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Sugar, acid and furfural quantification in a sulphite pulp mill: Feedstock, product and hydrolysate analysis by HPLC/RID

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ABSTRACT

Waste from pulp and paper mills consist of sugar-rich fractions comprising hemicellulose derivatives and cellulose by-products. A complete characterisation of the waste streams is necessary to study the possibilities of an existing mill. In this work, four chromatographic methods have been developed to obtain the most suitable chromatographic method conditions for measuring woody feedstocks, lignocellulosic hydrolysates and cellulose pulp in sulphite pulping processes.

The analysis of major and minor monosaccharides, aliphatic carboxylic acids and furfurals has been optimised. An important drawback of the spent liquors generated after sulphite pulping is their acidic nature, high viscosity and adhesive properties that interfere in the column lifetime. This work recommends both a CHO-782Pb column for the sugar analysis and an SH-1011 resin-based cross-linked gel column to separate low-molecular-weight chain acids, alcohols and furfurals. Such columns resulted in a good separation with long lifetime, wide pH operating range and low fouling issues.

1. Introduction

There is a growing demand for lignocellulosic materials used as feedstocks for chemical conversion into bio-based polymers, chemicals, biofuels or energy. Their high availability and low cost and the energetic demand problem suffered in Europe have placed a heavy emphasis on the need for rapid and reliable analysis methods for the complete characterisation of the aforementioned materials [1–3]. Many authors are currently working on improvements of all of the steps to transform lignocellulosic biomass into useful products, including fractionation [4–6], detoxification [7,8], hydrolysis and saccharification [6,9–11] and fermentation [12,13]. In addition, other factories using lignocellulosic biomass are being transformed into biorefineries because they have just some of these processes introduced in the plants. In this sense, pulp and paper mills are perfect candidates to convert lignocellulosic waste materials into several bio-products within the biorefinery concept.

Environmental friendly methods have been recently implemented in pulp mills to reduce their environmental impact and to compete in the current market, ensuring sustainable principles. Among the material valorisation alternatives are (i) sugar fermentation to high value-added products such as ethanol [14,15], single-cell protein [16–18], pharmaceuticals, paper pulp, compost or energy; (ii) xylooligomers having food and pharmaceutical applications; and (iii) chemical products such as lignin producing vanillin, and furfural, a chemical intermediate for the manufacture of polymers, furfuryl alcohol or tetrahydrofuran. All the aforementioned alternatives require an accurate quantitative method for monosaccharides and sugar-derived compounds analysis.

1.1. Overview of the procedures suitable for the carbohydrate analysis of lignocellulosic feedstocks

Because a consensus about the complete analysis of lignocellulosic carbohydrates does not exist, an overview of the main available characterisation techniques for these types of feedstocks is provided. The main methods reported are displayed in Table 1. An extensive variety of techniques was found.

Gas chromatography (GC) of alditol acetates constitutes the standardised method for carbohydrate biomass feedstocks [19]. The first application of GC to carbohydrates was reported in 1958, and it described the separation of fully methylated monosaccharides. GC of alditol acetates is widely used for determining the composition of monosaccharide mixtures, being better resolved than the other commonly used derivatives. In contrast, the current methods for preparing alditol acetates involve relatively long acetylation times at elevated temperatures [20] using hazardous reagents. The Gas Chromatography

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Nomenclature

HPLC	High performance liquid chromatography
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
PC	Paper chromatography
LC	Liquid chromatography
NLPC	Normal phase liquid chromatography
LEC	Liquid exchange chromatography
SFC	Super fluid chromatography
CE	Capillary electrophoresis
HILIC-ELSD	Hydrophilic interaction liquid chromatography with evaporative light scattering detection
HPAEC-PAD	High performance anion exchange chromatography with pulsed amperometric detection
HPSEC	High performance size exclusion chromatograph
HPLC-APCI-MS	High performance liquid chromatographic with atmospheric pressure chemical ionisation mass spectrometry
RP-HPLC-CEAD	Reverse phase-high performance liquid chromatography colorimetric electrode array detection
FT-Raman	Fourier transform raman spectroscopy
FTIR	Fourier transform infrared spectroscopy
NMR	Nuclear magnetic resonance
HPLC-RID	High performance liquid chromatography with refractive index detector
SSL	Spent sulphite liquor
LS	Lignosulphonate
DNS	3,5-dinitrosalicylic acid
HMF	5-hydroxymethylfurfural
WSSL	Weak spent sulphite liquor
TSSL	Thick spent sulphite liquor
LCBR	Lignocellulosic biorrefineries
CP	Crude pulps
BP	Bleached pulps
HWDK	Hardwood dissolving krafp pulp
TMP	Thermomechanic pulp
HWPK	Hardwood paper kraft pulp
SWA	Softwood aspen
HWE	Hardwood eucalypt
HWP	Hardwood parkia

Mass Spectrometry (GC–MS) of carbohydrate derivatives has also been extensively used, but there are some limitations generated by the low volatility of these derivatives [21]. Paper chromatography (PC) has also been traditionally applied for carbohydrate quantification in wood samples [22,23]. Nevertheless, PC and GC have the disadvantage of requiring extensive sample preparation, resulting in lengthy and tedious procedures. Currently, the analysis of monosaccharides and more complex carbohydrates is often performed by column liquid chromatography (LC) techniques. Normal-phase liquid chromatography (NPLC), ligand-exchange chromatography (LEC), supercritical fluid chromatography (SFC) and capillary electrophoresis (CE) have been reported by Karlsson et al. [24], who developed a method using hydrophilic interaction liquid chromatography with evaporative light scattering detection (HILIC-ELSD) to separate monosaccharides in glycoprotein.

