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A biorefinery strategy for the manufacture and characterization of oligosaccharides and antioxidants from poplar hemicelluloses

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ABSTRACT

A combination of biorefinery processes (hydrothermal and membrane processing) was performed for the manufacture of oligosaccharides and natural phenolic compounds from poplar hemicelluloses. Both, oligosaccharides and phenolics have multiple potential applications related to their bioactive properties. Firstly, non-isothermal autohydrolysis of poplar was performed to obtain oligosaccharides from the hemicellulosic fraction, while cellulose and lignin remained almost unaltered in the spent solid allowing their valorization in further processing stages. Operating at 210 °C and liquid to solid ratio of 6 kg/kg, solutions containing 28.71 g oligosaccharides/L were obtained. Secondly, membrane refining of the resulting solution and further freeze-drying resulted in a product with high purity (90 kg of substituted oligosaccharides/100 kg of non-volatile compounds). The yield of substituted oligosaccharides in the target product accounted 16.6 kg/100 kg of poplar wood. Product characterization was accomplished by HPLC, HPSEC, HPAEC-PAD and MALDI TOF-MS. The data confirmed the presence of backbones containing up to DP13 pentose units, with a complex substitution pattern by acetyl groups and *O*-methyl-uronic moieties. Additionally, the presence of natural phenolic compounds bounded to oligosaccharides conferred antioxidant properties to the target product. Substituted oligosaccharides and phenolics are postulated as valuable products with interest in food and pharmaceutical industries.

1. Introduction

The research on the production of value added products from biomass is booming, due to the eco-friendly nature and cost-effectiveness of the feedstock. Woody biomass is a feedstock of choice for the industry, as it does not compete with food applications (Naik et al., 2010; Singh et al., 2017). Woods show important advantages over other biomasses (such as herbaceous or agriculture residues), including the higher density (that facilitates logistics and transportation), and the year-round harvesting capability (Zhu et al., 2019).

Poplar wood (PW) is a favorable feedstock for chemical processing, due to its short rotation, high biomass yield, and favorable cell-wall chemistry (Sannigrahi et al., 2010; Rohde et al., 2018). PW utilization can be achieved following the biorefinery concept, based on the selective separation of the main feedstock components (cellulose, hemicelluloses and lignin), and their subsequent individual valorization (Hou et al., 2014; Geng et al., 2018). In this sense, the selection of

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an adequate technology for the fractionation of biomass is a crucial step, since it controls the products to be obtained. A number of methods have been used for this purpose, including hydrothermal processing and treatments based on the utilization of acids, alkalis, oxidants, organic solvents, and ionic liquids (Carvalheiro et al., 2008; Kumar et al., 2020). Autohydrolysis (AH) is a cost effective and eco-friendly hydrothermal technology based on the utilization of hot, compressed water as the sole fractionation agent. AH minimizes corrosion problems, and allows the selective separation of the hemicellulosic fraction from native lignocellulosic materials (including woods) (Carvalheiro et al., 2008, Qing et al., 2013). When performed under suitable conditions, AH results in extensive solubilization of hemicelluloses, leading to the formation of oligosaccharides as major reaction products, while most cellulose and lignin remain in the solid phase with little alteration (Garrote et al., 2002; Yáñez et al., 2009; Rigual et al., 2018).

Hardwood hemicelluloses, including those of poplar species, correspond mostly to heteroxylan, a polymer consisting of a backbone of β-1, 4-linked xylose units substituted with acetyl and O-methylglucuronic acid groups, with bonded phenolic residues (Sannigrahi et al., 2010; Yuan et al., 2010; Singh et al., 2015). The hemicellulose hydrolysis reactions happening in the AH media under typical conditions result in the formation of low molecular weight polysaccharides and oligosaccharides (OS) as major reaction products, together with monosaccharides and non-saccharide-products (including compounds derived from extractives and lignin) (Rico et al., 2018). The soluble saccharides derived from hardwood hemicelluloses can be used for a number of applications, some of them related to their prebiotic and antioxidant properties. The prebiotic properties are related to their non-digestible character, whereas the antioxidant activity is conferred by esterified phenolic residues (Ebringerová et al., 2008; Rivas et al., 2013; Singh et al., 2015).

The International Scientific Association for Prebiotics (ISAPP) updated in 2016 the definition of prebiotics to a "substrate that is selectively utilized by host microorganisms conferring a health benefit", on the understanding that the host can be human or animal (Gibson et al., 2017). The prebiotic effect of xylooligosaccharides (XOS) has been demonstrated, as well as their remarkable potential as food ingredients, based on economic competitiveness, stability to heat and pH, favorable organoleptic properties, and multieffect on human health and livestock (Amorim et al., 2019). Recently, process integration strategies for greener and more efficient production of prebiotics from costless lignocellulosic residues have been reviewed (Amorim et al., 2019). In this field, the prebiotic character of hardwood-derived OS has been established (Gullón et al., 2011; Gullón et al., 2014; Singh et al., 2015).

The phenolic compounds bound to OS represent an attractive resource of natural antioxidants. Singh et al. (2015) reviewed the antioxidant activity of xylan-derived saccharides from a number of substrates, including wheat bran, wheat stalk, corncob and sugarcane bagasse. The presence of natural phenolic compounds with antioxidant activity in hemicellulosic OS was also confirmed for different hardwoods (Rivas et al., 2013; Renault et al., 2014; Singh et al., 2015). The antioxidant activity is generally attributed to the presence of esterified hydroxycinnamic acids (Singh et al., 2015). This aspect is especially interesting as food and pharmaceutical industries need to provide the increasing demand of consumers of a natural alternative that replaces the synthetic compounds in the products formulation.

