussion

3.1. Composition of HS

The average composition of HS, listed in Table 1, was the same reported in a previous work performed with the same HS lot [5]. The major structural component of HS was lignin (40.08 wt% in oven-dry basis). Higher lignin contents, in the range 46–51.3 wt%, were reported for the same substrate [7,40,41]. Hemicelluloses accounted for 32.28% of the dry mass of HS, and were mainly constituted by xylan (22.69%), followed by uronic and acetyl substituents (overall content, 8.93%). Minor amounts of arabinan were also detected. Aydinli and Caglar (2012) [42] reported 28.9% of hemicelluloses, a value markedly higher than the 18.7% reported by Surek and Buyukkileci (2017) [7]. The glucan content of HS accounted 26.49% of the raw material, in the range reported by Aydinli and Caglar (2012) [42], and considerably higher than the 18.7% determined by Surek and Buyukkileci (2017) [7].

Table 1. Composition of HS expressed as g of component per 100 g of oven-dried raw material. The values are reported as the average of triplicate measurements $\pm$ standard deviation.

Component	Content (g/100 g of Dry HS)	
Glucan	26.49	± 0.26
Xylan	22.69	± 0.23
Arabinan	0.65	± 0.01
Acetyl groups	4.37	± 0.04
Uronic groups	4.56	± 0.17
Klason Lignin	40.08	± 0.21
Ash	0.78	± 0.05
Total Identified (%)	98.85	

3.2. Hydrothermal Pretreatment

The hydrothermal pretreatments were carried out under nonisothermal conditions up to reach temperatures in the range 190–225 °C. Once the target temperatures were reached, the reaction media were cooled immediately and filtered. The aqueous phases contained hemicellulose-derived oligomers, monosaccharides, sugar decomposition products, and acetic acid. The generation rate of these compounds depended on the severity of the autohydrolysis conditions [20,43,44], here measured by the maximal temperature.

Table 2 shows data regarding the effects of the hydrothermal pretreatments performed at diverse temperatures on both the solid yield and the composition of liquid phases. The solid yield decreased steadily with temperature, a trend that was more marked up to 210 °C. This behavior can be explained because increasing temperatures promote the progressive solubilization of xylan and acetyl and uronic groups in hemicelluloses. Although arabinan also makes part of hemicelluloses, its practical importance is limited owing to the low contents. The progressive removal of hemicelluloses from the raw material led to the production of AS with increased glucan and lignin content. At the highest temperature assayed (225 °C), the content of both lignin and glucan accounted for 93.13% of AS. The glucan and lignin contents of AS varied in the ranges of 25.47%–38.70% and 41.08%–54.43%, respectively, corresponding to recovery yields of glucan and Klason lignin in solid phase higher than 90% and 85%, respectively. These results are favorable for the subsequent processing of AS by other methods, thus enabling the integral valorization of the raw material [2,13,20].

Table 2. Solid yields (measured as g of oven-dry AS/100 g of oven-dry HS) and composition of AS (measured as g component/100 g of oven-dry AS) obtained in experiments performed up to the desired temperatures. The results are reported as the average of triplicate measurements $\pm$ standard deviation.

Temperature (°C)	Solid Yield (g of AS/100 g of Dry HS)	Composition (g of Component/100 g of AS)						
		Glucan	Xylan	Arabinan	Acetyl Groups	Uronic Groups	Klason Lignin	Others (by Difference)
190	88.92	25.47 $\pm$ 0.16	20.7 $\pm$ 0.09	0.16 $\pm$ 0.02	3.96 $\pm$ 0.26	3.34 $\pm$ 0.03	41.08 $\pm$ 0.15	5.29
200	78.5	31.42 $\pm$ 0.19	15.44 $\pm$ 0.64	0.09 $\pm$ 0.00	3.01 $\pm$ 0.05	2.31 $\pm$ 0.13	43.90 $\pm$ 0.22	3.92
210	70.85	33.67 $\pm$ 0.21	9.70 $\pm$ 0.04	0.08 $\pm$ 0.00	1.76 $\pm$ 0.06	2.12 $\pm$ 0.22	49.65 $\pm$ 0.16	3.02
220	66.23	37.51 $\pm$ 0.19	6.66 $\pm$ 0.18	0.00	1.20 $\pm$ 0.10	0.64 $\pm$ 0.04	52.14 $\pm$ 0.21	1.86
225	64.84	38.70 $\pm$ 0.41	4.62 $\pm$ 0.33	0.00	0.71 $\pm$ 0.04	0.48 $\pm$ 0.02	54.43 $\pm$ 0.39	1.05

