Microwave-assisted extraction of hemicellulosic oligosaccharides and phenolics from *Robinia pseudoacacia* wood

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ABSTRACT

Microwave-assisted autohydrolysis is an environmentally friendly intensification technology that permits the selective solubilization of hemicelluloses in form of oligosaccharides in a short time and with low energy consumption. The purpose of this work was to evaluate the suitability of microwave-assisted autohydrolysis to produce oligosaccharides and phenolics with potential prebiotic and antioxidant activities from *Robinia pseudoacacia* wood. The influence of treatment time (0–30 min) and temperature (200–230 °C) on oligosaccharide production was studied and conditions of 230 °C and 0.25 min resulted in maximum content of xylooligosaccharides (7.69 g XO/L) and more efficient energy consumption. Furthermore, under those conditions, liquors showed high contents of phenols (80.28 mg GAE/g of RW) and flavonoids (44.51 RE/g) with significant antioxidant activities (112.07 and 102.30 mg TE/g, measured by ABTS and FRAP tests, respectively). Additionally, the solubilized hemicelluloses were structurally characterized by HPAEC-PAD, MALDI-TOF-MS, FTIR and TGA/DSC, and HPLC-ESI-MS analysis allowed the tentative identification of 17 phytochemicals.

1. Introduction

Lately, the current nutritional lifestyle of consumers has been transformed due to the growth in awareness of the nutrition-health relationship (Gullón et al., 2018). Hence, the demand for functional food, that is healthier and provides beneficial features, for instance reducing the risk of diseases, has increased (Holdt & Kraan, 2011; Sagar et al., 2018). In this sense, the design of this functional food enriched with bioactive compounds (namely polysaccharides, soluble fiber, phenolic compounds, fatty acids or peptides) has gained interest in the nutritional market (Rico et al., 2018). Such bioactive compounds can be found naturally in vegetable biomass (Rico et al., 2018; Zhao et al., 2020), and specifically in lignocellulosic biomass (LCB), which has become one interesting feedstock to obtain these bioproducts (Dávila et al., 2016).

LCB comprises non-edible materials that present some beneficial features such as being renewable, available, ubiquitous, sustainable, and inexpensive (Chandel et al., 2020; Wagner et al., 2018), which promotes its use within a biorefinery in order to obtain high-added value chemicals, fuels and/or products (Rajesh Banu et al., 2021). Among the fast-growing trees that can be commercially exploited, *Robinia pseudoacacia* (black locust) is characterized by rapid growth, high regrowth potential, good adaptation to water scarcity, high resistance to stress conditions, and high nitrogen fixation capacity, leading to high biomass yields (14 t ha^{-1} yr^{-1}). Besides that, *Robinia* wood (RW) is mainly composed of structural polysaccharides (45–50 % cellulose and 10–35 % hemicelluloses) being an interesting source for multiple added-value compounds (Kamperidou et al., 2021; Martín-Sampedro et al., 2019; Straker et al., 2015).

In this sense, the hemicellulosic fraction is an important source of xylooligosaccharides (XO), a type of oligosaccharides that are of interest due to their prebiotic and immunostimulating effect, and anti-inflammatory and anti-infective properties (Amorim et al., 2019).

Nevertheless, since cellulose, hemicelluloses and lignin are complexly linked (Pinales-Márquez et al., 2021), a pretreatment to isolate and exploit the hemicelluloses is needed (del Río et al., 2020). Several sorts of pretreatments have been described in the literature for obtaining xylooligosaccharides, such as acidic, alkaline or enzymatic hydrolysis (Rico et al., 2018). However, autohydrolysis (also known as hydrothermal processing) has now been widely evaluated as the first

fractionation stage within a biorefinery concept. It is an environmentally sustainable technology, using only water as a reagent under subcritical conditions (150–230 °C and residence times of 30 to 90 min). Through this treatment, the selective solubilization of hemicelluloses in the form of oligosaccharides is achieved, leaving the cellulose and lignin in the solid phase (del Río et al., 2020; Morales et al., 2020). Nonetheless, autohydrolysis present some drawbacks such as high energy consumption, the hysteresis effect and the intense temperature gradients that take place in the reactor (Dávila et al., 2021). In this sense, the intensification of the autohydrolysis treatment by means of microwaves (MW) could suppose a solution. This technology enables a simultaneous and uniform heating of the sample (radiation is only absorbed by the solvent or biomass) allowing a faster and more efficient heat transfer, which translates into a reduction in reaction times and consumption energy (Aguilar-Reynosa et al., 2017b; Dávila et al., 2021; del Río, Gullón, Romaní, et al., 2021).

The main objective of this work was to evaluate the suitability of microwave-assisted autohydrolysis as the first step of *Robinia pseudoacacia* wood biorefinery. The influence of the temperature and the reaction time on the solubilization of the hemicelluloses was evaluated, the liquors were thoroughly characterized in carbohydrate content, and the energy consumption of the pretreatment was determined. The liquor from the optimal conditions for oligosaccharides (especially XO) recovery was subjected to total phenolic and flavonoids content, antioxidant capacity determination (ABTS, DPPH and FRAP), and structural characterization (FTIR, TGA/DSC, MALDI-TOF-MS, HPAEC-PAD and HPLC-ESI-MS of the ethyl acetate extract of the liquor).

2. Materials and methods

2.1. Feedstock

The feedstock used for this study was *Robinia pseudoacacia* wood (RW), supplied by a local company (Aserradero de Madera Hijos de J. González Groba, S.L., O Porriño, Pontevedra, NW Spain). Once air-dried, RW was milled to a particle size ≤1 mm, appropriate for MW treatment, and it was stored in a cool, dry and dark place until the analysis and its use in experiments.

2.2. Feedstock analysis

RW was chemically characterized by NREL analytical procedures: moisture, ash, extractives, and carbohydrates content. The acid insoluble residue after carbohydrate analysis (quantitative acid hydrolysis) was weighted and determined as Klason lignin. The liquid phase after the carbohydrate procedure was injected in HPLC (Agilent 1200 series model) to determine the monomers (glucose, xylose and arabinose) and acetic acid concentration, using a Rezex ROA-Organic acid H^+ column (Phenomenex) at 60 °C, mobile phase at 0.03 M H_2SO_4 at 0.600 mL/min, and a refractive index detector at 40 °C. Uronic acids were determined using the methodology proposed by Blumenkrantz and Asboe-Hansen (1973) using galacturonic acid standards. The determinations were assayed in three replicates and the chemical composition of the wood, expressed in g/100 g of wood on dry basis ± standard deviation, was as follows: glucan 34.51 ± 0.26, xylan 13.77 ± 0.17, arabinan 0.21 ± 0.04, acetyl groups 2.88 ± 0.04, Klason lignin 23.06 ± 0.36, extractives 13.81 ± 0.00, ashes 0.74 ± 0.00.