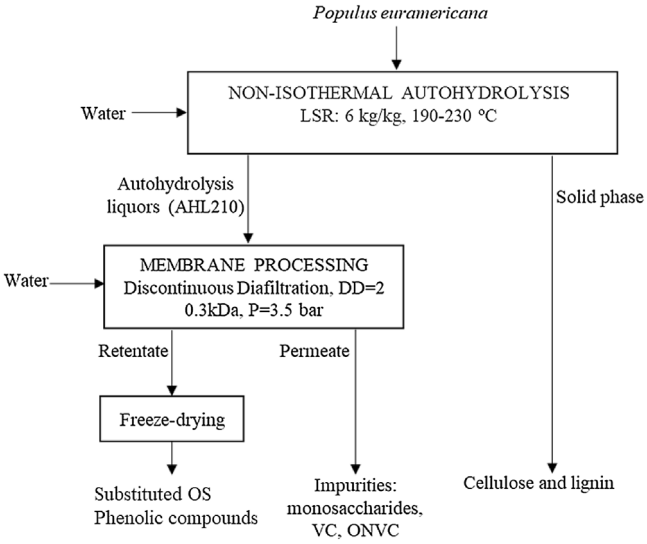

Fig. 1 – Processing scheme proposed for poplar wood utilization. Abbreviations: AH, autohydrolysis, AHL210, autohydrolysis liquors obtained at 210 °C; LSR, liquid to solid ratio; DD, discontinuous diafiltration; OS, Oligosaccharides; VC, volatile compounds; ONVC, other non volatile compounds.

Food grade OS must have high purities (typically in the range 75-95%) (Aachary and Prapulla, 2011; Rivas et al., 2017). In order to reach this threshold, unwanted components present in the liquid phase from AH (including monosaccharides and non-saccharide compounds) must be selectively removed. For this purpose, a number of separation technologies can be employed, including freeze-drying, chromatographic separation, solvent extraction, ion exchange or membrane processing (Gullón et al., 2008a; Conde et al., 2011a; Gullón et al., 2011; Rivas et al., 2017). Membrane processing has been reported to be one of the most promising downstream strategies for the industrial production of high-purity oligosaccharides, based in its advantages respect to other purification technologies such as low energy requirements, easiness of manipulating the critical operational variables or relatively easy scale-up (Qing et al., 2013).

This work provides a quantitative assessment on a biorefinery method for the valorization of PW hemicelluloses. A combination of environmentally friendly technologies (AH and membrane processing) allowed the manufacture of hemicellulosic oligosaccharides and natural antioxidants, leaving cellulose and lignin in solid phase. AH was conceived as the first step of a multistage biorefinery process suitable for yielding soluble (OS) and phenolic compounds (PC) from hemicelluloses. PW was subjected to AH at different severities using a low liquid to solid ratio (LSR), and the resulting phases were assayed for composition and yield to allow the formulation of material balances. The AH liquors (AHL) obtained under the optimal conditions that lead to achieve the maximum recovery of OS were subjected to membrane processing for refining, as shown in the process scheme proposed in Fig. 1. The target products were assayed for composition and characterization by High Performance Liquid Chromatography (HPLC), High-Performance Size Exclusion Chromatography (HPSEC), High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD), and Matrix-Assisted

Laser Desorption and Ionization Time of Flight Mass Spectrometry (MALDI TOF-MS). These streams were also assayed for total phenolic content (TPC), identification by HPLC-DAD and antioxidant activity.

2. Materials and methods

2.1. Raw material

PW (*Populus euramericana*) was kindly provided by CIFOR-INIA, Madrid, Spain. Wood chips were milled and sieved to obtain particles of 0.30 – 2.0 mm, homogenized and stored.

2.2. Autohydrolysis of PW

PW and water were mixed in a 450 mL Parr (Parr Instrument Company, Moline, IL, USA) at a liquid to solid ratio (LSR) of 6 kg water/kg dry PW, heated up to achieve the target temperature (in the range 190 – 230 °C), and cooled immediately. The liquid and solid phases were separated by vacuum filtration. The solid phase was washed with water before to be analyzed for yield and composition as described below. All experiments were performed by duplicate, except the ones performed near the temperature leading to the maximal concentrations of oligosaccharides.

The combined effects of temperature and time were measured using the "severity" parameter (S_0) (Overend & Chornet, 1987), defined as the logarithm of the severity factor R_0:

$$S_0 = logR_0 = \log\left[R_{0Heating} + R_{0Cooling}\right] = log\left[\int_0^{t_{Max}} e\left(\frac{T(t)-T_{Ref}}{\omega}\right)dt + \int_{t_{Max}}^{t_F} e\left(\frac{T(t)-T_{Ref}}{\omega}\right)dt\right] \quad (1)$$

where t_{Max} (min) is the time needed to reach the target temperature (T_{Max}, °C), t_F (min) is the cooling time, and T(t) and T′(t) represent the heating and cooling temperature profiles, respectively. Figure S1 (see Supplementary Information) shows the heating and cooling profiles corresponding to the diverse temperatures considered in this study. Literature data were assumed for the parameters ω and T_{Ref} (14.75 and 100 in the units considered here, respectively) (Overend & Chornet, 1987).

2.3. Membrane processing

Autohydrolysis liquors (AHL) obtained under optimal conditions were purified using membranes. On the basis of previous studies (Balboa et al., 2013; González-Muñoz et al., 2013; Rivas et al., 2017; Rico et al., 2018), AHL were subjected to discontinuous diafiltration in a 350 mL stirred Amicon cell (Millipore), using a 0.3 kDa cut-off membrane (GE Osmonics Inc., Minnetonka, MN, USA) with an area of 41.8 cm^2. Water was added to AHL at a mass ratio of 2 kg/kg AHL, and membrane processing was performed at 3.5 bar and room temperature to achieve a final retentate mass of 0.94 kg / kg of AHL. Experiments were performed by triplicate. Aliquots of retentate and permeate were assayed for compositional analysis as described in section 2.5. The retentate was freeze dried and stored to obtain the targeted OS concentrate.

2.4. Analysis of raw material and AH solids

PW was subjected to the following analysis: extractives (NREL/TP-510-42619 method) (Sluiter et al., 2008b), structural carbohydrates and Klason lignin and acid soluble lignin (NREL/TP-510-42618 method) (Sluiter et al., 2008a, 2012). The hydrolyzates from the structural carbohydrate determination stage were analyzed for monosaccharides, acetic acid, furfural and hydroxymethylfurfural (HMF) by HPLC using an Agilent Instrument 1260 Infinity System, equipped with a refractive index (RI) detector and a 300 × 7.8 mm ROA Organic Acid column (Phenomenex) operating at 60 °C with a mobile phase (0.005 M H_2SO_4) eluted at 0.6 mL/min. Additionally, the hydrolyzates were neutralized with barium carbonate (Rivas et al., 2012, 2013) and assayed for monosaccharides (glucose, xylose, mannose, galactose and arabinose) using a CARBOSep CHO-682 column with Micro-Guard cartridges (Transgenomic Inc., Omaha, USA) using water as mobile phase (flow rate of 0.4 mL/min) at 80 °C. Monosaccharides from Sigma-Aldrich (purity≥99%) were used as standards. Spent solids from AH were analyzed using the same methods employed for PW. All analysis were made in triplicate.

