PRODUCTION AND REFINING OF SOLUBLE PRODUCTS FROM *Eucalyptus globulus* GLUCURONOXYLAN

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Milled *Eucalyptus globulus* wood samples were subjected to hydrothermal treatment under a variety of operational conditions in order to cause the hydrolytic degradation of glucuronoxylan. Liquors contained both hemicellulose-derived products (soluble products from xylan, mainly corresponding to substituted oligosaccharides and monosaccharides) and non-saccharide compounds. Liquors obtained under selected conditions were concentrated and subjected to ethyl acetate extraction (3 steps) and ion exchange (with Amberlite IRA 400 or Amberlite IRA 96) to decrease the content of non-saccharide components. The various streams involved in the whole process were assayed for composition, and material balances were formulated for the best results. The highest purification degree was obtained with Amberlite IRA 400, which led to a final isolate (consisting of monosaccharides, substituted oligosaccharides and non-volatile, non-saccharide components) containing 92.2 wt.% of saccharides and 7.76 wt.% of non-saccharide components (mainly phenolic compounds).
Keywords: Autohydrolysis; *Eucalyptus globulus*; Hemicelluloses; Glucuronoxylan; Purification; Refining; Xylooligosaccharides; Wood hydrothermal treatment; Hydrolysis.

Lignocellulosic materials (LCM) are mainly made up of cellulose, hemicelluloses and lignin, as well as other fractions such as extractives, proteins and ashes.

Hemicelluloses are heteropolysaccharides containing pentoses (xylose, arabinose) and hexoses (glucose, mannose, galactose), which may be substituted with uronic acids (or their *O*-methyl derivatives), or with acetyl and phenolic groups.

The most important hemicellulosic polymers are xylans, made up of xylose units. Xylans represent an immense source of biopolymers for practical applications, accounting for 25–35% of lignocellulosic biomass, and occurring in some tissues of cereal grains up to 50%. The composition of xylans depends on the lignocellulosic material considered: hardwoods (like

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Eucalyptus globulus wood) contain mostly xylans substituted with uronic acids and with acetyl groups (acetylated glucuronoxylan), whereas many agricultural wastes (such as rice hulls or corncobs) are substituted with arabinose, uronic acid moieties and acetic, ferulic or coumaric groups[1].

When LCM are treated in aqueous media at temperatures 130–230 °C (hydrothermal processing or autohydrolysis reaction), hemicelluloses are depolymerised and converted mainly to soluble oligomers as well as into sugars and sugar-degradation products (furaldehyde and (hydroxymethyl)-furaldehyde). Simultaneously, acetyl groups are cleaved to give acetic acid. All these reactions are catalysed by hydronium ions generated from water autoionisation and from the acid species (uronic acids, acetic acid) present in the raw material or generated in the reaction medium[2,3].

Besides hemicellulose degradation, a variety of side-processes also take place in the reaction media, including removal of extractives from solid phase, partial dissolution of acid-soluble lignin and ashes, solubilisation of proteins and reaction of proteins and amino acids with sugars to give melanoidins.

Considered as a biomass fractionation technology, aqueous treatments present interesting technical and environmental features, including: (i) selectivity in cellulose decomposition (cellulose is recovered in solid phase almost untouched); (ii) generation of soluble reaction byproducts with antioxidant activity[4,5] and (iii) limited corrosion effects and the absence of reagents other than water and LCM [6].

According to the above ideas, the autohydrolysis liquors show a complex composition[7], yielding substituted xylooligomers (here denoted SXO) whose composition reflects to some extent that of the feedstock utilised. Kabel et al.[8] reported that the degree of polymerisation of SXO obtained by autohydrolysis of *Eucalyptus globulus* wood under optimal operation conditions varied in the range 4–10.

Xylooligosaccharides find applications as prebiotic food ingredients[9–11], causing a variety of positive health effects[9,12,13]. When xylooligosaccharides for food applications are produced by autohydrolysis, the reaction products have to be refined to decrease the contents of non-carbohydrate compounds. This work deals with the refining of *Eucalyptus globulus* autohydrolysis liquors to increase their content of soluble products derived from glucuronoxylan, and with the characterisation of the final products for application as food ingredients.

EXPERIMENTAL

Raw material. *Eucalyptus globulus* chips were collected in a local factory, air-dried, milled to pass a 1-mm screen, homogenised in a single lot to avoid compositional differences among aliquots, and stored until use.