2.3. Microwave-assisted autohydrolysis (MAA) of Robinia pseudoacacia

The MAA treatment was performed under an isothermal regime and using a solid loading (SL) of 6 (g of solid/100 g of total weight). The biomass and water mixture was introduced into 30 mL glass vials (Reaction Vial G30 from Anton Paar), specific for use in the microwave (Monowave 450 from Anton Paar GmbH). The blend was magnetically stirred at 900 rpm. During the microwave treatment, a heating period of

5 min was carried out (set for all experiments), and once reached the desired temperature, it was maintained for a set amount of time until cooling (around 5–6 min) by an air compressor coupled to the equipment.

The temperatures selected during the autohydrolysis process were 200, 215 and 230 °C; and the range of treatment time was from 0 to 30 min. These conditions were selected based on previous studies from the authors with a similar raw material (del Río, Gullón, Romaní, et al., 2021).

The severity (S_0) was calculated for each experiment in order to facilitate the comparison between the different pretreatment conditions. This parameter, which takes into account the effect of temperature and time, is considered a measure of the harshness of the treatment and is expressed as Aguilar-Reynosa et al. (2017a):

$$S_0 = logR_0 = log(R_{0\,HEATING} + R_{0\,ISOTHERMAL} + R_{0\,COOLING})$$
$$= log\left(\int_0^{t_H} exp \bullet \left(\frac{T(t) - T_{REF}}{\omega} \right) \right.$$
$$\bullet\, dt + exp \bullet \left(\frac{T_{ISOT} - T_{REF}}{\omega} \right) \cdot t_{ISOT} + \int_{t_H+t_{ISOT}}^{t_H+t_{ISOT}+t_C} exp \bullet \left(\frac{T'(t) - T_{REF}}{\omega} \right)$$
$$\left. \bullet\, dt \right)$$

$$(1)$$

where R_0 is the severity factor, $T(t)$ and $T'(t)$ are the temperature profiles in the heating and cooling stages, respectively, t_H (min) is the time required to achieve the maximum temperature T_{ISOT} (°C), the temperature that is selected for isothermal processing for an established time t_{ISOT} (min), t_C (min) is the time elapsed in the cooling period, ω represents the empirical parameter associated to the activation energy and T_{REF} is the reference temperature (values fixed according to literature: ω = 14.75 K and T_{REF} = 100 °C).

After the biomass was subjected to the hydrothermal treatment, the solid and liquid fractions were separated by filtration. The liquid phase was stored at 4 °C until the moment of its analysis and the solid phase was washed with distilled water until neutral pH and subjected to a gravimetric analysis to calculate the solid yield.

2.4. Chemical characterization of the liquid fraction resulting from the autohydrolysis treatment

RW liquors were subjected to three different analyses which were made in triplicate. First, an aliquot of this fraction was filtered through a 0.45 μm membrane filter and directly analyzed by HPLC for the quantification of monosaccharides (glucose, xylose, arabinose), acetic acid and degradation products (5-hydroxymethyl-2-furfural (HMF) and furfural (F)). For the determination of oligomers, another aliquot was subjected to quantitative post-hydrolysis (4 % H_2SO_4 at 121 °C for 20 min), filtered and injected in HPLC (method described in Section 2.2). The oligosaccharide concentration was calculated through the monosaccharide concentration difference observed after and before posthydrolysis and it was expressed as monosaccharide equivalents. The last analysis was the determination of non-volatile compounds (NVC), which was carried out by drying another aliquot in an oven at 105 °C until constant weight.

2.5. Total phenolic content (TPC) and total flavonoid content (TFC) assays

For the analysis of these two parameters, an aliquot of the autohydrolyzed liquid fraction was subjected to two different colorimetric methods, in triplicate. In the case of TPC, the Folin-Ciocalteu method (Singleton & Rossi, 1965) was used, and the results were expressed as mg of gallic acid equivalents (GAE)/g of RW. For the TFC, the aluminum chloride method was used as described by Blasa et al. (2006) and the

results were expressed as mg of rutin equivalents (RE)/g of RW of autohydrolysis liquor.

2.6. Antioxidant capacity assays

The determination of the antioxidant activity of autohydrolysis liquors was carried out through two complementary methods collected by Gullón et al. (2017), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation decolorization assay and ferric reducing antioxidant power (FRAP). The standard employed in the two types of tests was Trolox (6-hydroxy-2,5,7,8-tetramethylchromen-2-carboxylic acid) and the results were expressed as mg of Trolox equivalents (TE)/g of RW of autohydrolysis liquor (Gullón et al., 2014). Each of the methods was assayed in triplicate.

2.7. Fourier-transformed infrared spectroscopy (FTIR)

An aliquot of the lyophilized autohydrolysis liquor of RW was characterized via FTIR analysis using a Nicolet 6700 (Thermo-Scientific, USA). KBr was employed to prepare the sample and it was measured with an IR source and DTGS KBr detector. The spectral range evaluated varied from 400 to 4000 cm^{-1} using a resolution of 4 cm^{-1} and 32 scans per minute.

2.8. Thermogravimetric analysis and differential scanning calorimetry (TGA/DSC)

A SETSYS Evolution 1750 Simultaneous TGA DSC Thermal Analyzer (Setaram) was used to determine the TGA/DSC curves employing 5 mg of lyophilized liquor that was heated from 20 °C (room temperature) up to 600 °C, using a constant increment of 10 °C/min within a nitrogen atmosphere.

2.9. Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS)

As described by Gullón et al. (2014), an aliquot of the lyophilized liquor was analyzed via MALDI-TOF-MS using an autoflex TOF/TOF equipment (Bruker, MA, USA). 0.1 % trifluoroacetic acid was employed to reach a concentration of 1 mg of lyophilized liquor/mL, which was applied in a MALDI plate at a ratio of 1:1 with a matrix of 50 % 2,5-dihydroxy-benzoic acid 0.1 % acetonitrile/trifluoroacetic acid. A positive refractor was employed for the analyses, using a mass range of 500–3500 m/z, and a nitrogen laser ($\lambda = 337$ nm).

2.10. High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

The degree of polymerization (DP) of oligosaccharides was determined using HPAEC-PAD as explained by Gullón et al. (2014). ICS3000 chromatographic system (Dionex, Sunnyvale, CA, USA) combined with a CarboPac PA-1 column was employed for the measurement. XO with a DP between 2 and 6 (Megazyme) were used as calibration standards.