2.5. Analysis of liquid phases from AH and membrane processing

The composition of the liquid phases from AH and membrane processing was determined by HPLC before and after a quantitative posthydrolysis (performed with 4% H_2SO_4, at 121 °C). Samples were assayed for monosaccharides, furans, and organic acids using the same HPLC method described in section 2.4. Uronic acids (UA) were quantified as *per* Blumenkrantz and Asboe-Hansen (1973). The content of total non-volatile compounds (NVC) was measured by oven-drying liquor aliquots at 105 °C until constant weight. All analyses were carried out in triplicate.

2.6. Characterization of oligosaccharides from PW hemicelluloses

The hemicellulose-derived products in samples of AH210 and retentate from membrane processing were characterized by HPSEC for molecular weight distribution, using two TSKGel G3000PWXL and G2500PWXL columns in series combined with a PWX-guard column (Tosoh bioscience, Stuttgart, Germany) using the method reported by Rivas et al. (2013, 2017). Samples were also assayed by HPAEC-PAD using an ICS3000 chromatographic system (Dionex, Sunnyvale, USA) equipped CarboPac PA guard column and CarboPac PA-1 column as reported by Gullón et al. (2011). MALDI-TOF-MS analyses were performed using and Autoflex III Smartbeam Mass Spectrofotometer (Bruker Daltonik Instrument, 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 Daltonik), respectively, according to the method reported by Rivas et al. (2012). Xylooligosaccharides (XOS) with degrees of polymerization (DP) 2 to 6 (from Megazyme, Ireland) were used as external standards (Gullón et al., 2008b).

2.7. Total phenolic content and antioxidant activities

The Total phenolic content (TPC) was determined by the Folin-Ciocalteu assay (Singleton and Rossi, 1965; Rivas et al., 2013),

Table 1 – Composition of PW, expressed as g of component/100 g extractive-free material ± standard deviations.

Fraction	Weigh percent
Glucan	37.2 ± 0.3
Xylan	14.8 ± 0.6
Galactan	0.9 ± 0.0
Arabinan	0.4 ± 0.0
Mannan	2.9 ± 0.1
Acetyl groups	4.3 ± 0.1
Uronic groups	4.5 ± 0.1
Klason Lignin	27.4 ± 0.2
Acid Soluble Lignin (ASL)	6.7 ± 0.1
Ash	0.8 ± 0.0

and expressed as Gallic Acid Equivalents (GAE). Identification of phenolic compounds was carried out by HPLC-DAD as *per* Conde et al. (2008). The antioxidant activities were measured using the following methods: DPPH (von Gadow et al., 1997), TEAC (Re et al., 1999) and FRAP (Benzie and Strain, 1996). All analyses were made in triplicate.

2.8. *Error Assesment*

Standard deviations and relative errors were provided for the compositional data involved in the different stages of autohydrolysis and membrane processing proposed in this work. Relative errors were calculated in terms of the absolute values of the deviations with respect to their corresponding means, expressed as percentages, and denoted as ε.

3. Results and discussion

3.1. *Composition of the raw material*

Table 1 lists the average composition and the standard deviations obtained from the analysis by triplicate of PW. The contents of cellulose (37.2 wt%) and Klason lignin (27.4 wt%) were in the range reported for poplar hybrids (Dou et al., 2018). The hemicellulosic fraction (27.8 wt%) was mainly made up of xylosyl units, substituted by acetyl and uronic groups, following the general trends depicted in literature (Sannigrahi et al., 2010; Dou et al., 2018; Geng et al., 2018; Dou et al., 2019).

3.2. *Hydrothermal processing of PW*

AH is considered an environmentally friendly, economical and simple method for biomass fractionation, enabling the separation of hemicelluloses by splitting the glycosidic bonds to yield soluble fragments. Besides this, the interest of AH as a technology for fractionation is boosted by its selectivity, since cellulose and lignin remain in solid phase with little chemical and/or structural modification.

3.2.1. *PW solubilization*

Fig. 2 shows the average results concerning the dependence of the solid yield and solid composition on temperature of the hydrothermal processing. Additionally, the severities of the diverse operational conditions are also shown in the Fig. 2. The solid yield decreased progressively when the severity was increased, particularly at temperatures up to 220 °C that corresponded with $S_0 = 4.22$ (which led to 68.2% solid yield). This variation pattern is mainly ascribed to hemicellulose solubilization (Sannigrahi et al., 2010; Yuan et al., 2010; Singh et al., 2015). Xylan was completely removed from the solid phase under the harshest conditions assayed (230 °C, $S_0 = 4.53$), as well as mannan, galactan and arabinan. The contents of acetyl and uronyl groups also decreased with severity. Cellulose remained almost quantitatively in the spent solid, accounting for 41-51.8 wt% of this material. A similar behavior was observed for Klason lignin, which accounted for 28.9-39.3 wt% of the treated solids. The recovery yields of cellulose and Klason lignin in the solid phase were higher than 96% and 87% of the initial amounts, respectively, under all the conditions assayed. Oppositely, the acid-soluble lignin (ASL) was negatively affected by severity. The joint contributions of cel-