Table 3 lists the results determined for the composition of the reaction media in the same set of experiments. When the hydrothermal pretreatments were carried out at temperatures below 210 °C, the concentration of nonvolatile compounds (NVC) increased up to 26.46 g/L. This concentration remained fairly constant in the range 210–220 °C, and dropped at the highest temperature assayed. The data are expressed in terms of the identified NVC (INVC), calculated as the joint contributions of OS and monosaccharides. XOS, the most abundant components in AL, reached their highest concentration (16.24 g/L, accounting for 73.67% of the xylan present in the feedstock) at 210 °C. Harsher conditions resulted in decreased XOS concentrations, owing to the generation of xylose (and furfural under the most severe conditions assayed). Arabinoooligosaccharides (ArOS) were only found (in little concentrations) in assays performed under mild conditions. The corresponding monomer (arabinose) reached its highest concentration (0.45 g/L) at 210 °C. Glucoooligosaccharides (GOS) and glucose also reached limited concentrations, revealing the solubilization of a small fraction of glucan under the most severe conditions. Concerning the substituents, the concentration of acetyl groups (AG) bound to OS reached concentrations up to 3.55 g/L at 220 °C (corresponding to 77.05% of the amount present in the feedstock), whereas the maximal concentrations of uronic groups linked to OS (U) were found at a milder temperature (200 °C). From the results shown in Table 3, it can be calculated that the maximum concentration of substituted OS (including GOS, XOS, ArOS, AG, and U) was achieved at 210 °C, and reached 20.49 g/L. The concentrations of monosaccharides (maximum value, 2.68 g/L achieved at 220 °C) were comparatively low.

Table 3. Concentrations of products present in the liquid phase of hydrothermal treatments performed at temperatures ranging from 190 to 225 °C. Data are expressed in g/L.

		Temperature (°C)				
		190	200	210	220	225
INVC	Glucose	0.06	0.08	0.12	0.02	0.08
	Xylose	0.15	0.31	1.44	2.06	2.32
	Arabinose	0.17	0.25	0.45	0.34	0.28
	GOS	0.14	0.11	0.11	0.09	0.20
	XOS	3.18	9.35	16.24	15.71	14.33
	ArOS	0.22	0.17	0.01	0.00	0.00
	AG	0.74	2.24	3.09	3.55	3.32
	U	1.39	1.12	1.07	0.94	0.89
	Others *	2.48	3.15	3.92	3.75	4.41
	Total NVC	8.51	16.78	26.46	26.45	25.82
VC	Acetic acid	0.20	0.36	0.90	1.42	2.08
	Furfural	0.02	0.01	0.20	0.40	0.81
	HMF	0.00	0.00	0.01	0.02	0.03

GOS: glucoooligosaccharides; XOS: xyloooligosaccharides; ArOS: arabinoooligosaccharides; AG, acetyl groups linked to oligosaccharides; U: uronic acid linked to oligosaccharides; HMF: hydroxymethylfurfural. NVC: total nonvolatile compounds; INVC: identified nonvolatile components; VC: volatile compounds. * Others: measured as the difference of the total NVC and the INVC.

The concentrations of volatile compounds (VC) increased smoothly up to 210 °C, and then increased markedly as a result of reactions taking place under severe conditions (for example, cleavage of AG into acetic acid and furfural generation from pentoses).

3.3. Refining of Oligosaccharides by Membrane Treatment

Based on the results discussed above, 210 °C was selected as the optimal temperature because this experiment (denoted AL210) led to the highest concentration of substituted OS. However, unwanted compounds (including monosaccharides or nonsaccharide compounds) were also present in the liquid phase, and could limit the application of the NVC fraction in the food, cosmetic, or pharmaceutical industries. OS refining can be achieved using a number of separation techniques, including solvent extraction [21,45], adsorption [23,33], chromatography [45], and membrane filtration [21,23,25–27]. Membrane processing is currently seen as the most promising technology for the industrial manufacture of high purity, owing to the low energy requirements, easy manipulation of operational variables, and relatively easy scale up [25,45].