2.11. Identification of major phenolic compounds using HPLC-ESI-MS

Autohydrolysis liquor from RW was extracted with ethyl acetate at a liquor:solvent ratio of 1:1 (v/v) at room temperature during 30 min under magnetic stirring. A separatory funnel was employed for the isolation of the organic fraction, while the aqueous phase was extracted a total of 3 times. The final ethyl acetate aliquot containing the soluble compounds was vacuum-evaporated at 40 °C and dissolved in a known volume of methanol for subsequent phenolic compounds identification. In this sense, the sample was injected in a high performance liquid chromatography fitted with ion mobility spectrometry and time of flight high resolution mass spectrometry (HPLC-ESI-MS) equipped with a

ZORBAX Eclipse XDB-C18 rapid resolution HD (2.1X100mm 1.8 Micron of Agilent) column and an Elute UHPLC (Bruker Daltonics), employing a flow rate of 0.400 mL/min of solvent A (0.1 % formic acid) and solvent B (0.1 % formic acid in acetonitrile). The gradients employed entailed: 98 % of solvent A during 2 min, 98–70 % of solvent A during 13 min, 70–0 % of solvent A during 2 min, 0 % of solvent A during 4 min, 0–98 % of solvent A for 1 min, and 98 % of solvent A for 2 min. An ESI source in negative ion mode was employed to ionize the samples at operational conditions of 3000 V capillary voltage, 500 V end plate offset, 2 bar nebulizer pressure, 8 L/min dry gas, and 220 °C dry heater. Accurate mass data, isotopic pattern matching (mSigma value), retention time (using the available standards) and the compounds reported in the bibliography were employed for identifying the metabolites.

2.12. Statistical analysis

Hemicelluloses removal and energy consumption during the microwave processing (measured as mean values ± standard deviation) were statistically studied using the software R (version 4.1.0). Differences between each determination were tested with the analysis of variance based on the one-way ANOVA followed by Tukey's test. The statistical confidence level was set at 95 %.

3. Results and discussion

3.1. Effect of microwave-assisted autohydrolysis on the RW spent solids

After the microwave-assisted hydrothermal treatment, the solid fraction obtained was subjected to a chemical analysis, in order to recognize the suitability of this technology for the solubilization of hemicelluloses present in *Robinia pseudoacacia* wood.

Table 1 displays the effect of the severity of MAA treatment on the elimination of hemicelluloses (expressed as the sum of xylan, arabinan and acetyl groups) taking into account the temperature and residence time of each experiment. As expected, the solubilization of hemicelluloses increased at higher severities. For instance, the temperature of 200 °C, with residence times from 0 to 30 min (severities of 2.90–4.43) enabled the solubilization of 12 to 85 % of hemicelluloses in raw material (residence times from 0 to 30 min). Similar phenomena were observed by other authors using *Pinus radiata* wood (Santos et al., 2018), sweet sorghum bagasse (Kurian et al., 2015) or Paulownia wood (del Río et al., 2022).

Moreover, trials with similar severities reached similar hemicellulosic solubilization. As a case in point, $S_0 = 3.68$–3.71 provided hemicelluloses removal of 59–64 %, whereas at $S_0 = 4.01$–4.06 enable between 70 and 80 %, reaching slightly higher values at higher severities. Additionally, the highest value of hemicelluloses removal is achieved at 230 °C for 10 min ($S_0 = 4.85$), reaching values up to 97 %. These values can be compared to other feedstock, for instance, Paulownia wood subjected to microwave hydrothermal treatment resulted in a solubilization of 70 % of initial hemicellulose at $S_0 = 3.77$ (230 °C for 0 min), whereas harsher conditions of 230 °C for 6 min ($S_0 = 4.66$) enabled the removal of up to 94 % of the hemicelluloses (del Río, Gullón, Romaní, et al., 2021). In a similar way, Rigual et al. (2018) reached a complete solubilization of hemicelluloses (xylan, galactan, arabinan, mannan and acetyl groups) performing autohydrolysis to *Eucalyptus* wood at $S_0 = 4.42$, whereas a $S_0 = 4.90$ was necessary to remove 97 % of *Pinus radiata* hemicelluloses.

Therefore, these results demonstrate the effectiveness of MAA to solubilize the hemicellulosic fraction of RW, which depends on the synergetic effect of temperature and time of the treatment as already suggested by Remón et al. (2018) and del Río, Gullón, Pérez-Pérez, et al. (2021) leaving a solid fraction enriched in glucan and lignin, comprising an average of 88 % of the spent solids (data not shown).

Table 1

Hemicelluloses (sum of xylan, arabinan and acetyl groups) removal in the solid phase after microwave-assisted autohydrolysis at 200 °C, 215 °C and 230 °C. In each line, different letters indicate significant differences ($p \leq 0.05$).

$T = 200$ °C		
t (min)	S_0	Hemicelluloses removal (%)
0	2.90	11.99 ± 1.68^a
5	3.71	58.58 ± 0.39^c
8	3.90	62.52 ± 1.31^{de}
10	3.98	70.42 ± 0.45^f
12	4.06	69.59 ± 0.32^f
15	4.14	75.33 ± 0.70^{gh}
20	4.26	80.02 ± 0.23^j
30	4.43	84.65 ± 0.43^k

$T = 215$ °C		
t (min)	S_0	Hemicelluloses removal (%)
0	3.27	45.21 ± 1.26^b
1.25	3.68	60.78 ± 0.78^{cd}
2.5	3.90	73.10 ± 0.27^g
3	3.96	77.35 ± 0.29^{hj}
3.5	4.01	79.42 ± 0.46^{ij}
5	4.13	82.75 ± 0.44^k

$T = 230$ °C		
t (min)	S_0	Hemicelluloses removal (%)
0	3.62	63.76 ± 0.90^e
0.25	3.79	69.40 ± 0.95^f
0.5	3.87	73.82 ± 0.77^g
0.75	3.96	75.56 ± 0.71^{gh}
1	4.04	76.98 ± 0.63^{hi}
2	4.25	84.01 ± 0.77^k
5	4.57	93.79 ± 0.46^l
7	4.71	96.29 ± 0.02^{lm}
10	4.85	97.00 ± 0.50^m

3.2. Effect of the microwave-assisted autohydrolysis on the composition of RW liquid fraction

As explained before, the autohydrolysis pretreatment enables the breakdown of the lignocellulose, especially hemicelluloses in the form of oligosaccharides, monosaccharides and degradation products (Del Castillo-Llamosas et al., 2021).