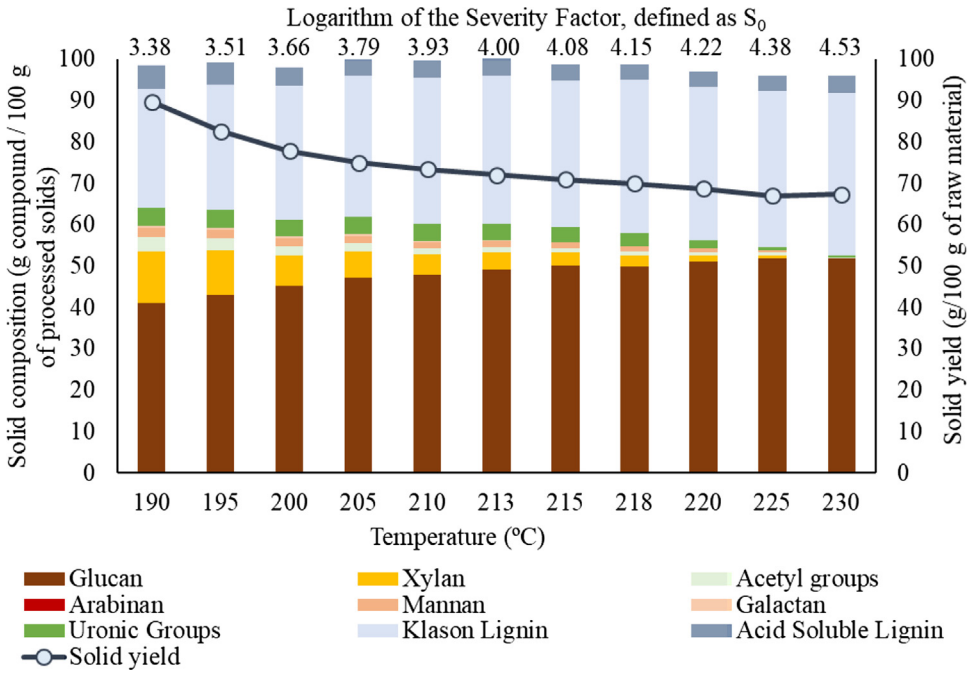

Fig. 2 – Dependence of the chemical composition of processed solids and solid yield on temperature and severity of autohydrolysis.

lulose and lignin accounted for 95 wt% of the processed solids obtained at 230 °C, confirming the suitability of AH for hemicellulose separation. The results obtained in this work are in the range reported for other hardwoods (Ko et al., 2015; Rivas et al., 2017).

The average relative errors determined from the data showed in Fig. 2 were 0.5, 1.0 and 1.4% for glucan, Klason Lignin and ASL, respectively. Respect to hemicelluloses, the value ε was 2.0, 5.1, 7.4, 4.9 and 5.9% for xylan, galactan, mannan, acetyl and uronic groups.

3.2.2. *Composition of the liquid phase from AH*

AH resulted in the formation of volatile and non-volatile compounds (denoted VC and NVC, respectively). Table 2 lists the average data concerning the identified non-volatile compounds (INVC, which include OS, OS substituents, and monosaccharides) and their standard deviations. The concentration of the non-saccharide, non-volatile components (ONVC) was measured by difference between NVC and INVC. Table S1 (Supplementary Information) lists data concerning the identified VC, expressed in terms of conversions.

NVC first increased with temperature up to achieve a maximum value (22.37 g/100 g PW or 35.70 g /L) at 210 °C (S_0 = 3.93), remained fairly constant in the range 210 – 220 °C, and decreased at higher temperatures. VC increased with temperature (Table S1) owing to the conversion of acetyl groups (AcG) into acetic acid (up to 6.64 g/L at 230 °C), and to the dehydration of monosaccharides into furans (Garrote et al., 2002; Gullón et al., 2010; Rico et al., 2018). The maximal concentration of substituted OS (28.71 g /L) was reached at 210 °C (S_0 = 3.93). Soluble xylan fragments (ascribed to XOS) were the major AH products: at 210 °C (S_0 = 3.93), 76% of xylan was converted into XOS, yielding AHL containing 18.33 g XOS/L. Harsher treatments resulted in decreased XOS yields owing to the increased participation of XOS hydrolysis reactions into xylose. This sugar reached a maximum concentration (3.70 g/L) at 225 °C, and decreased at higher temperatures by dehydration into furfural (which reached a maximum concentration of 3.94 g/L at 230 °C). In comparison, the maximum MnOS concentration (2.99 g/L) was achieved at 220 °C, whereas GaOS and ArOS were found at concentrations below 1 g/L.

The compositional data obtained for the aqueous phase from autohydrolysis (see Table S1 in Supplementary Information) confirmed the resistance of glucose polymers to AH, with conversions into GlOS and glucose that never exceeded 2.6% and 0.9%, respectively, whereas HMF (generated from hexose dehydration) achieved concentrations below 0.46 g/L.

On the basis of the above discussion, 210 °C was selected as the optimal AH temperature. AHL samples obtained at this temperature (denoted AHL210) were used as feedstocks for membrane purification. AHL210 contained a limited proportion of ONVC, and showed a remarkable relative amount of the target products (75.2 g of substituted OS/100 g of solubilized compounds, or 80.4 g of substituted OS/100 g of NVC).

3.3. *Membrane processing*

The manufacture of high purity OS from the solution AH210 by membrane processing was assayed as a way to increase their added value for the food and pharmaceutical industries. The objective of the AHL210 refining was to remove the non-volatile, non-saccharide components selectively. Since the volatile components in AH210 can be removed by evaporation after membrane refining, the removal of this type of components was considered of scarce importance for the purposes of this work.

Fig. 3 shows the average compositional data for the streams involved in the membrane stage. The OS concentrations of the feed, retentate and permeate were 38.16, 31.7 and 3.5 g/L. The NVC content of retentate (31.2 g/L) was similar to the determined for AHL210, while a much lower concentration (2.6 g/L) was found in the permeate. Upon membrane processing, the concentration of monosaccharides dropped from 2.2 g/L in AHL210 to 0.71 g/L in the retentate. The most important finding was the significant ONVC removal upon diafiltration, with concentrations decreasing from 4.83 g/L in AHL210 to 2.43 g/L in the retentate. Overall, more than 90% of the OS contained in the feed stream were recovered in the retentate, confirming the suitability of diafiltration for OS refinining. As a result of membrane processing, VC, monosaccharides and ONVC were partially removed in the permeate. The retentate contained 90 g OS/100 g of NVC (or 88.4 g OS/100 g of solubilized compounds in the retentate). Similar conclusions were reported by González-Muñoz et al. (2013) in a study dealing with consecutive membrane processing, in which the final stage (performed with a 0.3 kDa cut-off membrane) led to a retentate containing decreased contents of monosaccharides and ONVC, and oligosaccharides of diverse molecular masses. In related studies reported for hardwood processing, the manufacture of purified concentrates containing over 90% OS has been reported (Gullón et al., 2011; Chen et al., 2016; Rivas et al., 2013, 2017).

Considering the results shown in Fig. 3, the average value ε determined for the OS contained in the retentate, varied from 2.3% for XOS, the most abundant component, in comparison with 7.5% for ArOS (which appeared in low concentrations). The values of ε determined for the rest of the target OS (GlOS, GaOS, MnOS, AcG and UA) varied in a range of 3.3-4.8%.