Figure 2 shows the scheme of the purification method used in this work, including data regarding the chemical characterization of streams and material balances. The solution AL210 (content of total OS, 20.49 g/L, accounting for 77.41% of the NVC) was employed as a feed. This stream also contained some unwanted components that should be removed, including monosaccharides (2.01 g/L), ONVC (3.97 g/L), and VC (1.11 g/L). The feed solution was refined by DD, which led to a retentate (containing 17.44 g NVC/L) and permeate (NVC concentration, 4.02 g/L). As expected, the retentate showed an increased proportion of nonvolatile solutes corresponding to OS (90.87 g/100 g of NVC, in comparison to 77.41 g OS/100 g of NVC determined for the feed solution AL210). This finding confirmed the suitability of membrane processing for OS purification, keeping a good balance between the concentrations of the target products in retentate (15.85 g/L) and in permeate (1.76 g/L). It can be noted that most unwanted compounds were present in permeate (i.e., 1.63 g ONVC/L and 0.63 g monosaccharides/L).

The available data allow the comparison of the molar ratios of oligomer components (XOS:AG:U) between the feed and the retentate (1:0.47:0.05 and 1:0.43:0.04, respectively). The molar ratio XOS:AG was slightly higher than the one reported in literature for autohydrolysis liquors from hazelnut shells [7]. A comparative molar ratio XOS:AG of 1:0.56 was also reported for membrane processing of autohydrolysis liquors from peanut shells [24].

AL210 also contained VC (1.11 g/L), a fraction mainly made up of acetic acid (0.90 g/L) and minor amounts of HMF and furfural. The VC concentration decreased considerably in the retentate (0.29 g/L) relative to the feed content.

From the above data, it can be concluded that DD of AL210 allowed a selective recovery of substituted OS in retentate, in which the target products accounted for 90.87% of the NVC fraction, while most ONVC, monosaccharides, and VC were removed in the permeate. This finding is in agreement with the results reported in literature [24], with an increasing purity of oligomers from autohydrolysis liquors of peanut shells from 55.70% up to 72.4% using DD. In a related study, Singh et al. (2019) [26] obtained XOS of low degree of polymerization from almond shells using enzymatic treatments and membrane assisted refining. To our knowledge, no previous studies reported on the membrane refining of HS-derived OS.