There are also chromatography techniques such as high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), high-performance size exclusion chromatography (HPSEC), high-performance liquid chromatographic with atmospheric pressure chemical ionisation mass spectrometry (HPLC-APCI-MS) and reverse phase-high performance liquid chromatography colorimetric electrode array detection (RP-HPLC-CEAD).

There are semi-quantitative, qualitative and quantitative non-chromatographic techniques, also summarised in Table 1, such as

Table 1
Review of analytical techniques to carbohydrates and degradation products determination in lignocellulosic feedstocks.

Sample	Technique	Detector	References
Chromatographic techniques for sugar and derived products analysis			
wood and pulp samples	GC Gas Chromatography	MS Mass Spectrometry	[19,39–41]
lignocellulosic feedstocks	HPAEC High Performance Anion Exchange Chromatography	PAD Pulsed Amperometric Detection	[26,42–45]
eucalypts, corn cob, brewery's spent grain	HPSEC High Performance Size Exclusion Chromatography	MS Mass spectrometry	[26,44]
standard mixtures	HILIC Hydrophilic Interaction Liquid Chromatograph	ELSD Evaporative Light Scattering Detection	[25]
wood kraft black liquors	HPLC-APCI High Performance Liquid Chromatography with Atmospheric Pressure Chemical Ionisation	MS Mass Spectrometry	[46]
food plants	RP-HPLC Reverse Phase High Performance Liquid Chromatography	CEAD Colorimetric Electrode Array Detection	[47]
lignocellulosic feedstocks	HPLC High Performance Liquid Chromatography	RID Refractive Index Detector	[1,22,25–38]
softwood, hardwood species & kraft liquors	HPLC High Performance Liquid Chromatography	UV Ultraviolet detector	[48–50]
eucalypt extracts, bagasse hydrolysates & orange juice samples	HPLC High Performance Liquid Chromatography	DAD Diode Array Detector	[33,43,51]
Non-Chromatographic techniques for sugar and derived products analysis			
eucalyptus nitens,trabutii, camaldulensis, globulus	FT-Raman Raman Spectroscopy		[52]
softwood and hardwood hydrolysates	FTIR Fourier Transform Infrared Spectroscopy		[48,53,54]
wood and spent liquors	NMR Nuclear Magnetic Resonance		[17]

Fourier transform Raman spectroscopy (FT-Raman), Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) to identify functional groups and empirical and structural formulas.

Among the analytical techniques highlighted in Table 1, HPLC coupled with refractive index detector (RID) is the most promising, rapid and reliable analytical technique for the sugar quantification of lignocellulosic hydrolysates. In addition, an overview of the chromatographic columns suitable for sugars, acids and furfurals was also carried out. Among the chromatographic columns used within the HPLC analysis technique, Bio-Rad columns were previously used for the neutral sugar, uronic, furan derivative and organic acid quantification of softwoods and hardwoods [1,12,20–22], hydrolysates [22–25] and other types of lignocellulosic feedstocks [21,25–30]. Lead Pb^{+2} columns are better candidates in the case of monosaccharide characterisation, as are hydrogen H^{+} columns in the case of acids and furfurals [31]. Nevertheless, such columns do not resist too much under acidic conditions. In this paper, other columns based on lead Pb^{+2} and hydrogen H^{+} were tested and proposed as the best options for the quantitative analysis of lignocellulosic carbohydrates.

1.2. Framework and objectives

This research contemplates the development of suitable and efficient analysis procedures to quantify monosaccharides and other derivative compounds of woody biomass generated in a pulp mill. The main components of the lignocellulosic residue provided from a sulphite pulp mill were analysed. Once the pulp is formed, subsequent wood digestion under acidic conditions produces lignin and hemicellulose, which pass through the residual aqueous phase. The spent sulphite liquor (SSL) is a renewable source containing a large proportion of lignin in the form of lignosulphonates, depolymerised hemicelluloses, acids, tannins and furfurals [4]. Nevertheless, the characterisation of the SSL can introduce problems due to the acidic and corrosive nature of the liquor, caused by the residual SO_2 content that reduces the column lifetime. Additionally, the high lignosulphonate (LS) concentrations presented in the SSL cause fouling problems. The columns must be subjected continuously to cleaning and regenerating cycles because of the high viscosity and sticky properties of the LS. Another issue inherent to carbohydrate characterisation is the separation of the sugar peaks. Wood monosaccharides have a quite similar structure, and therefore much effort is needed to achieve a correct separation of the five major monosaccharides dissolved in the lignocellulosic hydrolysates. Additionally, SSL samples have a strong brown colour, which makes them difficult to analyse colorimetrically, i.e., total or reducing sugars by phenol-sulphuric and 3,5-dinitrosalicylic acid (DNS) methods.