3.4. *Total Phenolic Content (TPC)*

According to literature (Ebringerová et al., 2008; Conde et al., 2011a; Rivas et al., 2013; Gullón et al., 2017), when autohydrolysis is carried out in a single stage, phenolic compounds (including soluble lignin and free phenolics from esterified acids) are solubilized, contributing to the TPC of the liquid phases and to their antioxidant activities.

Average composition of TPC and the correspondent standard deviations of triplicates from AHL210 and retentate from membrane processing were reported in Table 3. In both cases, deviations were lower than 5% to their respective means. The AHL210 sample showed an average composition of TPC of 1.73 g GAE/L, which is in the range reported for AH liquors from other lignocellulosic materials, including eucalypt wood (1.64-1.98 g GAE/L), corncobs (1.91-2.82 g GAE/L) (Conde et al., 2011a), pruning residues (1.33 g GAE/L) (Gullón et al., 2017), and peanut shells (1.58 g GAE/L) (Rico et al., 2018). Membrane purification yielded a retentate of decreased TPC (with 23% removal of phenolics). This result is in agreement with the data reported by Rivas et al. (2013) in a study dealing with the refining of OS from AHL of rice husks, eucalypt and pine. It can be noted that the TPC determined for both retentate and AHL210 could be slightly overestimated owing to the presence of furan derivatives, which can react with the Folin-Ciocalteu reagent (Tang et al., 2015). HPLC-DAD analysis allowed the identification of furan derivatives and simple phenolics, including acids (4-hydroxybenzoic, vanillinic, siringic, and gallic acids) and

Table 2 – Effect of AH in the chemical composition of the solubilized compounds ± standard deviations.

Composition of AH liquors (g/L)		Temperature (°C)										
		190	195	200	205	210	213	215	218	220	225	230
		Logarithm of the Severity factor (S_0)										
		3.38	3.51	3.66	3.79	3.93	4.00	4.08	4.15	4.22	4.38	4.53
INVC	Glucose	0.07 ± 0.00	0.19 ± 0.00	0.20 ± 0.00	0.23 ± 0.00	0.20 ± 0.00	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.00	0.29 ± 0.01	0.40 ± 0.01	0.52 ± 0.02
	Xylose	0.08 ± 0.00	0.17 ± 0.01	0.37 ± 0.02	0.60 ± 0.03	1.00 ± 0.01	1.26 ± 0.05	1.80 ± 0.06	2.25 ± 0.07	3.04 ± 0.02	3.7 ± 0.02	3.28 ± 0.12
	Galactose	0.14 ± 0.00	0.21 ± 0.01	0.31 ± 0.02	0.35 ± 0.03	0.38 ± 0.03	0.39 ± 0.03	0.39 ± 0.02	0.46 ± 0.03	0.47 ± 0.02	0.39 ± 0.02	0.27 ± 0.02
	Arabinose	0.22 ± 0.01	0.32 ± 0.03	0.41 ± 0.06	0.46 ± 0.03	0.43 ± 0.02	0.39 ± 0.01	0.35 ± 0.03	0.26 ± 0.02	0.23 ± 0.02	0.12 ± 0.01	0.00 ± 0.00
	Mannose	0.01 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.09 ± 0.01	0.14 ± 0.01	0.17 ± 0.01	0.26 ± 0.01	0.36 ± 0.03	0.50 ± 0.02	0.68 ± 0.06	0.64 ± 0.04
	GlOS	0.78 ± 0.05	0.84 ± 0.00	0.97 ± 0.02	1.05 ± 0.02	1.23 ± 0.01	1.22 ± 0.00	1.37 ± 0.01	1.47 ± 0.03	1.55 ± 0.01	1.50 ± 0.05	1.49 ± 0.01
	XOS	4.67 ± 0.12	10.47 ± 0.44	14.39 ± 0.17	16.86 ± 0.15	18.33 ± 0.25	17.24 ± 0.24	16.12 ± 0.91	15.46 ± 0.34	13.66 ± 0.10	8.08 ± 0.15	3.11 ± 0.07
	GaOS	0.77 ± 0.03	0.86 ± 0.00	0.89 ± 0.04	0.92 ± 0.04	0.86 ± 0.04	0.74 ± 0.02	0.64 ± 0.04	0.60 ± 0.03	0.52 ± 0.03	0.26 ± 0.03	0.12 ± 0.01
	ArOS	0.44 ± 0.04	0.39 ± 0.03	0.28 ± 0.04	0.17 ± 0.01	0.14 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.05 ± 0.00	0.10 ± 0.00	0.00 ± 0.00
	MnOS	1.84 ± 0.09	2.13 ± 0.03	2.33 ± 0.05	2.57 ± 0.09	2.79 ± 0.05	2.83 ± 0.02	2.91 ± 0.13	2.98 ± 0.11	2.99 ± 0.05	2.30 ± 0.02	1.89 ± 0.01
	AcG	1.39 ± 0.09	3.04 ± 0.02	3.71 ± 0.34	4.48 ± 0.08	4.60 ± 0.05	4.41 ± 0.05	4.43 ± 0.01	4.15 ± 0.15	3.68 ± 0.11	2.61 ± 0.09	1.20 ± 0.05
	UA	0.43 ± 0.01	1.30 ± 0.03	1.12 ± 0.01	0.70 ± 0.02	0.76 ± 0.05	0.30 ± 0.00	0.35 ± 0.01	0.30 ± 0.01	0.26 ± 0.01	0.23 ± 0.02	0.10 ± 0.01
	Other	5.64	5.32	6.14	5.51	4.83	6.28	6.43	7.18	6.62	7.19	11.73
	Total NVC	16.47 ± 0.08	25.26 ± 0.18	31.16 ± 0.11	33.99 ± 0.27	35.70 ± 0.10	35.55 ± 0.00	35.39 ± 0.40	35.81 ± 0.06	33.87 ± 0.01	27.55 ± 0.29	24.34 ± 0.12
VC	Acetic acid	0.55 ± 0.02	0.77 ± 0.02	1.09 ± 0.01	1.36 ± 0.02	1.91 ± 0.02	2.23 ± 0.05	2.64 ± 0.09	3.07 ± 0.10	3.72 ± 0.09	4.72 ± 0.12	6.64 ± 0.20
	Furfural	0.03 ± 0.00	0.04 ± 0.00	0.17 ± 0.00	0.29 ± 0.02	0.46 ± 0.02	0.74 ± 0.05	1.06 ± 0.06	1.32 ± 0.04	1.71 ± 0.13	2.9 ± 0.17	3.94 ± 0.20
	HMF	0.02 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.07 ± 0.01	0.10 ± 0.00	0.14 ± 0.01	0.08 ± 0.01	0.22 ± 0.01	0.30 ± 0.02	0.46 ± 0.02	0.00 ± 0.00

GlOS: glucose units in OS; XOS: xylose units in OS; GaOS: galactose units in OS; MnOS: mannose units in OS; AcG, acetyl groups linked to OS; UA: uronic acid linked to OS; HMF: hydroxymethylfurfural.
Others: measured as the difference between NVC and INV.