Analysis of the raw material. Aliquots of stored samples were subjected to moisture determination (ISO 638 method), to ash determination (ISO 776 method) and to quantitative acid hydrolysis (TAPPI T13m method). HPLC analysis of liquors from quantitative acid hydrolysis allowed the determination of the contents of cellulose, hemicellulose constituents and acetyl groups[6]. In HPLC chromatograms, xylose, mannose and galactose were eluted in a single peak. The products from eucalyptus hydrothermal treatments contain minor amounts of mannose and galactose[14], but the whole peak was integrated as xylose due to the higher proportions of this sugar. The oven-dry weight of the solid phase from quantitative hydrolysis is here denoted acid-insoluble residue. Uronic acids were determined by the method of Blumenkrantz and Asboe-Hansen[15] using galacturonic acid as a standard for quantification.

Autohydrolysis. Wood samples and water were mixed in the desired proportions and reacted in a Parr reactor under non-isothermal conditions at a liquor-to-solid ratio of 8 kg/kg oven-dry solid, following the standard temperature profile until the desired temperature was reached. At the end of treatments, liquors were recovered by filtration, analysed and processed as described below.

Analysis of autohydrolysis liquors. For analytical purposes, samples of liquors were filtered through 0.45 μm membranes and used for direct HPLC determination of glucose, xylose, arabinose, furfural, and acetic acid using the same method as employed in the analysis of the raw material. An aliquot of liquors was subjected to quantitative posthydrolysis (with 4% sulfuric acid at 121 °C for 20 min), and the reaction products were assayed by the same HPLC method. The increase in the concentrations of monosaccharides and acetic acid caused by posthydrolysis provided a measure of the oligomer concentration and their degree of substitution with acetyl groups[16]. In order to have a more real composition of liquors, xylooligosaccharides were measured as equivalents of oligomers with DP = 7, the average degree of polymerisation reported for SXO obtained under the same conditions[8]. The furaldehyde and (hydroxymethyl)furaldehyde concentrations determined in this work were negligible for practical purposes. Uronic acids in soluble products were assayed as reported for the raw material. The contents of raw or processed liquors in non-volatile compounds (NVC) were measured by oven-drying at 105 °C to constant weight. All the determinations were made in triplicate, and the average values are reported.

Elemental analysis. Elemental analysis of soluble products was carried out using a Thermo Finnegan Flash EA™ 1112 Analyzer at 130 and 100 ml/min of He and O_2 and an oven temperature of 50 °C. Determinations were made in triplicate, and the average values are reported.

Acid-soluble lignin. Selected soluble fractions were assayed spectrophotometrically for acid-soluble lignin after hydrolysis with 4% sulfuric acid at 121 °C for 20 min using the method of Maekawa et al.[17] Determinations were made in triplicate, and the average values are reported.

Concentration of liquors. Autohydrolysis liquors were concentrated to the desired volume ratio using a centrifugal evaporator (Savant SC210A SpeedVac Plus), operating at the minimum heating power to avoid sample degradation.

Solvent extraction of liquors. Aliquots of autohydrolysis liquors were extracted with ethyl acetate in three sequential steps at a liquor:solvent weight ratio of 1:1 (kg/kg) per step. The organic phases were mixed and vacuum-evaporated to remove both solvent and volatile dissolved compounds, and the remaining solid phase was used to calculate the yield of the autohydrolysis-extraction process. The aqueous phases were assayed for composition using the same methods as described for the autohydrolysis liquors.

Ion exchange processing of liquors. The aqueous phases from the ethyl acetate extraction steps were treated with Amberlite IRA 400 (a strong anion exchanger, quaternary ammonium, gel-type resin, supplied in Cl^- form) and Amberlite IRA 96 (a weak anion exchanger, polyamine, macroreticular resin, supplied in free-base form). Both resins were obtained from Rohm and Hass. Liquors and resin were contacted for 24 h with gentle agitation at room temperature using 1 kg resin (oven-dry weight)/20 kg liquor. Samples were processed in triplicate, and the average values are reported.

RESULTS AND DISCUSSION

Raw Material and Autohydrolysis Treatment

Table I shows the composition of the wood lot utilised in experiments. Hemicelluloses are made up of acetylated glucuronxylan with minor substitution with arabinose, and accounted for 27.96% of the oven-dry weight of the samples (calculated as the sum of the contents of xylan, arabinan, uronic acid groups and acetyl groups).