Based on the study of the state of the art and the problems surrounding the sulphite process, this research attempts to find the best chromatographic methods to efficiently analyse lignocellulosic feedstocks, products and waste streams. The available Bio-Rad columns and other cross-linked columns such as SH 1011Shodex and CHO-782 Transgenomic were tested and corroborated. The alternatives checked were suitable for sugar and derived-sugar inhibitor separation and quantification. Because the use of the sugar-rich residues generated in the pulp mill is very important within the biorefinery concept, the present work establishes efficient and fast HPLC methods for wood derivative quantification. Sugars such as hexoses (D-glucose, D-mannose and D-galactose), pentoses (L-arabinose and D-xylose) and deoxyhexoses

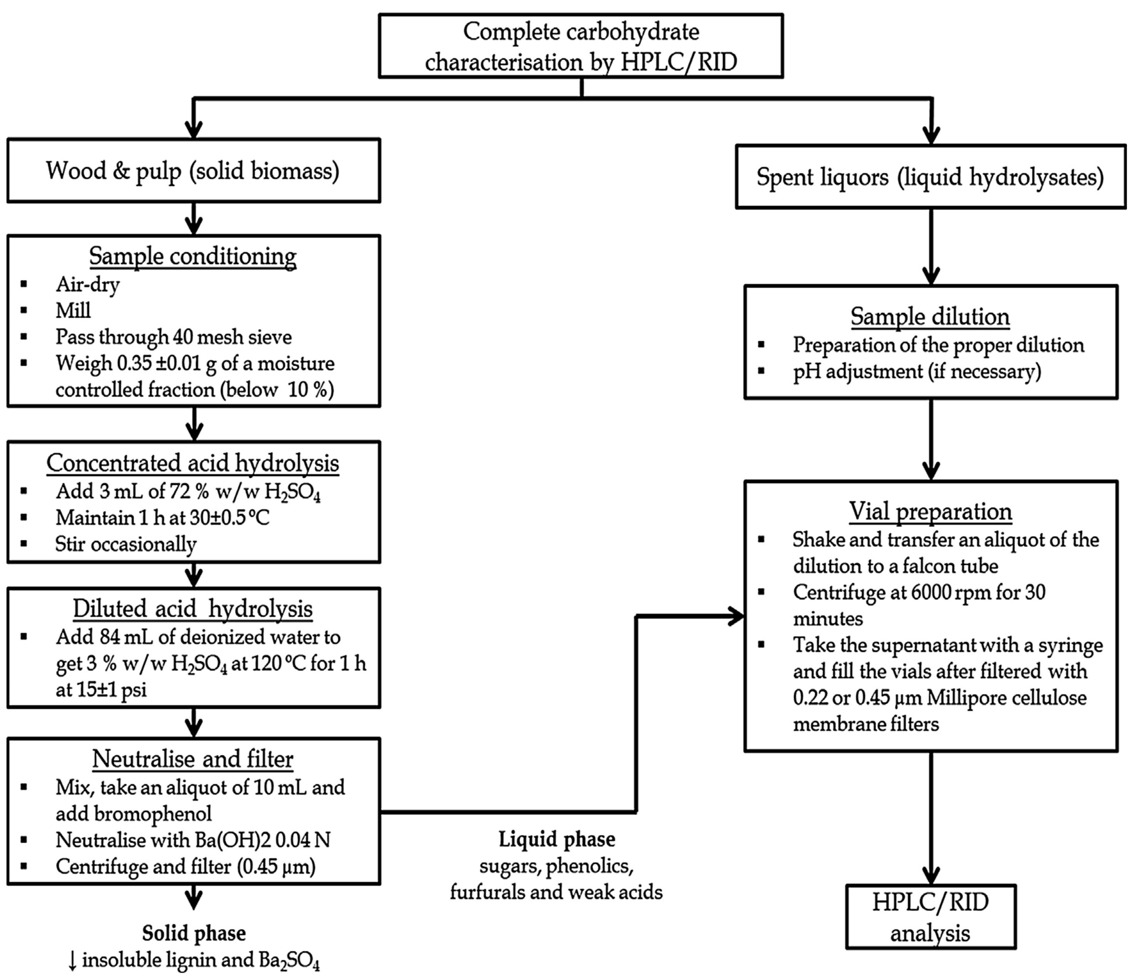

Fig. 1. Methodological approach for total carbohydrate analysis of lignocellulosic samples.

(L-fucose); furfurals, such as furfural and 5-hydroxymethylfurfural (HMF); and aliphatic acids, such as acetic acid, levulinic acid and formic acid, were measured by HPLC/RID.

This paper achieves the complete carbohydrate characterisation of lignocellulosic feedstocks, cellulose pulps and residual hydrolysates. Four chromatographic columns under several conditions were studied to establish the most suitable methods to separate all wood-derived sugars and related compounds in lignocellulosic biomass, which is the most abundant natural feedstock on earth.

2. Materials and methods

2.1. Chromatography system

The HPLC system used was a Shimadzu Prominence LGE-UV (low-pressure gradient system) equipped with a CMB-20A control system, a DGU-20-A5 inline degasser channel, an LC20AD isocratic pump, and an SIL-20AHT auto sampler with thermostatic cooling (samples held at 4 °C), a CTO-20ASVP column oven and an RID-10A refractive index detector. Four cationic exchange columns were employed: (i) two lead Pb^{+2} columns: Aminex HPX-87P, Bio-Rad Inc. (300 mm × 7.8 mm, 9-µm particle size) in combination with a Micro-Guard column and a Transgenomic CHO-782 column (300 mm × 7.8 mm, 7-µm particle size) coupled with a Micro-Guard column and (ii) two hydrogen H^+ columns: Bio-Rad Aminex HPX-87H (300 mm × 7.8 mm, 9-µm particle size) with a Micro-Guard cartridge and a Shodex SH-1011 (300 mm × 8 mm, 6-µm particle size) with a Micro-Guard pre-column. Monosaccharides were quantified using Lead Pb^{+2} columns and acids, and furfurals were quantified using Hydrogen H^+ columns.