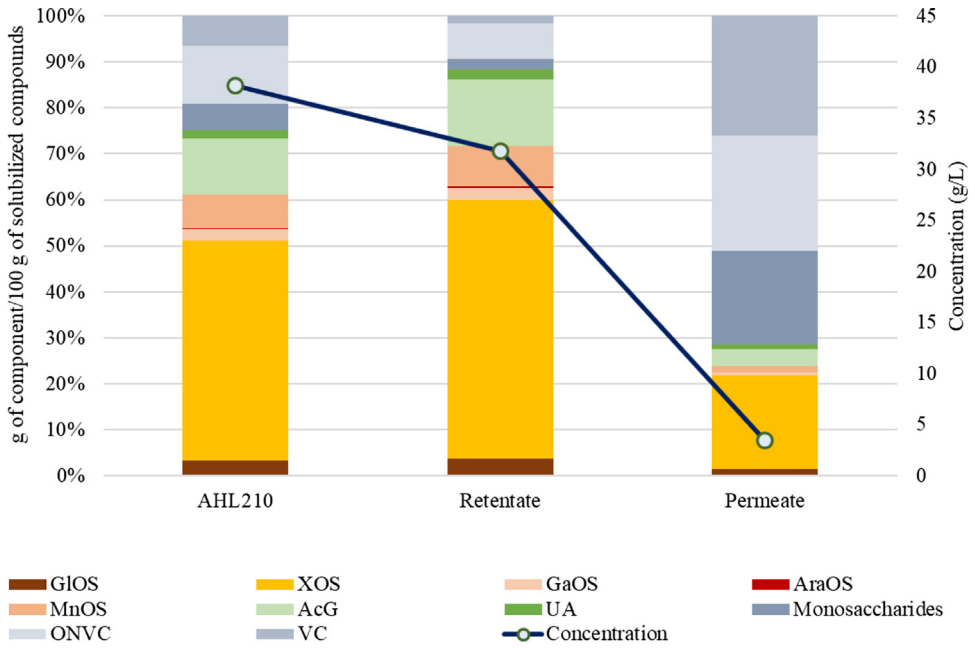

Fig. 3 – Composition of the streams (AHL210, retentate and permeate) involved in the membrane processing, expressed as g of component/100 g of solubilized compounds, and total concentration of each stream, as g/L.

Table 3 – Total Phenolic Content of AHL210 and retentate from membrane processing ± standard deviations.

Sample	AHL210	Retentate
TPC, g GAE/L	1.73 ± 0.03	1.39 ± 0.03

Table 4 – Antioxidant activities determined by TEAC, DPPH and FRAP assays of AHL210 and retentate from membrane processing ± standard deviations.

Sample	TEAC	DPPH	FRAP
AHL210	4.76 ± 0.42	0.28 ± 0.01	3.00 ± 0.05
Retentate	4.11 ± 0.47	0.27 ± 0.01	2.46 ± 0.03

TEAC, g Trolox/L; DPPH (EC_{50}), g GAE/L; FRAP, g $FeSO_4 \cdot 7H_2O$/L.

aldehydes (syringaldehyde, vanillin, and dihydroxybenzaldehyde).

In order to highlight the results achieved with the combination of autohydrolysis and membrane processing, Fig. 4 shows the mass balances calculated for the operational conditions considered as optimal. The data indicate the yields of the target products, 16.6 g of substituted oligosaccharides and 0.8 g of natural phenolic compounds in 100 g of dry poplar. The corresponding amounts of AH processed solids, mainly made up of cellulose and lignin, are also indicated. AH processed solids could be employed for further valorization in additional processing stages.

3.5. *Antioxidant activity*

The evaluation of the antioxidant activity of AHL210 and retentate has been performed using multiple tests (DPPH, TEAC and FRAP) based on diverse reactions, since the compounds or fractions contributing to the antioxidant activity may present various modes of action (Dudonné et al., 2009; Conde et al., 2011a). The average antioxidant activities of assays performed by triplicate and the standard deviations are listed in Table 4. The activities determined for AHL210 were in the range reported in studies dealing with diverse feedstocks (Conde et al., 2011a; Rivas et al., 2013; Gullón et al., 2017). Jesus et al. (2017) obtained a lower TEAC activity (247.05 mg Trolox/L) in AHL from vine prunings treated at $S_0 = 4.13$; whereas Rico et al. (2018) reported TEAC, DPPH and FRAP activities of 5.01, 1.18 and 2.57 g Trolox/100 g for AHL from peanut shells treated at 210 °C. The assays showed a higher antioxidant capacity for the raw hydrolysate (AHL210) in comparison with the retentate, as expected from the respective TPC. The maximum difference between the antioxidant capacity of these streams was observed for the FRAP and TEAC assays, which presented 18% and 13.6% less activity in the retentate than in the feed, respectively (Table 4). Minor differences were found in DPPH assays (3.6% decrease in the retentate respect to the feed stream), a result in agreement with the data reported by Rivas et al. (2013). Conde et al. (2011b) reported a similar variation pattern for the products present in AHL from diverse lignocellulosic materials subjected to refining with polymeric resins.

3.6. *Characterization of oligosaccharides from PW hemicelluloses*

Looking for additional information regarding the potential applications of the target products, oligomeric compounds present in AHL210 and retentate were assessed by HPSEC, HPAEC-PAD and MALDI TOF-MS. Qing et al. (2013) reviewed the techniques for xylooligosaccharides characterization, including the direct one by HPLC and HPAEC-PAD. Additionally, valuable information was obtained by MALDI-TOF MS.