Fig. 1 depicts the oligomers content in the MAA liquors of RW treated at 200, 215 and 230 °C. The oligosaccharides represent the main components in the liquid phase highlighting the presence of xylooligo-saccharides, which vary from 0.16 g XO/L up to 7.69 g XO/L (representing of 79 % regarding the initial xylan content of RW). Specifically, shorter processing times enabled the solubilization of smaller amounts of XO (1.44–7.11 g XO/L depending on the temperature employed) up to a maximum, to afterwards be hydrolyzed to xylose or converted into degradation products, such as furfural. The maximum content in XO was achieved at different severities for each temperature, acquiring the largest content at the temperature of 230 °C during 0.25 min ($S_0 = 3.79$). Similar behavior was observed for acetyl groups, which appeared as acetyl groups linked to oligosaccharides at lower severities, and as acetic acid at higher severities. The other oligosaccharides appeared in smaller amounts. Comparatively, Pino et al. (2019) reached XO concentrations up to 8.37 g/L from agave bagasse hydrothermally treated in a horizontal bioreactor at $S_0 = 3.73$ (190 °C for 10 min). Moreover, Dávila et al. (2021) also reported that shorter reaction times during higher temperature pretreatments were needed to reach the maximum recovery of oligosaccharides.

Fig. 2 reflects the monosaccharides, glucuronic and galacturonic acid concentration in the liquors of RW after MAA at 200, 215 and 230 °C.

Regarding the monosaccharides content, the xylose content accounted for 0.38 g xylose/L at $S_0 = 2.90$ (200 °C) and augmented up to 2.26 g xylose/L at $S_0 = 4.41$ (200 °C), representing the up to 23 % of the initial xylose content. Analogous behavior was observed when using both 215 and 230 °C, however, higher severities (4.71–4.85) in the temperature of 230 °C revealed the hydrolysis of xylose, probably triggering the formation of degradation products, such as furans. Conversely, glucose content was lower than 0.03 g/L, except for the highest severity (4.57–4.85) at the temperature of 230 °C, where the concentration increased up to 0.42 g glucose/L. Regarding the glucuronic acid content, it varied in a narrower range, from 0.01 at lower severities up to 0.67 g/L at $S_0 = 4.41$ (200 °C). The arabinose and galacturonic acid reached concentrations lower than 0.16 g/L in all cases.

Fig. 3 plots the acetic acid and furans content of the microwave liquors from RW. Acetic acid concentration ranged 0.12–2.73 g/L, whereas furfural varied between 0.00 and 1.84 g/L. On the other hand, HMF reached values lower than 0.41 g/L. The data reflect the hydrolysis of acetyl groups linked to oligosaccharides and the degradation of pentoses and hexoses, producing furfural and HMF respectively. In this sense, the severest conditions lead to the highest values in these three species.

A similar performance was observed in the microwave hydrothermal processing of corn stover, where the increment of temperature and time implied the cleavage of oligomeric hemicelluloses, triggering the formation of monosaccharides and degradation products (namely HMF and furfural) (Aguilar-Reynosa et al., 2017a) as well as in the microwave autohydrolysis of walnut shell (Ahorsu et al., 2019). Overall, these results are in line with those reported by del Río et al. (2022) for the MAA of Paulownia wood (4.86 g/L of total degradation products) under similar operational conditions to the ones used here ($S_0 = 4.66$) and were lower than that found for vine shoots (7.72 g/L of these compounds in the experiment performed at $S_0 = 4.65$) (Dávila et al., 2016).

Taking into account the chemical composition of the liquor and the energy consumed during the pretreatment, the condition of 230 °C for 0.25 min ($S_0 = 3.79$) was selected as optimal for the maximal recovery of xylooligosaccharides with a lesser consumption of energy if compared with the other temperatures.