2.2. Reagents and standards

HPLC-grade D(+)-glucose, D(+)-galactose, D(+)-xylose, L(+)-arabinose, D(+)-mannose, formic acid, acetic acid and furfural were from Panreac (Barcelona, Spain). Levulinic acid was from Fluka Analytical-Sigma Aldrich (Steinheim, Germany). 5-Hydroxymethyl-2-furaldehyde (HMF) and L(−)-fucose were from Sigma Aldrich (Steinheim, Germany). Sodium hydroxide pellets and sulphuric acid were from Panreac (Barcelona, Spain).

2.3. Samples

Twenty industrial samples of spent liquor, weak spent sulphite liquors (WSSL) before the evaporation step and thick spent sulphite liquors (TSSL) after the multiple-effect evaporation step were analysed. In addition, the solid feedstock (*Eucalyptus globulus* timber) and dissolved pulps were also analysed.

All samples were previously diluted to be within the detection limits and at the same time to adjust the pH. Then, the samples were centrifuged at 5000 rpm and filtered through 0.22 µm filters. Fig. 1 describes the main stages carried out in this research for the complete carbohydrate characterisation of the solid biomass (wood & pulps) and liquid hydrolysates (WSSL & TSSL).

3. Results and discussion

3.1. HPLC-RID methods for sugars, weak acids and furans

A preliminary stage in the pulp and paper (P & P) mill transformation into lignocellulosic biorefineries (LCBR) is to perform an accurate analysis of the lignocellulosic streams generated throughout the process. Therefore, four methods have been developed by testing four chromatographic columns. The optimal conditions have been obtained based on the literature [31,35,36], experimental work carried out in the laboratory, and the threshold limit values shown in Table 2.

The flow, pressure, temperature and injection volume were optimised. The mobile phase was fixed in ultrapure water (HPX-87P and CHO-872 columns) and 0.005 M H_2SO_4 (HPX-87H and SH1011 columns). The mobile phase flow, injection volume and column oven temperature were optimised. Such chromatographic parameters significantly affect the residence times and peak resolution. The longitudinal diffusion of the solute in the mobile phase and low mass transfer between the solute and the mobile phase might contribute to band broadening. Nevertheless, a compromise solution was found for each method, giving good peak separation at acceptable retention times.

The calibration curves are shown in Fig. 2. An external standard method was used in all cases. A lineal adjustment force through zero with regression factors (R^2) up to 0.999 was obtained. Standards were prepared in the range of 0.1–3 g/L for furfural, HMF and methanol; from 0.1 up to 10 g/L for acetic, levulinic and formic acids; and in the range of 0.5–20 g/L for sugars.

Ligand exchange is the preferred method for the separation of the tested columns using deionized water (sugars separation) or diluted sulphuric acid (acids and furfurals separation) as the eluent. The negatively charged hydroxyl groups on the carbohydrate molecule interact with the positively charged loaded metal groups. Monosaccharides are eluted by the polar water eluent mobile phase, which competes for sites on the metal ion. Other secondary mechanisms are also involved in the separation of carbohydrates, including size exclusion and normal phase partitioning.

Table 3 shows the main results. The HPX-87P and CHO-782Pb

Table 2
Operating guidelines and specifications of the tested columns.

	HPX-87P	CHO-782Pb	HPX-87H	SH-1011
Resin ionic form	Lead	Lead	Hydrogen	Hydrogen
Support	Sulfonated divinyl benzene-styrene copolymer	Poly styrene-divinylbenzene copolymer	Sulfonated divinyl benzene-styrene copolymer	Poly styrene-divinylbenzene copolymer
Max. Pressure	1500 psi	1100 psi	1500 psi	725 psi
Max. Flow	1 mL/min	0.7 mL/min	Unknown f(Pmax)	1.5 mL/min
Max Temp.	85 °C	95 °C	65 °C	95 °C
Mobile phase	Ultrapure Water	Ultrapure Water	0.005 M H_2SO_4	0.005 M H_2SO_4
pH range	5–9	1–14	1–3	0–14
Guard column	Micro-guard cartridge 125–0119	CARBOSep CHO-99-2354	Micro-guard cartridge 125-0129	SH-G SUGAR
Cleaning solvent (reverse column)	30% CH_3CN in water, 4 h, 25 °C, 0.2 mL/min	50% CH_3CN in water 0.1 mL/min 65 °C	65 °C, 0.2 mL/min 1) 4 h 5% CH_3CN in 0.005 M H_2SO_4 2) 12 h 30% CH_3CN in 0.005 M H_2SO_4	1 mL/min 0.005 M H_2SO_4, 15 min