In hydrothermal treatments, xylooligosaccharides (denoted XO) are produced from xylan and simultaneously converted to xylose and xylose-degradation products[6,18]. As typical reaction intermediates, XO reach maxi-

TABLE I
Composition of oven-dry material

Component	wt.%
Cellulose	42.38
Xylan	16.01
Arabinan	0.38
Acetyl groups	3.05
Uronic acids	8.52
Acid-insoluble residue	27.89
Ashes	0.30
Other (by difference)	1.47

mal concentrations under medium severity reaction conditions. As the optimal operational conditions depend to some extent on the sample employed (and particularly, on its acetyl group content, which may vary slightly from sample to sample), preliminary non-isothermal autohydrolysis treatments were carried out following the standard heating profile of the reactor to reach final temperatures in the range 190–202 °C.

Figure 1a shows the dependence of the composition of solids from hydrothermal processing on the final temperature of treatments. Under non-isothermal conditions, the severity of the operational conditions increases owing to the combined effects of time and temperature. The weight percentage of cellulose increased steadily with the final temperature reached in assays, owing to its low susceptibility to aqueous treatments and to the progressive dissolution of other fractions. The acid-insoluble residue (mainly made up of Klason lignin) increased slightly owing to the combined effects of selective hemicellulose degradation, extractive removal and lignin hydrolysis to soluble fragments. Finally, as expected, the content of xylan decreased steadily with the severity of treatments owing to the higher conversion achieved by hydrolytic reactions.

Figure 1b shows that XO (measured from the increase in xylose concentration after quantitatiave acid hydrolysis and corrected for hydration, assuming DP = 7) were the major components of liquors, with concentrations up to 13 times higher than other products such as glucooligosaccharides (denoted GO). The concentration of XO first increased and then decreased with the final temperature of treatments, with optimal conditions corresponding to the experiment carried out to reach 198 °C, a finding in agreement with reported data[18]. Acetyl groups linked to oligosaccharides (denoted AcO) showed a closely related behaviour, confirming that the acetylation degree was fairly constant in all the experiments. The concentration of GO increased steadily with the final temperature, owing to the progressive hydrolysis of low-DP glucose polymers, whereas the concentrations of uronic acid groups (UA) decreased with the severity of the treatments. The low content of arabinan in the raw material and possible decomposition reactions during treatments made arabinooligosaccharides (ArO) undetectable.

Figure 1c presents the concentration profiles of monosaccharides (generated from oligomers) and acetic acid (produced from acetyl groups). The xylose concentration increased sharply with temperature due to XO cleavage. Under the same conditions, GO were not significantly affected by temperature, leading to flat glucose concentration profiles. Acetic acid concentration increased with temperature, reflecting the higher extent of acetyl

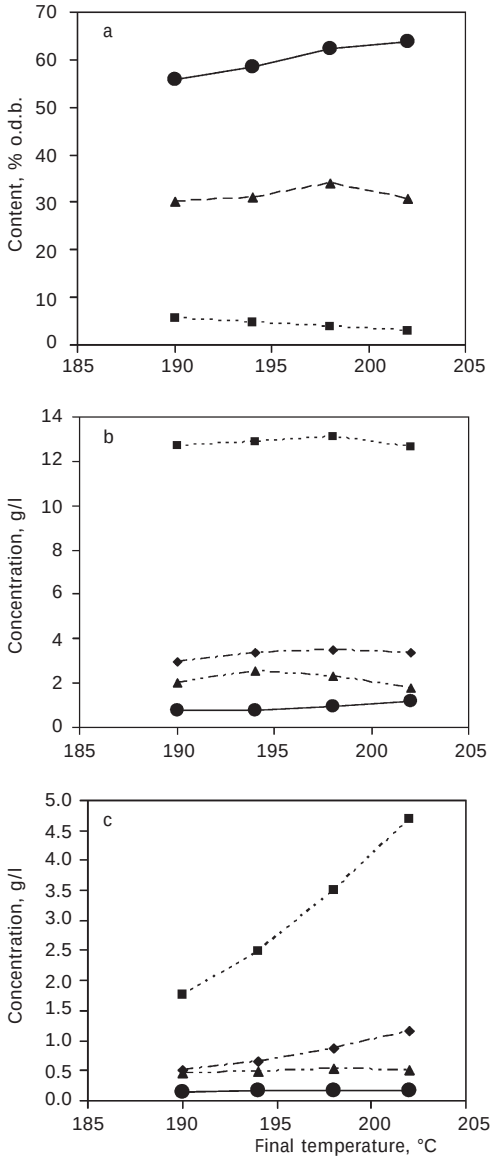

FIG. 1

Composition of solid and liquid phases from autohydrolysis. a Composition of spent solids from treatments (AIR, acid-insoluble residue): ● cellulose, ■ xylan, ▲ AIR. b Contents of xylooligosaccharides (XO, ■), glucooligosaccharides (GO, ●), acetyl groups in oligosaccharides (AcO, ◆) and uronic acids (UA, ▲) in liquors. c Contents of monosaccharides and acetic acid in liquors: ● glucose, ■ xylose, ▲ arabinose, ◆ acetic acid

group removal from xylan and XO, whereas low arabinose concentrations were observed in liquors.