In a complementary manner, the TPC, TFC and two complementary antioxidant capacity tests (ABTS and FRAP) were evaluated in the liquor of RW selected as optimal. Specifically, the TPC was 80.28 ± 0.44 mg GAE/g of RW, TFC 44.51 ± 0.44 mg RE/g of RW, whereas ABTS assay reached a value of 112.07 ± 1.21 mg TE/g of RW, and FRAP assay 102.30 ± 3.05 mg TE/g of RW. These values can be compared to others from the literature. For instance, 50 % (v/v) methanol ultrasound-assisted extraction of black locust (*Robinia pseudoacacia*) flowers for 60 min enabled the obtainment of up to 37.8 mg GAE/g measured with TPC assay (Boskov et al., 2021). On the other hand, black locust bark extracted via ultrasonic bath with ethanol (50 % v/v) for 60 min at 50 °C permitted the recovery of phenolics of 25.3 mg GAE/g of bark, flavonoids of 15.6 mg catechin equivalent/g of bark and 44.2 mg TE/g bark measured through FRAP assay (Vangeel et al., 2021). Moreover, black locust bark extracted with 50 % v/v ethanol via conventional extraction (1 h, 50 °C) enabled the obtainment of 584.85 mg TE/g dried bark extract measured via ABTS assays, which is similar to that obtained in this work (491.00 mg/g extract). However, FRAP assay lead to 146.11 mg TE/g dried bark extract (Sillero et al., 2019) which is a lower value compared with this work (449.36 mg TE/g extract). Additionally, Sillero et al. (2021) evaluated the extraction of phenolic compounds from black locust wood using 50 % v/v ethanol via conventional extraction (1 h, 50 °C), reaching 142 mg of GAE/g of dried extract, which can be compared with the extraction of 352.65 mg of GAE/g of extract obtained in the current study. In addition, results from this work can be positively compared to those obtained by Ballesteros et al. (2017) who optimized the extraction of antioxidant phenolics from spent coffee grounds via autohydrolysis (220 °C, 50 min and liquid-solid ratio of 15 mL/g), reaching values of TPC of 40.36 mg GAE/g and ABTS of 31.46 mg TE/g.
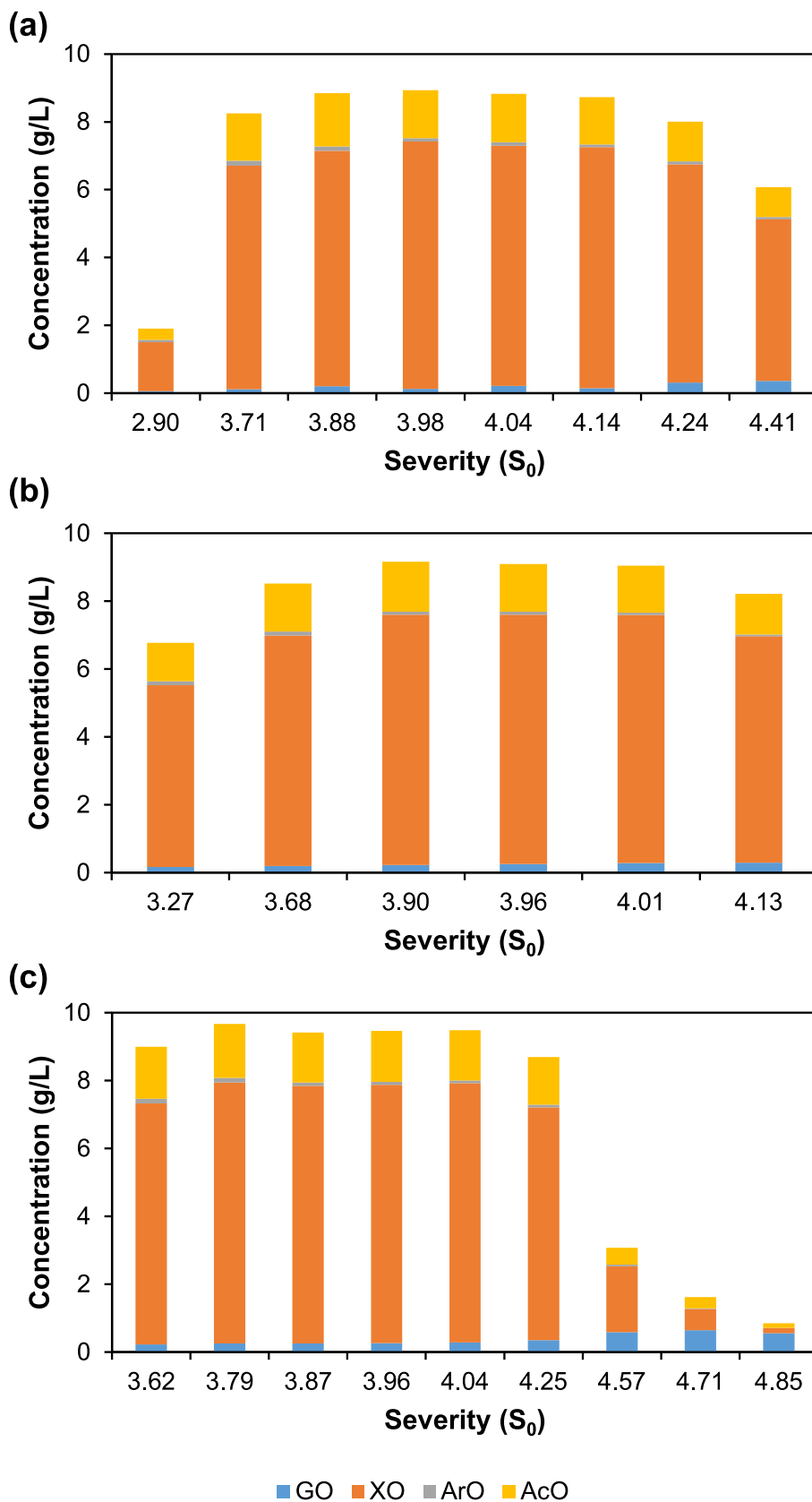
Fig. 1. Oligomers (GO, glucooligosaccharides; XO, xylooligosaccharides; ArO, arabinooligosaccharides; AcO, acetyl groups linked to oligosaccharides) content of the liquors after microwave-assisted autohydrolysis at (a) 200 °C, (b) 215 °C and (c) 230 °C.
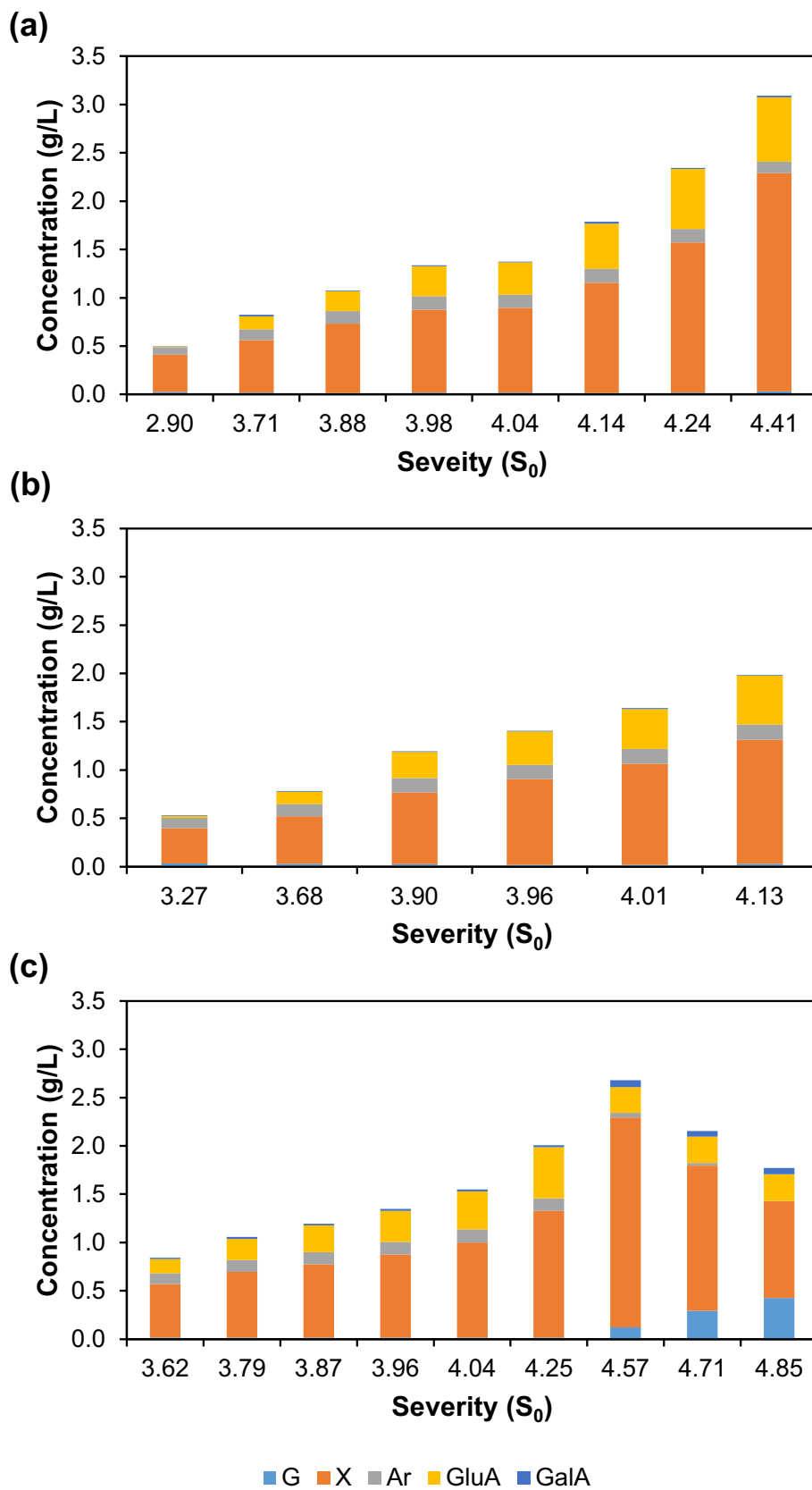
Fig. 2. Monosaccharides (G, glucose; X, xylose; Ar, arabinose), glucuronic acid (GluA), and galacturonic acid (GalA) content of the liquors after microwave-assisted autohydrolysis at (a) 200 °C, (b) 215 °C and (c) 230 °C.
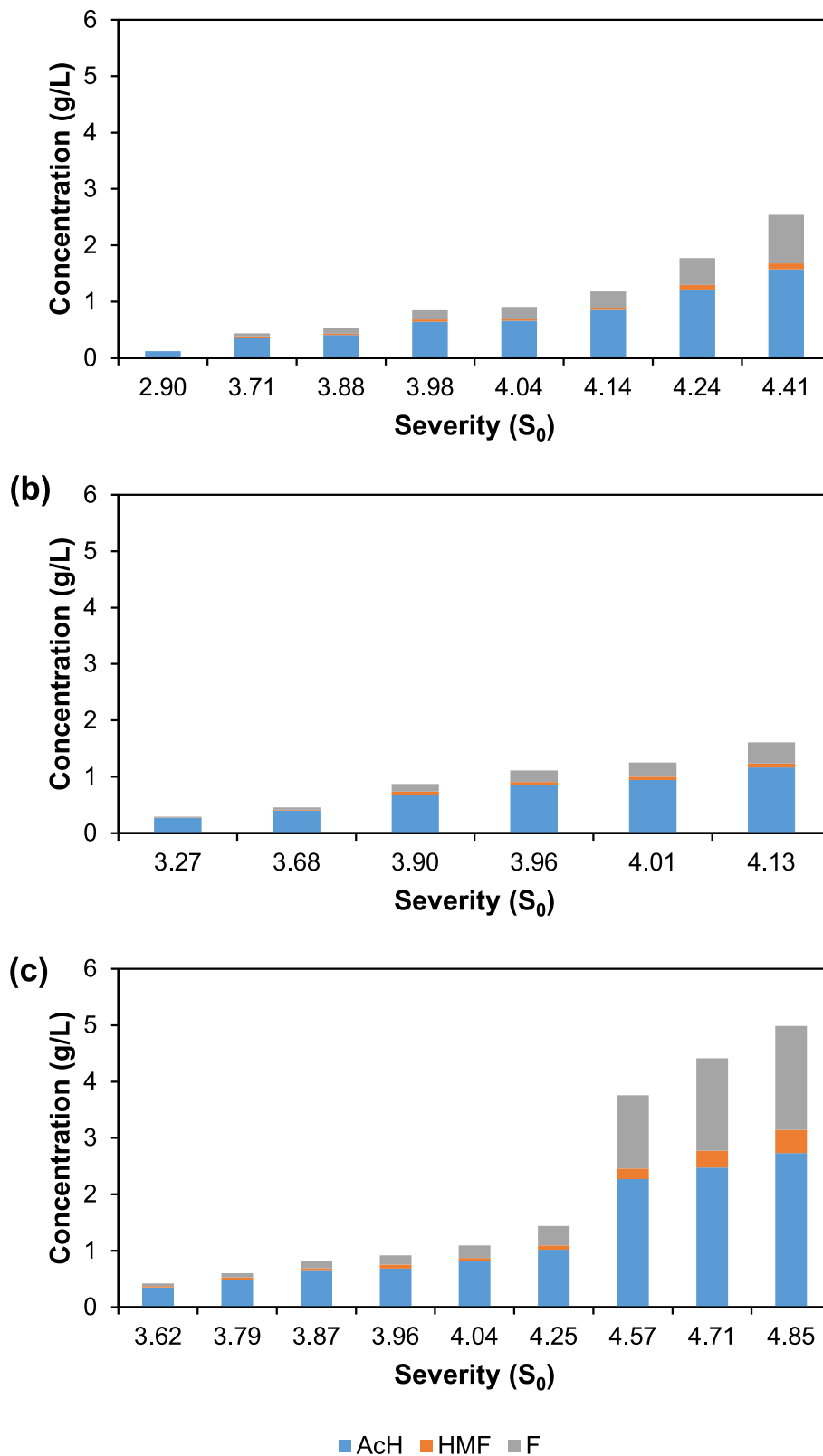
Fig. 3. Acetic acid (AcH) and furans (HMF, hydroxymethylfurfural; F, furfural) content of the liquors after microwave-assisted autohydrolysis at (a) 200 °C, (b) 215 °C and (c) 230 °C.