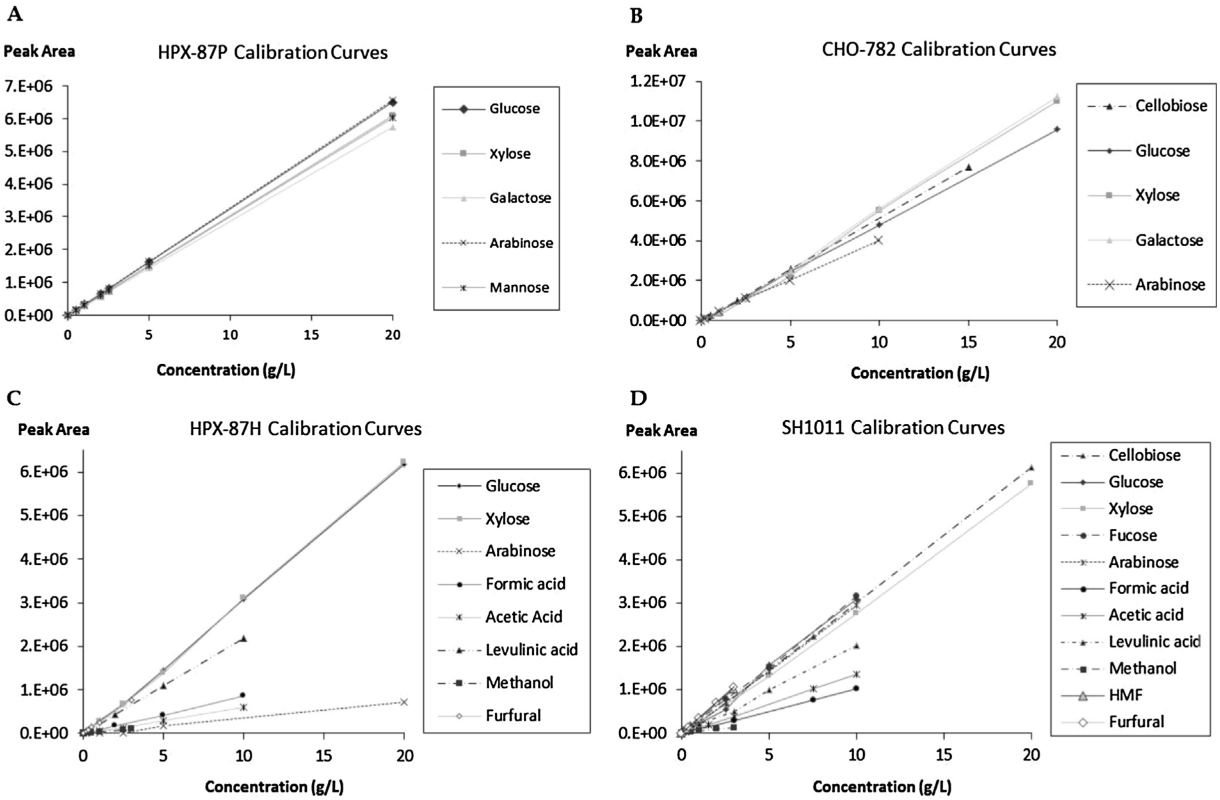

Fig. 2. Calibration curves for sugars, acids, methanol and furfurals.

columns separate the major sugars adequately. The major C6 sugars, such as glucose, galactose and mannose, and major C5 sugars, such as xylose and arabinose, could be integrated and separated in both the standards and liquor samples. The HPX-87H and SH-1011 columns are suitable to analyse cellobiose and sugars such as glucose, xylose and arabinose qualitatively. However, a quantitative approximation value of only xylose could be calculated by means of those columns because the peaks of galactose, mannose, and xylose co-eluted at the same time. In this case, it can be assumed that the peak is mostly xylose, the major sugar presented in the WSSL and TSSL samples. In addition, the HPX-87H and SH-1011 columns are capable of the analysis of acetic, formic and levulinic acids, as well as methanol and ethanol. Furfural and HMF are separated mainly by an SH1011 column because it has lower detection limits. In the case of using HPX-87H, good regression factors could be obtained at concentrations higher than 0.2 g/L; however, the furan con-centration in SSL is under 0.2 g/L in most cases.

It can be concluded that CHO-782Pb and SH-1011 are the most adequate for measuring monosaccharides and other hydrolysis by-products in the studied samples. CHO-782Pb operates at a wider pH range in comparison to HPX-87P. Taking into account that the liquor samples are acidic (pH = 1–3), working with the HPX-87P column, it is necessary to neutralise the sample, which can interfere in the liquor analysis (soluble sugars might precipitate and not be detected). Of the hydrogen-based columns, SH-1011 is preferred because of the detection limits, regression coefficients and wider pH interval. In addition, the fouling of lead ionic columns (CHO-782Pb and HPX-87H) occurs frequently, increasing the pressure system, making cleaning and regeneration protocols necessary to take care of the columns over their lifetime. Depending on the components of interest, it is preferable to analyse with SH-1011 or HPX-87H, which provide more information on separating acids, furfurals, alcohols and some major monosaccharides and avoid the fouling problems.

A correct separation of organic aliphatic acids, alcohols and furfurals is possible with the SH-1011 column. The only concern is in the sugar separation. Xylose, the major pentose contained in *Eucalyptus globulus* and consequently the SSL, co-eluted with mannose and

Table 3
Standards and method conditions.