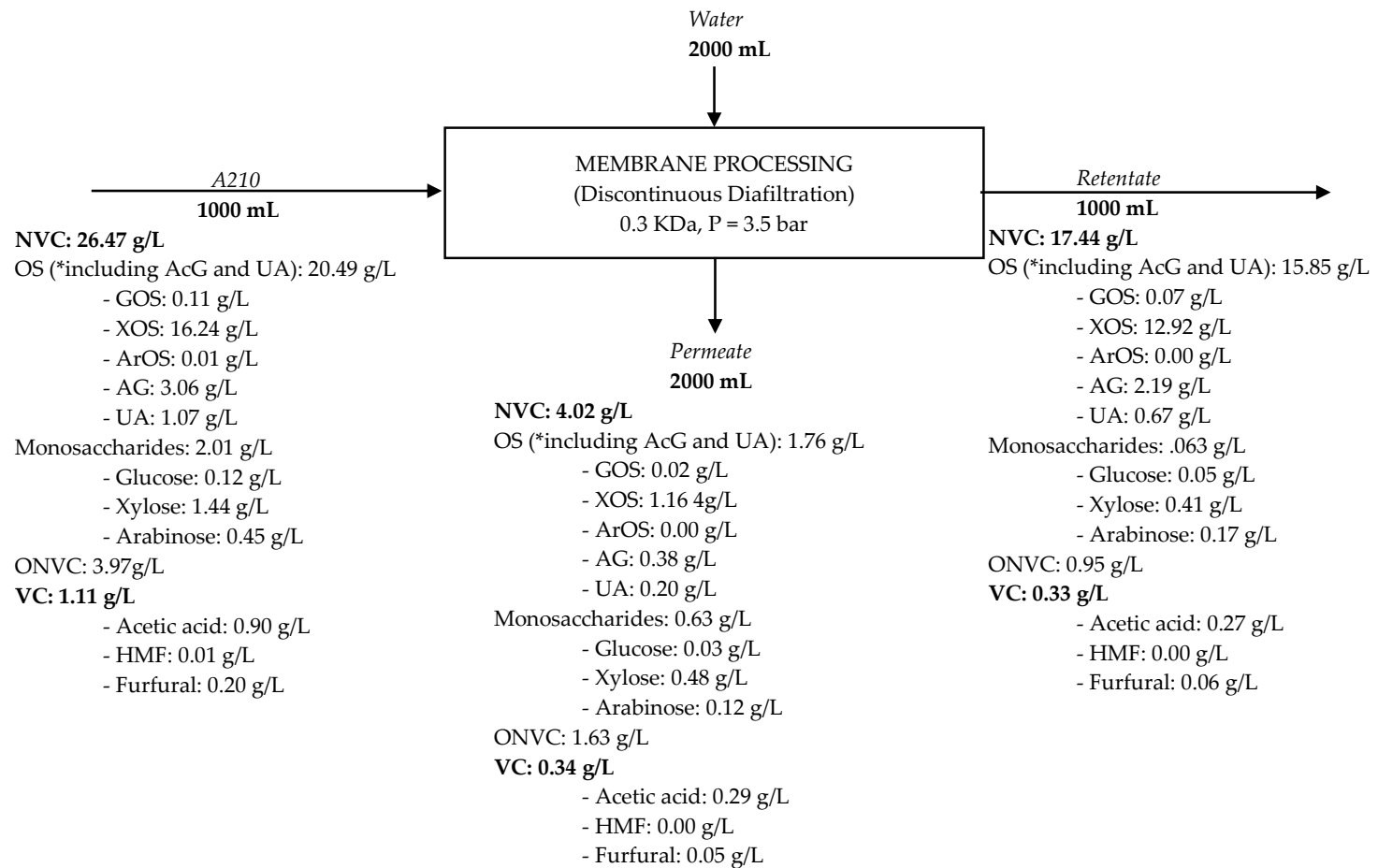


Figure 2. Diagram of membrane processing and composition of the involved streams: AL210, retentate, and permeate.

3.4. Structural Characterization of OS

Figure 3 shows the HPAEC–PAD elution profiles corresponding to AL210 and retentate. Data concerning commercial XOS in the range 2–6 are also included for comparison. It must be noted that the alkaline mobile phase used for HPAEC–PAD analysis caused the saponification of AG. Because of this, this technique provided useful information about the DP distribution of the oligomers, but not about the substitution pattern [33,43]. The elution profiles of AL210 and retentate showed similar patterns for compounds with DP > 3, peaks of oligomers with the same size. Oppositely, the peaks observed for DP2 and DP3 compounds were smaller in the case of retentate. This finding is in agreement the high recovery of XOS in the retentate, as discussed above.

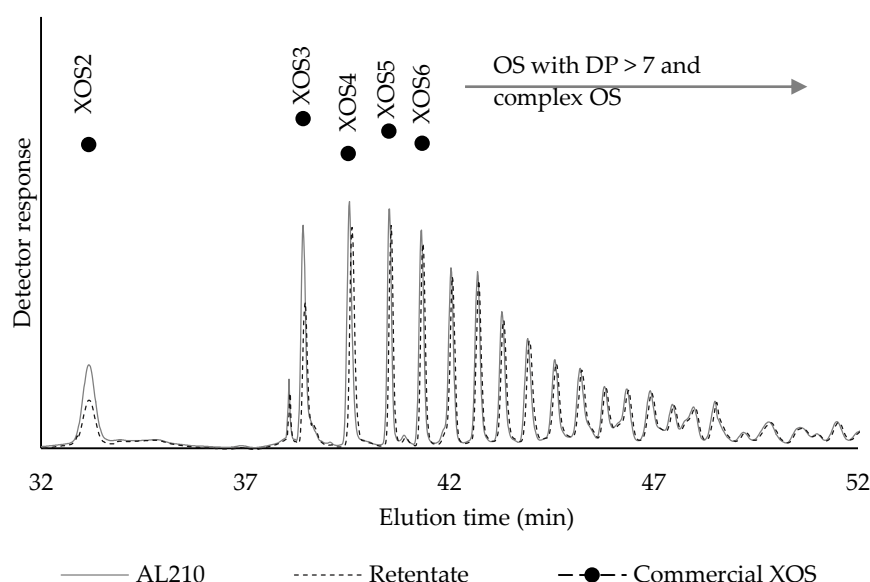


Figure 3. HPAEC–PAD elution profiles for the autohydrolysis liquors at 210 °C and retentate.