Figure S2 (see Supplementary Information) shows the HPSEC chromatograms determined for AHL210 and retentate. The complex elution profiles confirmed the presence of multiple saccharides in the media. A comparison between both chromatograms confirmed similar elution patterns for the oligosaccharides appearing after 20-47 minutes; whereas purification effects achieved upon membrane processing (par-

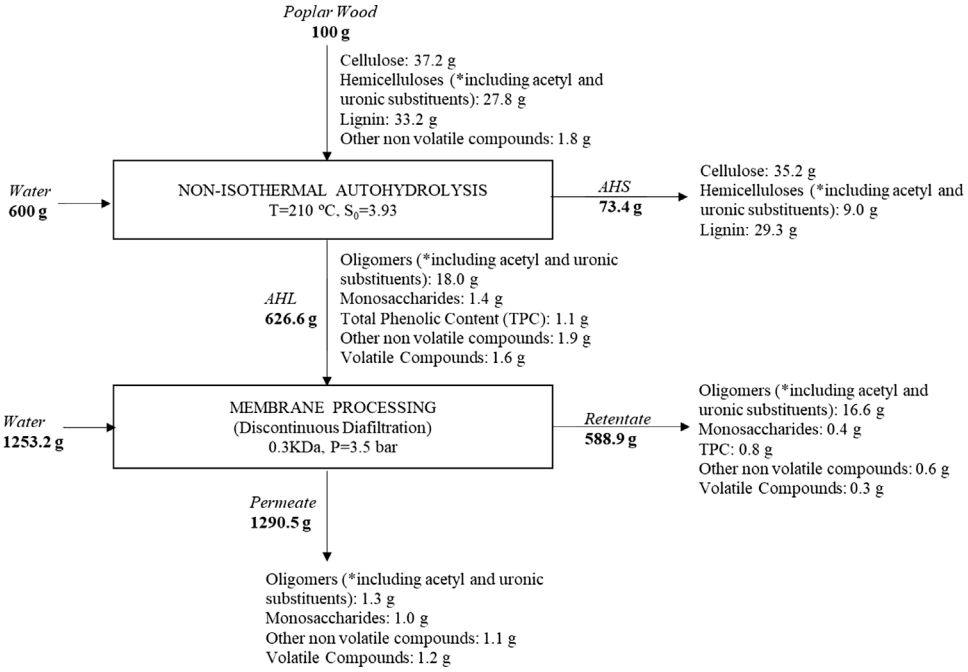

Fig. 4 – Mass balances corresponding to the condition considered as optimal for the manufacture of oligosaccharides and phenolic compounds.

tial removal of monosaccharides from AHL210) was evident from the different areas of the peaks eluted at 48 min.

Fig. 5 shows the HPAEC-PAD corresponding elution profiles, as well as the ones observed for XOS standards of DP 2-6. The OS were eluted in the range of 32-55 min, and the chromatograms confirmed the predominance of low- or medium-DP compounds. Concerning the substitution pattern, it must be noted that the HPAEC-PAD mobile phase (strongly alkaline) caused the saponification of the acetylated oligomers during the elution of samples (Gullón et al., 2011; Dávila et al., 2019). As expected, AHL210 and retentate showed similar HPAEC-PAD elution profiles, with close coincidences between peaks of oligomers eluted at correspondent times, in agreement with the high recovery of substituted OS in the retentate deduced from HPLC data.

In contrast to HPAEC-PAD, MALDI-TOF MS allowed the identification and elucidation of acetylated oligomers. Table 5 lists the data determined for the sodium adducts of compounds presents in the retentate, together with the potential composition assigned and the mass error in ppm (as comparison of the theoretical m/z and the experimentally observed m/z). Compounds with m/z below 500 could not be identified due to interferences with 2, 5-dihydroxybenzoic acid, which was used as a matrix for sample preparation (Gullón et al., 2008b; Rivas et al., 2012). On the basis of the compositional data in Table 2, it can be seen that pentose (P) chains must correspond to xylose, the most abundant monomer the hemicelluloses of PW. The pentose chains were highly substituted by AcG [mP nAcG] or AcG and UA [mP nAcG oUA], being m, n and o, the units of pentoses (P), acetyl groups (AcG) and uronics (UA), respectively. The structures formed by hexoses (H) may correspond to compounds made up of galactose, mannose, or glucose. The data showed that the soluble products from AH corresponded mainly to fragments of acetylated glucuronoxylan, with backbones of 3 - 13 pentose units. Most oligomeric backbones were substituted with 1 or 2 units of *O*-methylglucuronic acid (UA), whereas heavy substitution by AcG was observed in some cases (for example, DP10 - DP13 compounds may bear up to 6 AcG). These results were in good agreement with the compositional data determined for AHL210 (see Table 2). The calculated molar ratio XOS:AcG:UA (1:0.628:0.030) was in the range reported in literature: for example, Hou et al. (2014) reported a XOS:AcG molar ratio of 1:0.589 for poplar OS; whereas the MALDI-TOF-MS data obtained in this study followed the general features reported for products isolated from other hardwoods (Kabel et al., 2002; Chemin et al., 2015; Rivas et al., 2017).

4. Conclusions

The combination of autohydrolysis and membrane processing enabled an efficient production and refining of hemicellulose-derived oligosaccharides containing natural phenolic compounds with antioxidant activity. Optimized autohydrolysis conditions led to extensive hemicellulose solubilization, leading to an aqueous phase (AHL210) with a remarkable concentration of soluble oligosaccharides (28.71 g/L). Diafiltration of AHL210 removed monosaccharides and undesired non-saccharide, low molecular weight compounds, yielding a refined product containing high purity OS (90 kg OS/100 kg NVC). Additionally, the target fraction presented a good antioxidant activity, ascribed to the presence of natural phenolic compounds (equivalent amount, 3.66 g of GAE/100 g of NVC). The characterization of hemicellulose-derived saccharides showed backbones constituted by xylose units with a wide DP distribution and a rich substitution pattern by acetyl and *O*-methylglucuronic acid moieties. The results reported in this study provide a quantitative basis for assessing the manufacture and refining of oligosaccharides from poplar wood hemicelluloses containing phenolics with antioxidant properties. The compositional and material balance data provided in this study allow a sound assessment on a new biorefinery method for PW valorization, based on