On the other hand, Đurović et al. (2018) processed yellow soybean seeds with microwave achieving 18.77 mg GAE/g by TPC. The values of our study also are considerably higher than those reported for the liquors obtained via microwave hydrothermal treatment of Paulownia wood

(PW) under conditions that led to the maximum XO recovery (230 °C for 0.5 min and liquid-solid ratio of 15.67 mL/g): TPC: 25.6 mg GAE/g raw PW, TFC: 29.4 mg RE/g raw PW, DPPH: 8.18 mg TE/g raw PW, ABTS: 27.8 mg TE/g raw PW and FRAP: 26.5 mg TE/g raw PW (del Río et al.,

2022).

3.3. Structural characterization of the oligosaccharides from microwave-assisted autohydrolysis of RW

In order to obtain a more in-depth interpretation of the structure of oligosaccharides, the liquid fraction obtained in the MAA carried out at 230 °C for 0.25 min was analyzed by HPAEC-PAD, MALDI-TOF-MS, FTIR and TGA/DSC.

The HPAEC-PAD elution profile of the liquor corroborated the predominance of oligosaccharides with low- or medium-DP (data shown in Fig. S1 from Supplementary Information). Regarding the substitution pattern, HPAEC-PAD does not allow to determine the presence of acetylated oligomers, because the mobile phase used (highly alkaline solution) during elution causes the saponification of the sample (Dávila et al., 2019; Rivas et al., 2020).

The structure of the solubilized oligosaccharides was also analyzed by MALDI-TOF-MS. Table 2 presents a tentative assignment of the mass signals of the MALDI-TOF-MAS to specific oligomeric structures (all compounds were identified as potassium adducts). The predominant oligosaccharides corresponded mainly to pentose chains with a DP in the range of 5–17 (made up of xylose units according to the composition

Table 2
MALDI-TOF MS oligomer profile of XO from RW treated with MAA at 230 °C for 0.25 min. Numbers following compound names indicate the number of molecule units. Ac, acetyl group; Hex, hexose; Pent, pentose; MeUr, O-methyl-uronic acid.