Additional information on the structures of oligomeric saccharides contained in retentate was obtained using MALDI–TOF MS (see Table 4). This technique is a powerful tool widely used for carbohydrate characterization, as oligomers can be investigated with minimal fragmentation [45,46]. The experimental data allowed the assessment of the pentose chain lengths, as well as their substitution pattern. The data reported correspond to sodium and potassium adducts. As 2,5 dihydroxybenzoic acid (DHB) was used in the analyses as a matrix, the components with $m/z < 500$ could not be identified with this method [23,24,34].

Based on the compositional information shown in Figure 2, the OS in the retentate were constituted mainly by pentose units. Based on the HPLC data obtained for arabinose and xylose, it can be concluded that the pentoses in OS backbones corresponded to xylose. The pentose chains were highly substituted by acetyl groups (AG) and/or *O*-methylglucuronic groups (U), following the patterns [mP nAG] or [mP nAG oU]. The spectra confirmed the presence of OS composed by pentose chains with DP in the range 3–16, containing up to 6 AG groups, and 1 or 2 U groups. These results are in agreement with the compositional data and with the molar ratios XOS:AG:U discussed above.

Table 4. OS structures in the retentate stream identified by MALDI–TOF MS.

<i>m/z</i>	Structure	<i>m/z</i>	Structure	<i>m/z</i>	Structure
537.14	3P 2AG+K ⁺	1133.37	7P 4AG+Na ⁺	1529.54	7P 4AG U+K ⁺ /10P 4AG+Na ⁺
627.19	4P AG+K ⁺	1149.33	6P 3AG U+Na ⁺	1545.51	9P 3AG U+Na ⁺
653.23	4P 2AG+Na ⁺	1191.36	6P 4AG U+Na ⁺	1587.53	10P 5AG+K ⁺ /9P 4AG U+Na ⁺
669.19	3P AG U+Na ⁺	1197.37	8P 2AG+K ⁺ /7P AG U+Na ⁺	1629.55	10P 6AG+K ⁺
711.20	4P 3AG+K ⁺	1207.34	5P 3AG 2U+Na ⁺	1635.59	11P 3AG+K ⁺
801.23	5P 2AG+K ⁺ /4P AG U+Na ⁺	1223.42	8P 3AG+Na ⁺	1645.55	9P 5AG U+K ⁺
843.24	5P 3AG+K ⁺ /4P 2AG U+Na ⁺	1233.36	7P 6AG+K ⁺	1661.60	8P 4AG U+K ⁺
885.25	5P 4AG+K ⁺	1239.37	7P 2AG U+Na ⁺	1677.59	10P 3AG U+Na ⁺
901.25	4P 3AG U+K ⁺	1265.42	8P 4AG+Na ⁺	1719.59	10P 4AG U+Na ⁺
917.33	6P 2AG+Na ⁺	1281.39	7P 3AG U+Na ⁺	1809.65	11P 3AG U+Na ⁺
933.26	5P AG U+Na ⁺	1297.39	7P 3AG U+K ⁺	1851.63	11P 4AG U+Na ⁺
959.31	6P 3AG+Na ⁺	1323.41	8P 5AG+K ⁺	1941.70	13P 5AG+K ⁺
9752.70	5P 2AG U+Na ⁺	1339.41	7P 4AG U+Na ⁺	1983.67	12P 4AG U+Na ⁺
991.28	4P AG 2U+Na ⁺	1371.43	9P 3AG+K ⁺ /8P 2AG U+Na ⁺	1993.65	11P 6AG+K ⁺
1017.29	6P 4AG+K ⁺ /5P 3AG U+Na ⁺	1381.41	7P 5AG U+K ⁺	2067.69	13P 6AG+K ⁺
1033.29	5P 3AG U+K ⁺	1397.48	9P 4AG+Na ⁺	2115.74	14P 5AG+K ⁺
1059.31	5P 4AG U+Na ⁺	1413.45	8P 3AG U+Na ⁺	2199.70	14P 6AG+K ⁺
1065.31	7P 2AG+K ⁺ /6P AG U+Na ⁺	1455.46	9P 5AG+K ⁺	2289.72	14P 5AG U+Na ⁺
1075.30	5P 4AG U+K ⁺	1471.46	8P 4AG U+K ⁺	2331.71	15P 6AG+K ⁺
1091.36	7P 3AG+Na ⁺	1503.50	10P 3AG+K ⁺ /9P 2AG U+Na ⁺	2421.73	15P 5AG U+Na ⁺
1107.32	6P 2AG U+Na ⁺	1513.47	7P 4AG 2U+Na ⁺	2463.70	16P 6AG+K ⁺