Column	Components	Standards (g/L)	Retention times (min)	R^2	Method Conditions
HPX 87P	Sugars	0.5–5	25.01–33.07	0.99940–0.99993	0.3 mL/min ultrapure water, 79 °C, 20 μL, 940 psi
CHO-782Pb	Sugars	0.2–10	22.07–35.70	0.99984–0.99999	0.3 mL/min ultrapure water, 68 °C, 20 μL, 453 psi
HPX-87H	Sugars	0.1–10	9.21–13.66	0.99936–0.99988	0.5 mL/min H_2SO_4 0.005 M, 30 °C, 20 μL, 975 psi
	Acids	0.2–10	17.48–21.64	0.99925–0.99998	
	Alcohols	0.2–10	22.67–25.26	0.99950–0.99953	
SH-1011	Sugars	0.2–10	13.33–18.04	0.99922–0.99992	0.5 mL/min H_2SO_4 0.005 M, 60 °C, 20 μL, 198 psi
	Acids	0.2–1.0	21.03–24.11	0.99931–0.99998	
	Alcohols and Furfurals	0.5–5	27.36–66.46	0.99980–0.99997	

Table 4
Results of sugars, intermediates and inhibitors in SSL.

WSSL	Col. HPX-87P[a]	Col. CHO-782[b]	Col. HPX-87H[c]	Col. SH-1011[d]
Cellobiose (g/L)	–	2.24 ± 0.18	–	2.36 ± 0.90
Glucose (g/L)	4.53 ± 1.63	4.12 ± 0.72	1.67 ± 0.45	2.35 ± 0.72
Xylose (g/L)	23.6 ± 9.69	15.6 ± 3.05	26.2 ± 3.87	25.0 ± 6.23
Galactose (g/L)	3.70 ± 1.67	2.93 ± 0.89	–	–
Arabinose (g/L)	3.07 ± 1.88	1.53 ± 0.60	1.02 ± 0.89	1.67 ± 0.39
Mannose (g/ L)	1.56 ± 1.66	1.45 ± 0.87	–	–
Fucose (g/L)	–	1.10 ± 0.59	–	0.63 ± 0.08
Formic acid (g/L)	–	–	0.032 ± 0.005	0.029 ± 0.002
Acetic acid (g/L)	–	–	9.56 ± 1.53	6.93 ± 1.87
Levulinic acid (g/L)	–	–	0.0154 ± 0.003	0.0123 ± 0.001
Methanol (g/L)	–	–	2.03 ± 0.38	0.5542 ± 0.10
HMF (g/L)	–	–	< DL	0.022 ± 0.01
Furfural (g/L)	–	–	0.43 ± 0.014	0.170 ± 0.06
TSSL	**Col. HPX-87P[a]**	**Col. CHO-782[b]**	**Col. HPX-87H[c]**	**Col. SH-1011[d]**
Cellobiose (g/L)	–	23.0 ± 1.87	–	16.0 ± 3.04
Glucose (g/L)	27.6 ± 10.8	23.8 ± 7.29	9.36 ± 3.38	14.9 ± 2.21
Xylose (g/L)	114 ± 16.7	138 ± 17.1	145 ± 13.7	164 ± 19.4
Galactose (g/L)	17.8 ± 3.94	22.8 ± 7.22	–	–
Arabinose (g/L)	17.5 ± 7.75	12.7 ± 4.20	1.98 ± 0.23	11.4 ± 1.22
Mannose (g/L)	9.05 ± 8.72	10.8 ± 6.45	–	–
Fucose (g/L)	NM	10.1 ± 7.75	–	3.68 ± 0.40
Formic acid (g/L)	–	–	0.341 ± 0.071	0.228 ± 0.090
Acetic acid (g/L)	–	–	7.79 ± 1.27	5.03 ± 0.90
Levulinic acid (g/L)	–	–	0.151 ± 0.03	0.111 ± 0.02
Methanol (g/L)	–	–	3.63 ± 1.43	1.04 ± 0.16
HMF (g/L)	–	–	< DL	0.13 ± 0.05
Furfural (g/L)	–	–	0.20 ± 0.05	0.12 ± 0.09

[a] Method: 0.3 mL/min H_20, 79 °C, 20 µL,940psi.
[b] Method: 0.3 mL/min H_2O, 68 °C, 20 µL,450psi.
[c] Method: 0.5 mL/min 0,05 M H_2SO_4, 30 °C,20 µL,975psi.
[d] Method: 0.5 mL/min 0,05 M H_2SO_4, 60 °C,20 µL,198psi.

galactose, and the only solution is to consider this peak as only xylose. For all these reasons, both the CHO-782 and SH-1011 columns are recommended in this work as the most adequate solutions for the separation of monosaccharides and low molecular weight organic derivatives in lignocellulosic samples.

3.2. WSSL and TSSL characterisation

Twenty samples of industrial liquors were analysed: weak spent sulphite liquors (WSSL) collected at the inlet of the evaporation plant and thick spent sulphite liquors (TSSL) collected at the end of the plant. The average results of sugars, organic acids and furfurals in g/L of the twenty samples collected are shown in Table 4. The heterogeneity of the liquor samples depends on many factors such as the wood used as raw material and the cooking conditions (residence time, pressure and temperature reached all over the process).The results do not depend strongly on the chromatographic method applied in every single case. The best average values are obtained using the two proposed methods, with the CHO-782Pb and SH-1011 columns. Comparing the results of Table 4 with those of other authors, similar results were obtained, and therefore the chromatographic methods tested are adequate for these types of samples. Total monosaccharide contents in the range of 29.1–43.2 g/L for WSSL and 75.6–145.2 g/L for TSSL; total acid contents in the range of 8.2–10.3 g/L for WSSL and 4.2–12.6 g/L for TSSL; and total furfural contents in the range of 0.1–0.2 g/L in WSSL and lower than 0.06 g/L in TSSL were found in the literature [15,55,56]. The industrial liquor samples collected registered total monosaccharide contents in the range of 26.7–36.5 for WSSL and 185–214 g/L for TSSL; total acid contents in the range of 8.75–9.61 g/L for WSSL and 8.19–8.28 g/L for TSSL; and total furfural contents between 0.43–0.52 g/L for WSSL and 0.20–0.27 g/L for TSSL.