K adducts	m/z	Intensity
$Pent_5Ac_2K$	801.29	2555.76
$Pent_5Ac_3K$	843.31	2214.61
$Pent_5Ac_4K$	885.33	996.08
$Pent_6Ac2K$	933.35	1559.96
$Pent_6Ac_3K$	975.37	3112.44
$Pent_6Ac_4K$	1017.40	1692.95
$Pent_6Ac_5K$	1059.41	712.49
$Pent_4Ac_3MeUr_2K$	1091.47	901.85
$Pent_7Ac_3K$	1107.45	2458.54
$Pent_7Ac_4K$	1149.47	2130.71
$Pent_7Ac_5K$	1191.49	1870.60
$Pent_8Ac_2K$	1197.49	624.08
$Pent_8Ac_3K$	1239.52	2054.50
$Pent_8Ac_4K$	1281.54	3405.78
$Pent_8Ac_5K$	1323.56	2622.78
$Pent_9Ac_2K$	1329.55	374.31
$Pent_3Hex_3Ac_5MeUrK$	1339.55	380.48
$Pent_8Ac_6K$	1365.57	981.04
$Pent_9Ac_3K$	1371.58	1153.43
$Pent_9Ac_4K$	1413.61	2274.38
$Pent_9Ac_5K$	1455.63	2191.90
$Pent_9Ac_6K$	1497.64	1241.14
$Pent_{10}Ac_4K$	1545.67	1921.97
$Pent_{10}Ac_5K$	1587.69	2691.04
$Pent_{10}Ac_6K$	1629.71	1627.07
$Pent_{10}Ac_7K$	1671.73	855.80
$Pent_{11}Ac_4K$	1677.74	1122.88
$Pent_{11}Ac_5K$	1719.76	1743.51
$Pent_{11}Ac_6K$	1761.78	1550.20
$Pent_{11}Ac_7K$	1803.79	1111.30
$Pent_{12}Ac_5K$	1851.82	1140.08
$Pent_{12}Ac_6K$	1893.83	1261.73
$Pent_{12}Ac_7K$	1935.96	827.57
$Pent_{12}Ac_8K$	1977.87	500.86
$Pent_{13}Ac_5K$	1983.87	531.54
$Pent_{13}Ac_6K$	2025.89	698.88
$Pent_{13}Ac_7K$	2067.90	595.16
$Pent_{13}Ac_8K$	2109.93	440.43
$Pent_{14}Ac_6K$	2157.94	490.52
$Pent_{14}Ac_7K$	2199.95	495.33
$Pent_{14}Ac_8K$	2241.98	321.96
$Pent_{15}Ac_6K$	2289.97	248.51
$HexPent_{16}K$	2332.00	386.21
$HexPent_{16}Ac_2K$	2416.02	175.62
$HexPent_{17}K$	2464.07	192.84

analysis of the liquor mentioned above) highly substituted by acetyl groups. Furthermore, MALDI-TOF-MS spectra (Fig. S2 in Supplementary Information) also suggested the presence of oligomers partially substituted with some hexoses (mainly glucose) and methylglucuronosyl groups.

Overall, the profile of the oligomeric compounds obtained from Robinia was similar to what it has been published for other oligosaccharides generated by hydrothermal pretreatment of different raw materials such as poplar wood, vine shoots or chestnut shells (Dávila et al., 2019; Gullón et al., 2018; Rivas et al., 2020).

The FTIR spectrum (Fig. 4) obtained for oligosaccharides from Robinia presented the typical transmittance bands of hemicellulose-derived compounds (Dávila et al., 2016, 2021; Gullón et al., 2018; Jiang et al., 2014; Rico et al., 2018). The band located at 1038 cm^{-1} was assigned to the stretching and bending vibrations of C—O, C—C, C—OH and the glycosidic C—O—C, confirming the prevalence of xylooligosaccharides, which was supported by the compositional data of the liquor. The three peaks at 1164, 1076 and 992 cm^{-1} indicated the presence of arabinosyl side chains in the structure of XO (Dávila et al., 2021; Rico et al., 2018). The signal observed at 897 cm^{-1} was associated with the β-(1–4)-glycosidic linkages between xylose units, as reported by Rico et al. (2018) and Dávila et al. (2016). The bands situated at 1724, 1373 and 1240 cm^{-1} confirmed the acetylation of the backbone of oligosaccharides, in agreement with posthydrolysis results discussed above. The signals observed at 1517 and 1602 cm^{-1} can be attributable to the C=C stretching of aromatic rings, suggesting the presence of a small amount of lignin associated with the hemicellulosic oligosaccharides (Dávila et al., 2016; Jiang et al., 2014). Finally, the wide band situated at 3355 cm^{-1} was ascribed to the stretching vibrations of aromatic and aliphatic OH groups (Dávila et al., 2021; Rico et al., 2018).

Regarding the thermogravimetric analysis, TGA/DSC curves of the liquor are plotted in Fig. 5. This evaluation is related to the thermal stability that solubilized oligosaccharides possess after MAA of RW at 230 °C for 0.25 min. The data acquired from both TGA and DTG curves show that three main degradation processes happened. Firstly, two peaks, at 87 and 138 °C may be related to the evaporation of moisture and light volatile compounds with a mass loss of 6.23 % until to 150 °C (Ankona et al., 2021; Bhaumik et al., 2014). The largest mass loss of the freeze-dried liquor occurred between 170 and 400 °C, achieving a value of 54.08 % between those temperatures. As observed in the DTG curve, the peak at the temperature of 215 °C is commonly accredited to polymers degradation, in this case mainly hemicellulosic oligosaccharides (Gullón et al., 2018). At higher temperatures (<400 °C) there was a mass loss of 18.01 % that is normally attributed to the degradation of residues into gaseous compounds (namely carbon monoxide, carbon dioxide, methane, acetic acid or formic acid) (Wang et al., 2016). Above 800 °C, only 21.68 % of the mass remained unchanging as char, mainly composed of inorganic matter, reporting similar values to those obtained after thermogravimetric analysis of the autohydrolysis liquors from vine shoots (Dávila et al., 2016) and avocado peel (Del Castillo-
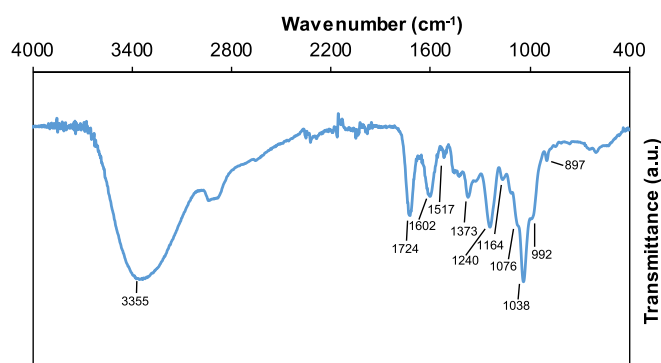