Based on the above information, hydrothermal experiments were carried out to reach a final temperature of 198 °C, and the liquors were used in further refining experiments. Table II summarises the effects caused by treatments carried out under these conditions in the raw material: about 29% of the initial oven-dry weight of the feedstock was lost in treatments, leading mainly to non-volatile compounds (denoted NVC). Xylan (measured as potential xylose) was solubilised to a high extent (86%) and arabinan was quantitatively removed, whereas small effects on cellulose were observed. XO (accounting for 65% of the initial xylan) were the main reaction products, whereas GO accounted for 1.85% of the initial cellulose. The limited content of GO was related to the low susceptibiliy of glucose polymers to hydrolytic degradation. Some inconsistencies of minor importance for the purposes of this study were observed when calculating the degree of arabinan conversion to arabinose (the experimental concentration was slightly higher than the stoichoiometric one, owing to the incidence of the experimental error when integrating very small peaks in chromatograms).

Refining of Liquors: Objectives and Processing Stages

Autohydrolysis liquors obtained under optimal conditions were collected, mixed with the washing waters of the spent solids, concentrated by evaporation to increase their NVC content and subjected to physicochemical treatments (solvent extraction followed by ion-exchange) for refining the oligosaccharide mixture (Fig. 2).

TABLE II
Results of non-isothermal autohydrolysis (final temperature, 198 °C)

Oven-dry solid solubilised in treatments, wt.%	29.05
Solid conversion to non-volatile compounds (NVC), oven-dry wt.%	24.21
Solid conversion to volatile compounds (VC), oven-dry wt.%	4.84
Cellulose conversion to glucose, %	0.41
Xylan conversion to xylose, %	21.18
Cellulose conversion to glucooligosaccharides, %	1.85
Xylan conversion to xylooligosaccharides[a], %	64.95
Acetyl group conversion into acetic acid, %	21.24

[a] Corrected for hydration considering an average polymerisation degree of 7.

Table III shows the NVC composition of concentrated liquors (stream CL in Fig. 2). The fraction "other non-volatile components" (ONVC) corresponded to the difference between the total NVC content (determined by oven-drying of liquors) and the joint contribution of the monosaccharides, oligosaccharides and oligosaccharide substituents listed in this table. The ONVC fraction was made up of non-saccharide products. The refining process intended to reduce the mass fraction of ONVC in concentrates.

For this purpose, three steps of ethyl acetate extraction were carried out to selectively remove ONVC components derived from extractives and lignin, including fatty acids, alcohols, esters, waxes and low-molecular-weight phenolics[19,20]. Owing to the solubility of water in ethyl acetate, the