P: pentoses; AG: acetyl groups; U: uronic groups. Na⁺: sodium; K⁺: potassium.

3.5. Total Phenolic Content and Antioxidant Activity

Pérez-Armada et al. (2019) [5] indicated that HS show a great potential as a source of natural antioxidants. These authors solubilized a part of the phenolics in HS by autohydrolysis, and recovered the target products using polymeric resins. In our work, the retentate from DD was subjected to acid hydrolysis to release a number of valuable compounds, including gallic, vanillic, and p-coumaric acids; aldehydes such as vanillin; and flavonoids such as catechin and (-) epicatechin.

Figure 4 shows the results achieved for the three streams involved in the membrane processing. The methods employed for this purpose included the total phenolic content (TPC, expressed as gallic acid equivalents/L or GAE/L), antioxidant activities determined using the methods TEAC (measured as g Trolox/L), FRAP (measured as g FeSO₄·7H₂O/L), and DPPH (measured as g GAE/L needed for EC₅₀) [35–38].

The composition of phenolics (TPC) in AL210 was 1.63 g GAE/L, which is in the range reported for AL of other biomasses, i.e., eucalypt (1.64–1.98 g GAE/L) [47], vine shoots (1.33 g/L) [48], or peanut shells (1.58 g/L) [24]. The TPC of retentate decreased by 53.74% relative to the AL210 stream, and followed a behavior in agreement with reported literature concerning OS purification by membrane processes [23,24,27]. The TPC in the retentate accounted for 0.87 g/L, which implies a contribution of 5.00% relative to the NVC (17.44 g/L).

Despite the decreased phenolic content of retentate, the presence of phenolics is interesting due to their antioxidant activity, as these provide additional value to the target products as functional food ingredients [23,24,27]. According to the data in Figure 4, AL210 showed acceptable FRAP, TEAC, and DPPH activities. Interestingly, the DPPH radical scavenging capacity was not affected by DD, leading to the same EC₅₀ value in feed and retentate. On the contrary, the ABTS radical scavenging activity determined by the TEAC assay dropped by 58.19% in retentate relative to the value measured for AL210. However, this antioxidant activity is still an interesting contribution to the functional properties of the retentate.

The assays based on radical scavenging reactions frequently show a dose-dependent response [23,49,50]. The data in Figure 5 show that the DPPH assay presented this type of behavior in the AL210 and retentate streams.