3.3. The complete carbohydrate analysis through the pulp mill by HPLC/RID

The final standards and sample chromatograms are presented in Fig. 3. Peaks 1–13 correspond to (1) cellobiose, (2) glucose, (3) xylose, (4) galactose, (5) fucose, (6) arabinose, (7) mannose, (8) formic acid, (9) acetic acid, (10) levulinic acid, (11) methanol, (12) HMF, and (13) furfural. Biorad HPX-87P and Transgenomic CHO-782Pb columns were adequate to separate the sugars. The major C6 sugars, such as glucose, galactose and mannose, and major C5 sugars, such as xylose and arabinose, could be integrated and separated from mixed standards and liquor samples. Biorad HPX-87H and Shodex SH-1011 columns are not the best choice for sugar quantification since the peaks of galactose, mannose, and xylose co-eluted and overlapped, and it was only possible to assume that the peak belonged to xylose, the major sugar of the SSL samples. Nevertheless, HPX-87H and SH-1011 separate furfurals and carboxylic acids. Furfural and HMF are separated mainly by the SH1011 column because it has lower detection limits.

Once the methods were performed and optimised for the spent liquors (SSL), Eucalypt hardwood (HW), crude pulps (CP), and bleached pulps (BP) were analysed with the selected columns: Shodex SH1011 and Transgenomic CHO-782.

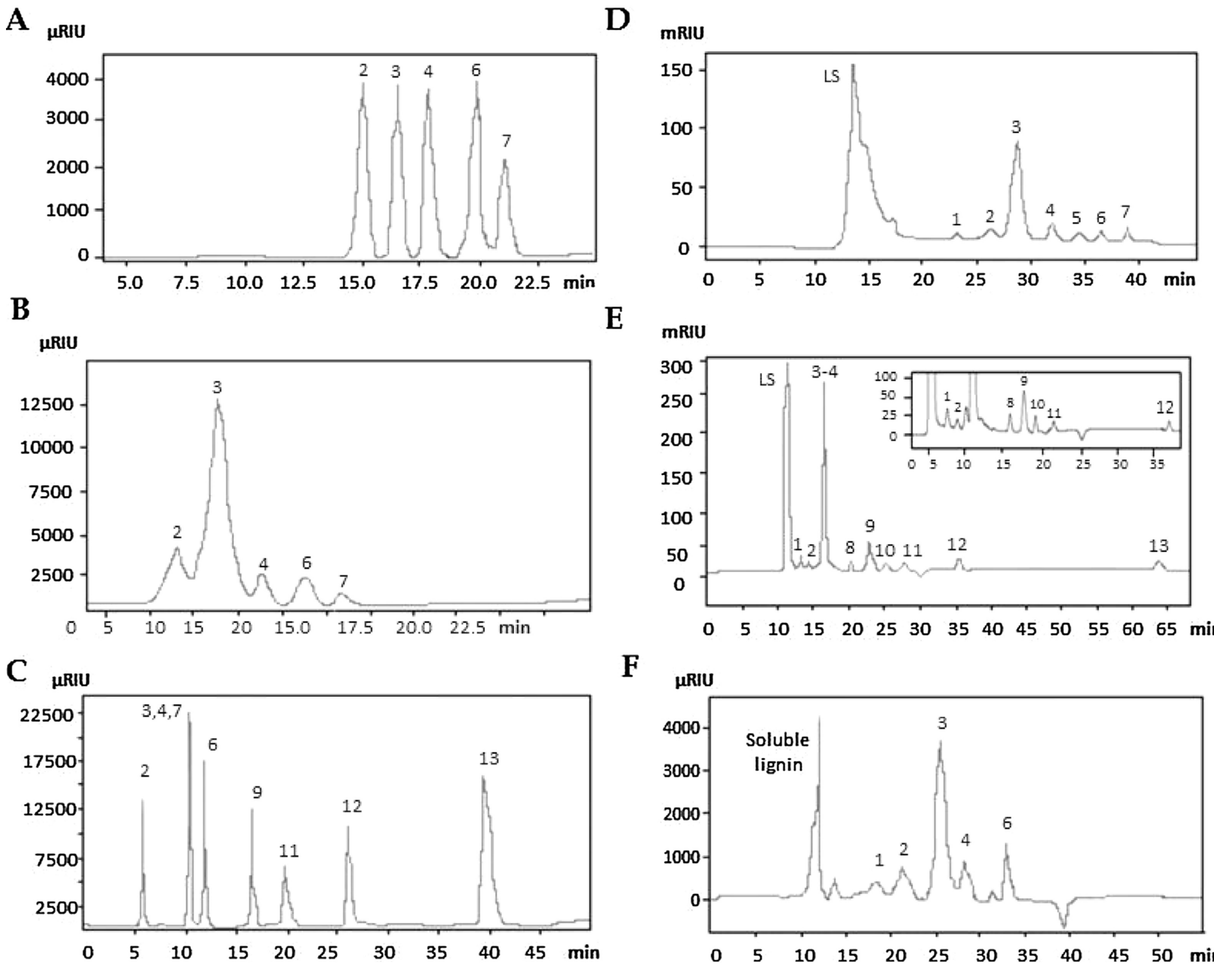

Fig. 3. (a) Chromatogram of monosaccharides passed through the HPX-87P column; (b) chromatogram of the SSL using the HPX-87P column; (c) sugars, acids and furfurals standards in the HPX-87H column; (d) chromatogram of the SSL using the CHO-782 column; (e) chromatogram of the SSL using the SH 1011 column; (f) chromatogram of wood and pulp hydrolysates using the CHO-782 column.