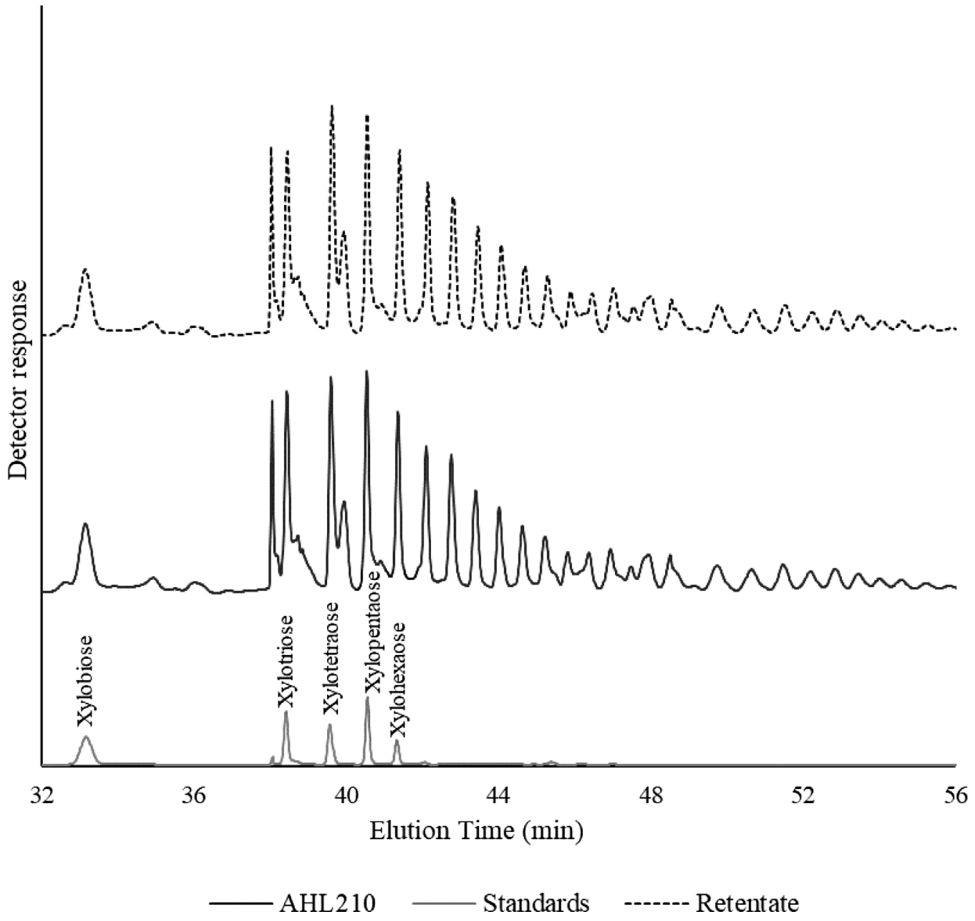

Fig. 5 – HPAEC-PAD elution profiles determined for AHL210 and retentate.

Table 5 – MALDI-TOF results determined for oligomers and ascribed potential composition of retentate.

m/z	Mass error (ppm)	Potential Compound	m/z	Mass error (ppm)	Potential Compound	m/z	Mass error (ppm)	Potential Compound
521.17	38.38	3P 2AcG	1175.37	22.12	7P 5AcG	1529.55	54.27	10P 4AcG
537.15	13.03	2P AcG UA	1191.36	18.47	6P 4AcG UA	1539.51	42.22	8P 6AcG UA
611.20	32.72	4P AcG	1197.37	16.70	7P AcG UA	1545.50	37.53	9P 3AcG UA
627.19	33.48	3P UA	1207.35	10.77	5P 3AcG 2UA	1555.80	41.15	7P 5AcG 2UA
653.22	42.86	4P 2AcG	1223.43	48.23	8P 3AcG	1571.56	56.63	10P 5AcG
689.22	13.74	4H	1233.36	8.92	6P 5AcG UA	1587.53	39.69	9P 4AcG UA
711.21	18.28	3P 2AcG UA	1239.38	12.10	7P 2AcG UA	1603.54	51.14	8P 3AcG 2UA
785.27	36.93	5P 2AcG	1265.42	36.35	8P 4AcG	1629.55	42.34	9P 5AcG UA
801.23	9.98	4P AcG UA	1275.39	25.88	6P 6AcG UA	1645.55	47.40	8P 4AcG 2UA
843.24	7.12	4P 2AcG UA	1281.39	13.27	7P 3AcG UA	1671.58	52.65	9P 6AcG UA
917.33	62.14	6P 2AcG	1297.40	20.81	6P 2AcG 2UA	1677.55	44.71	10P 3AcG UA
933.27	0.00	5P AcG UA	1307.42	22.95	8P 5AcG	1687.55	41.48	8P 5AcG 2UA
959.32	36.49	6P 3AcG	1323.42	24.94	7P 4AcG UA	1719.60	50.01	10P 4AcG UA
975.28	0.00	5P 2AcG UA	1339.42	30.61	6P 3AcG 2UA	1761.60	46.55	10P 5AcG UA
991.28	11.10	4P AcG 2UA	1365.42	20.51	7P 5AcG UA	1778.22	38.24	9P 4AcG 2UA
1001.32	22.97	6P 4AcG	1371.44	24.79	8P 2AcG UA	1803.61	43.80	10P 6AcG UA
1017.29	2.95	5P 3AcG UA	1381.42	22.44	6P 4AcG 2UA	1809.65	61.34	11P 3AcG UA
1049.36	38.12	7P 2AcG	1397.49	47.23	9P 4AcG	1819.59	37.37	9P 5AcG 2UA
1059.31	13.22	5P 4AcG UA	1413.46	28.30	8P 3AcG UA	1851.64	45.91	11P 4AcG UA
1065.32	7.51	6P AcG UA	1439.47	27.79	9P 5AcG	1893.68	61.26	11P 5AcG UA
1091.37	39.40	7P 3AcG	1455.47	29.54	8P 4AcG UA	1935.66	45.98	11P 6AcG UA
1107.33	7.22	6P 2AcG UA	1471.47	31.26	7P 3AcG 2UA	1983.68	42.35	12P 4AcG UA
1133.37	30.00	7P 4AcG	1497.49	38.07	8P 5AcG UA	2025.68	39.00	12P 5AcG UA
1149.34	3.48	6P 3AcG UA	1503.50	35.25	9P 2AcG UA	2067.69	37.72	12P 6AcG UA
1165.34	6.87	5P 2AcG 2UA	1513.48	31.72	7P 4AcG 2UA	2199.71	24.09	13P 6AcG UA

[a]Mass signals were identified <– –>for specific sodium adducts.

the manufacture of valuable compounds with interest in food and pharmaceutical markets.

5. Declaration of competing interest

There are no conflicts of interest to declare

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fbp.2020.07.018.

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