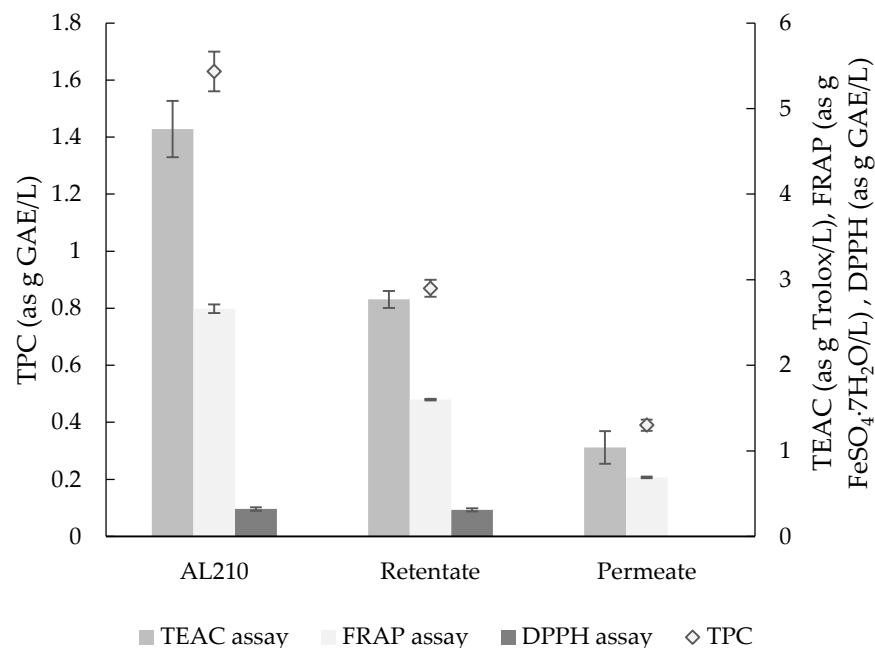


Figure 4. Total phenolic content (TPC) and antioxidant activity determined by TEAC, DPPH, and FRAP assays of AL210, retentate, and permeate. TPC, expressed as g GAE/L; TEAC, as g Trolox/L; FRAP, as g FeSO₄·7H₂O/L; DPPH (EC₅₀), as g GAE/L.

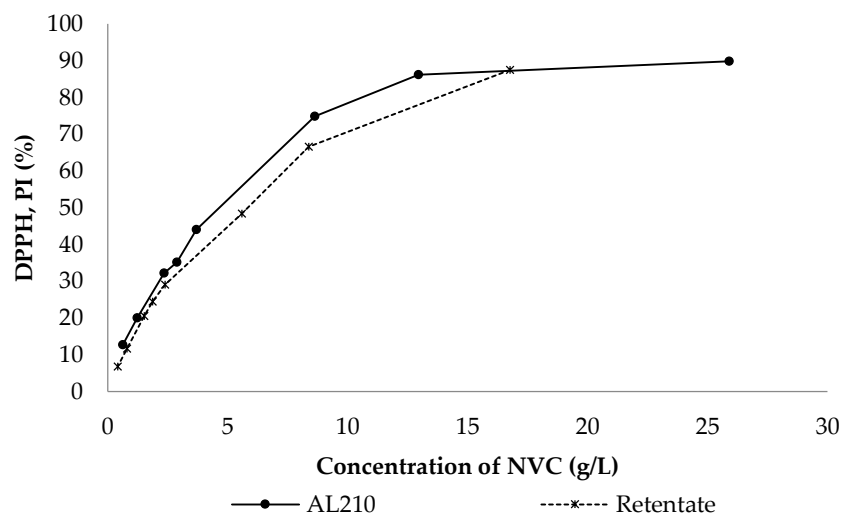


Figure 5. Effects of the concentration on the DPPH radical scavenging activity, expressed as percentage of inhibition, PI (%), for autohydrolysis liquors at 210 °C (AL210) and retentate.

In general terms, the data obtained in this work for antioxidant activities follow general trends similar to the ones reported in studies dealing with other lignocellulosic feedstocks (such as rice husks, pine and eucalypt woods, peanut shells or almond shells) [23,24,26].

4. Conclusions

This study deals with the hydrothermal pretreatment (autohydrolysis) of HS, conceived as the first step of an overall utilization of this feedstock in the scope of biorefineries. Operating at the temperature considered as optimal (210 °C), most hemicelluloses were broken down into soluble fragments, appearing in liquid phase as oligosaccharides. Unwanted, nonsaccharide products present in the liquid phase from autohydrolysis treatments were removed by DD. The target products (refined OS present in the retentate) accounted for 90.87% of the NVC fraction. OS presented a wide DP distribution, and the backbones were made up of xylose structural units substituted with acetyl and uronic groups. Phenolics bound to OS accounted for 5% of the total NVC, and showed dose-dependent antioxidant activity. The phenolics identified included gallic, vanillic, and *p*-coumaric acids; vanillin; and catechin. These results confirm the potential of the target products obtained in this study for applications in a number of fields, including the food, cosmetic, and pharmaceutical industries.

The autohydrolysis stage also produced solids containing up to 93.13% of glucan and Klason lignin, which represented recovery yields of these components above 90% and 85%, respectively. These data confirm the interest of hydrothermal processing as a first stage of an integrated process for the global valorization of HS.

Author Contributions: S.R. and A.M. designed the research. S.R. performed experiments and analyzed the data. S.R. and J.C.P. prepared the original draft. All authors discussed the data. All authors have read and agreed to the published version of the manuscript.

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