The monosaccharide composition is presented in Fig. 4a. Fig. 4b shows a comparison with different paper-grade [58,59,63] or dissolving-grade pulps [64,65] produced from hardwood or softwood. HWDK, SWDK, TMP, HWPK were hardwood dissolving grade from the kraft process, softwood dissolving grade from the kraft process, and thermomechanical pulp (the worst quality) and hardwood paper grade from the kraft process, respectively. It can be observed that glucan is the predominant homopolymer in all types of pulp regardless of their quality. However, TMP barely reaches 64.4% of the total carbohydrates because of the high amount of lignin that still remains in the pulp.

Fig. 4c shows a comparison between different softwood [1] and hardwood [25,61] species. SWA, HWE and HWP were softwood Aspen, hardwood Eucalyptus and hardwood Parkia, respectively. It should be noted that Fig. 4c does not show any content higher than 70% w/w because lignin is not graphed. Only the carbohydrate fraction (hemicellulose and cellulose) was considered. It can be assumed that the chromatographic methods evaluated in this research are also suitable for the wood and pulp carbohydrate quantification. These methods were successfully applied within different lignocellulosic samples: wood, pulp and bleached pulps [60,11], detoxified liquors [62,63], weak and thick liquors [64], paper and dissolving grade liquors [65,66].

4. Conclusions

The analysis of sugar and other decomposition products from cellulose and hemicellulose quantification have always been a complex issue, especially in the case of acid sulphite pulping samples, because of the acidic nature of the samples, their high viscosity, colour, high amount of suspended solids, adhesive properties of the lignosulphonates and heterogeneity. In this study, four chromatographic methods for separating monosaccharides, organic acids and furfurals in the effluent streams of a sulphite pulp mill have been developed.

The results showed that these methods are able to analyse not only the wastewater streams but also the feedstock and main product of the factory in a quick and reliable way. Such methods permit the analysis of the following compounds: cellobiose, glucose, xylose, galactose, arabinose, mannose and fucose; levulinic, formic, and acetic acids; HMF and furfural.

The structures of the sugars and their physico-chemical properties are quite similar, which posed a challenge for separating the C5 and C6 peaks. The best integration of the sugar results was obtained with HPX-87P Bio-Rad and CHO-782Pb Transgenomic columns. The HPX-87H Bio-Rad and SH-1011 Shodex columns, which operate with diluted sulphuric acid as the mobile phase, were also demonstrated to be more adequate to separate low molecular weight chain acids, alcohols and

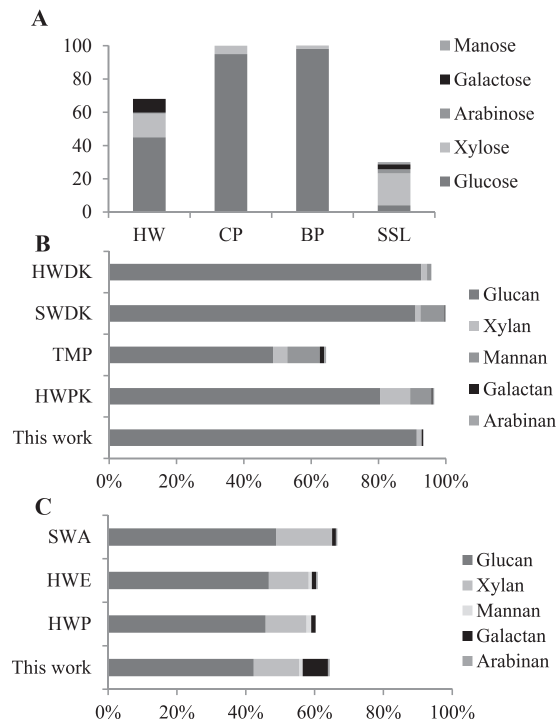

Fig. 4. (a) Monosaccharides composition within the pulp mill: hardwood (HW) feedstock, crude pulp (CP), bleached pulp (BP) and the hydrolysate so-called spent sulphite liquor (SSL); (b) Comparison with different quality pulps: Hardwood Dissolving Kraft Pulp (HDWK), Softwood Dissolving Kraft Pulp (SWDK), Thermomechanic pulp (TMP), Hardwood Paper Kraft Pulp (HWPK); (c) Comparison with different wood species: softwood aspen (SWA), hardwood eucalypt (HWE) and hardwood parkia (HWP).

furfurals in the samples studied. This work recommends the CHO-782Pb and SH-1011 columns because of their longer lifetimes, wider pH operating ranges and lower fouling effects in comparison with the HPX-87P and HPX-87H columns.

Declaration of interest

All authors of paper entitled "SUGAR, ACID AND FURFURAL QUANTIFICATION IN A SULPHITE PULP MILL: FEEDSTOCK, PRODUCT AND HYDROLYSATE ANALYSIS BY HPLC/RID" declare to do not have conflict of interest.

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