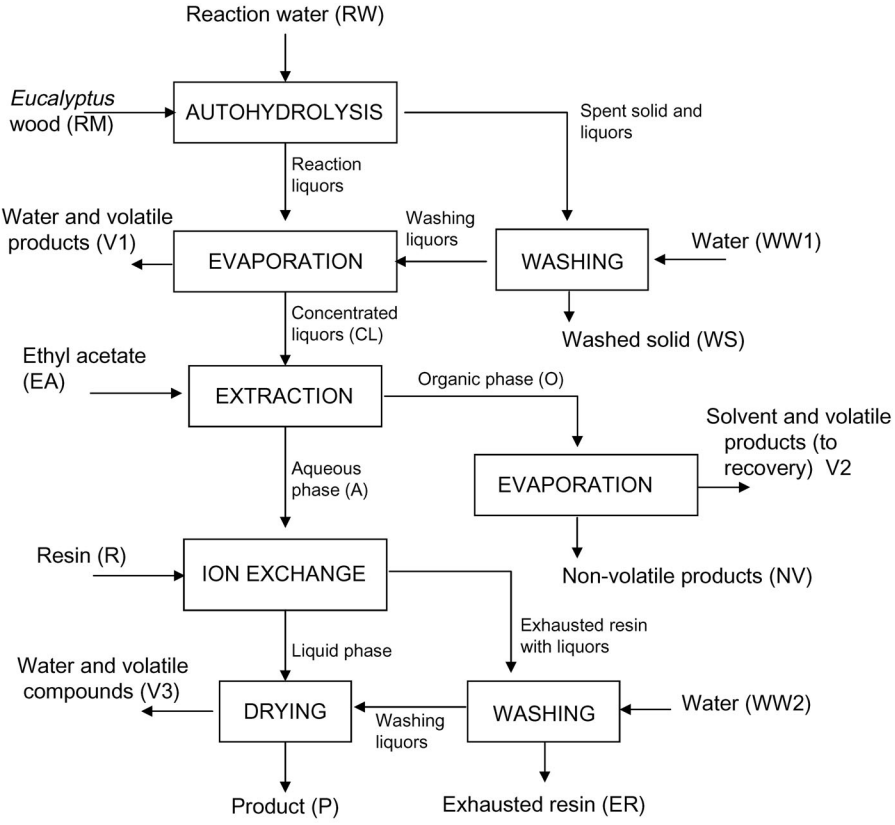

FIG. 2
Scheme of the process considered

weight of the aqueous phase from cross-flow extraction (stream A in Fig. 2) accounted for a part of the CL weight, and ethyl acetate was incorporated into the solid phase to reach the solubility concentration. However, it can be noted that the presence of volatile compounds in stream A is not relevant for the purposes of this work because they are removed when drying the refined product (see Fig. 2).

The compositional data in Tables III and IV show that ethyl acetate extraction resulted in the partial removal of the ONVC fraction contained in the feed stream (stream CL), causing a reduction of the ONVC mass fraction from 0.1667 in stream CL to 0.0919 in stream A. Interestingly, the ethyl acetate-soluble fraction from hydrolytic processing of biomass shows antioxidant properties with potential food applications[4,5]. Ethyl acetate extraction also resulted in complete removal of acetic acid, a compound that could be separated in the solvent recovery stages. A small part of SXO (3.66% of the amount contained in stream CL) was lost in the organic phase.

Refining of ethyl acetate-extracted liquors was assayed by ion exchange using the resins mentioned above (Amberlite IRA 400 and Amberlite IRA 96). Table V shows the composition of the streams obtained after treaments with these resins (denoted P in Fig. 2). The content of SXO was similar in both cases (with weight fractions of 0.7283 and 0.7179 for Amberlite IRA 400 and Amberlite IRA 96, respectively), whereas the percentage of NVC recovery for Amberlite IRA 400 was 93.5% in comparison with 91.2% for

TABLE III
Composition of the NVC components of the concentrated liquors (stream CL in Fig. 2)

Component	Weight fraction, kg/kg NVC
Glucose	0.0076
Xylose	0.1340
Arabinose	0.0205
Glocooligosaccharides	0.0346
Xylooligosaccharides	0.4510
Arabinooligosaccharides	0.0000
Acetyl groups in oligosaccharides	0.1160
Uronic acid groups	0.0696
ONVC[a]	0.1667

[a] Other non-volatile components.

Amberlite IRA 96. Minor comparative advantages were also confirmed for Amberlite IRA 400 in relation to the lower losses of xylooligosaccharides in resins (2.35% in comparison with 7.63% for Amberlite IRA 96) and to the superior purification effect (with a lower ONVC weight fraction in the case

TABLE IV
Composition of the NVC fraction of the aqueous phase from ethyl acetate extraction (stream A in Fig. 2)

Component	Mass fraction, kg/kg NVC
Glucose	0.0078
Xylose	0.1521
Arabinose	0.0188
Glocooligosaccharides	0.0384
Xylooligosaccharides	0.4973
Arabinooligosaccharides	0.0000
Acetyl groups in oligosaccharides	0.1192
Uronic acid	0.0745
ONVC[a]	0.0919

[a] Other non-volatile components.

TABLE V
Composition of the final product (stream P in Fig. 2)

	Amberlite IRA 400 weight fraction kg/kg NVC	Amberlite IRA 96 weight fraction kg/kg NVC
Glucose	0.0077	0.0074
Xylose	0.1633	0.1636
Arabinose	0.0232	0.0233
Glocooligosaccharides	0.0413	0.0417
Xylooligosaccharides	0.5118	0.5001
Arabinooligosaccharides	0.0000	0.0000
Acetyl groups in oligosaccharides	0.1207	0.1208
Uronic acid groups	0.0545	0.0553
ONVC[a]	0.0776	0.0879

[a] Other non-volatile components.

of Amberlite IRA 400). The weight fraction of ONVC in stream P in the experiment with Amberlite IRA 400 was 0.0776, a value that compares well with the results reported for other xylooligosaccharide-refining processes[19–21]. For example, autohydrolysis liquors from eucalyptus wood have been processed by two-step reaction, ethyl acetate extraction, solvent precipitation and freeze-drying extraction, to yield concentrates with up to 86.2 wt.% of substituted oligosaccharides, but with comparatively low recovery yields[22]. Starting from crop residues, a process based on two-stage extraction, chromatographic separation and ion exchange led to concentrates with similar composition[21]. Additional information on xylooligosaccharide purification processes is available in a recent review[23].

Material Balances

Table VI shows material balances for the whole processing scheme shown in Fig. 2 with the data determined for IRA 400, based on 100 kg of oven-dry wood containing 9.55 kg moisture, and assuming the generation of 4.84 kg of volatile reaction compounds and 24.21 kg of non-volatile reaction compounds in hydrothermal treatments. This table provides information on the distribution of the components of interest (volatile compounds, non-volatile compounds, monosaccharides, total oligosaccharides and ONVC) at the various processing stages. Inconsistencies of minor importance (caused by experimental error) were observed when the monosaccharide content of stream ER was calculated from material balances.

Further Characterisation of the ONVC Fraction

Considering that additional information on the composition of the ONVC fraction might provide valuable information concerning its behaviour as a potential food ingredient, the visible-UV spectra of aqueous solutions of the product P obtained with IRA 400 were recorded. The spectra showed typical absorption maxima for phenolic compounds, which were quantified as acid-soluble lignin after treatment with 4% sulfuric acid to estimate an acid-soluble lignin content of 4.05%. It can be noted that bonded phenolics show antioxidant properties, which could confer additional functional properties to the concentrate.

On the other hand, elemental analysis showed the presence of nitrogen in the final isolate. Expressed as a protein equivalent, it corresponded to 0.85 wt.% of stream P but, under the operational conditions employed, proteins must have reacted with carbohydrates to give Maillard reaction prod-

ucts with an undefined stoichiometry, which should account for a higher percentage of the final product.

TABLE VI
Material balance for the purification process with Amberlite IRA 400 (based on 100 kg oven-dry wood containing 9.55 kg water as moisture), assuming the generation of 4.84 kg volatile reaction products and 24.21 kg non-volatile reaction products in hydrothermal treatment

Stream[a]	NVC kg	VC kg	Total kg	Monosaccharides kg	TO[b] kg	ONVC kg
RW	790.45	0	790.45			
WW1	800.00	0	800.00			
WS	182.68	70.95	253.62			
CL	202.41	24.21	226.62	3.93	16.25	4.04
V1	1219.76	0	1219.76			
EA	679.86	0	679.86			
A	185.09	21.47	206.56	3.84	15.66	1.97
NV	0	2.75	2.75	0.09	0.59	2.06
V2	697.18	0	697.18			
R	3.38	10.33	13.71			
WW2	206.56	0	206.56			
ER	15.62	11.71	27.33	~0	1.03	0.42
V3	379.41	0	379.41			
P	20.080	0	20.08	3.90	14.62	1.56

[a] Streams of the considered process (see Fig. 2). [b] TO, total oligosaccharides.

CONCLUSIONS

Eucalyptus globulus wood samples were subjected to hydrothermal treatments for converting their hemicelluloses (acetylated glucuronoxylan) into soluble products. Oligosaccharides were the main reaction products, but other saccharide and non-saccharide compounds were also present in the reaction media. The liquors obtained in hydrothermal treatment carried out under optimal conditions were concentrated, extracted with ethyl acetate (3 steps) and subjected to ion exchange with Amberlite IRA 400 or Amberlite IRA 96 to increase their oligosaccharide content. The weight fractions of the non-volatile components of liquors (NVC) were measured, and

material balances were carried out to assess the distribution of the components of interest (volatile compounds, non-volatile compounds, monosaccharides, oligosaccharides and ONVC) in streams of each processing stage. The highest purification degree corresponded to operation with Amberlite IRA 400, which led to a final isolate (made up of monosaccharides, oligosaccharides and non-volatile, non-saccharide components ONVC) containing 92.2 wt.% of saccharides and 7.76 wt.% of non-saccharide components (mainly components of the acid-soluble lignin fraction).

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