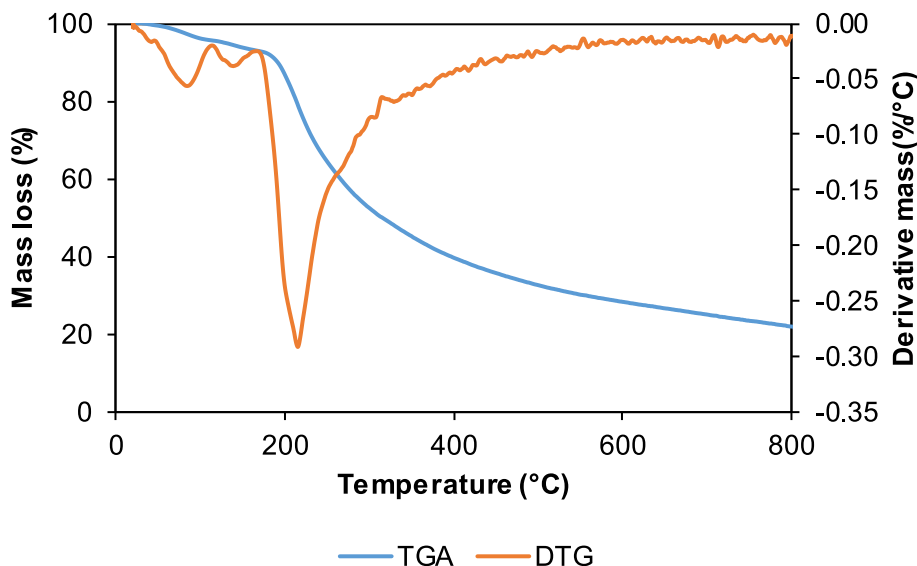**Fig. 4.** FTIR spectra of the RW liquors after MAA at 230 °C for 0.25 min.

Fig. 5. Thermogravimetric analysis spectra (TGA) and derivative DTG curves of the RW liquor after MAA at 230 °C for 0.25 min.

Llamosas et al., 2021).

3.4. Qualitative identification of polyphenols from RW microwave-assisted autohydrolysis liquor

As far as the authors know, this is the first tentative identification of polyphenolic constituents extracted by hydrothermal treatment (S_0 = 3.79) from RW. In this study, HPLC-ESI-MS was used to elucidate the metabolites of the ethyl acetate extract of the liquor (see Table 3 and Fig. S3 from Supplementary Information), which were determined based on the following criteria: (i) the measurement of the accurate mass of pseudomolecular ion [M-H]⁻ compared to the theoretical mass (with a deviation between the theoretical and the measured mass lower than 5 ppm), (ii) the mSigma value lower than 50, and (iii) the scientific data reported in the literature.

Altogether, 17 metabolites were tentatively identified belonging as to different families of compounds, including phenolic acids, flavonoids and stilbenes. Regarding the first group, Robinia wood extracts contained caffeic acid, chlorogenic acid, ferulic acid, *p*-cumaric acid, gallic acid, ellagic acid, *p*-hydroxybenzoic acid and protocatechuic acid. Among the different flavonoids identified include di-*O*-methylquercetin B, quercetin, epigallocatechin, catechin, kaempferol and procyanidin dimer. Besides, two stilbenes, i.e. resveratrol and piceatannol were also

found in this extract. The presence of a wide variety of phytochemicals in Robinia wood extract was already shown by Agarwal et al. (2021) and Sergent et al. (2014). In the study conducted by Agarwal et al. (2021), using HPLC-MS/MS detected ellagic acid, catechin, and procyanidin dimer, among other compounds in extracts from black locust obtained by ultrasonic extraction. Sergent et al. (2014) identified for the first time, the presence of resveratrol and piceatannol in *Robinia pseudoacacia* wood extractives. Multiple works have confirmed that these metabolites display interesting physiological effects. For example, phenolic acids have been reported to have antioxidant, anti-inflammatory, antidiabetic, antiobesity and anticarcinogenic activity (Monteiro Espíndola et al., 2019; Tajik et al., 2017; Zhang et al., 2020). Regarding flavonoids, Seo et al. (2015) using a mouse model demonstrated the inhibitory effect of quercetin in adipogenesis and inflammation. A preclinical study showed that catechin improves the functioning of the immune system (Ganeshpurkar & Saluja, 2018). Resveratrol and piceatannol have a plethora of beneficial health effects, being cardioprotective, antitumor, estrogenic, antiplatelet and anti-inflammatory (Agarwal et al., 2021).

3.5. Evaluation of the energy consumption during microwave-assisted autohydrolysis of RW

The energy consumed during the processing of biomass is one of the

Table 3
Tentative identification of phytochemicals in RW liquor after MAA at 230 °C for 0.25 min.

Compound	Class/subclass[a]	Molecular formula	*m/z* meas.	Δ*m/z* [ppm]	mSigma
Caffeic acid	PA/HC	$C_9H_8O_4$	179.0348	−0.639	9.2
Chlorogenic acid	PA/HC	$C_{16}H_{18}O_9$	353.0876	−0.404	1.7
Ferulic acid	PA/HC	$C_{10}H_{10}O_4$	193.0497	−1.257	8.9
p-Cumaric acid	PA/HC	$C_9H_8O_3$	163.0398	−4.951	11.1
Gallic acid	PA/HB	$C_7H_6O_5$	169.0139	−1.411	3.3
Ellagic acid	PA/HB	$C_{14}H_6O_8$	300.9986	−1.172	9.4
p-Hydroxybenzoic acid	PA/HB	$C_7H_6O_3$	137.0242	−1.023	7.2
Protocatechuic acid	PA/HB	$C_7H_6O_4$	153.0193	−0.207	2.4
Protocatechuric acid methyl ester	PA/HB	$C_8H_8O_4$	167.0342	−4.603	5.8
Di-*O*-methylquercetin B	FL/flavonol	$C_{17}H_{14}O_7$	329.0666	0.839	19
Quercetin	FL/flavonol	$C_{15}H_{10}O_7$	301.0354	0.161	2.1
Epigallocatechin	FL/flavanol	$C_{15}H_{14}O_7$	305.0664	−0.703	17.1
Catechin	FL/flavanol	$C_{15}H_{14}O_6$	289.0714	−1.057	4.4
Kaempferol	FL/flavanol	$C_{15}H_{10}O_6$	285.0405	−1.099	5.6
Procyanidin dimer	FL/flavonol oligomer	$C_{30}H_{26}O_{12}$	577.1343	−0.681	19.9
Resveratrol	Stilbene	$C_{14}H_{12}O_3$	227.07025	−4.936	2.3
Piceatannol	Stilbene	$C_{14}H_{12}O_4$	243.0661	−0.395	5.4

[a] Abbreviations: PA: phenolic acid; HB: hydroxybenzoic acid; HC: hydroxycinnamic acid; FL: flavanoids.

main thresholds to take into account within second generation bio-refineries since it is considered the most expensive step (Yang & Wyman, 2008).

Table 4 shows the energy consumed during the pretreatment per mass of processed RW (MJ/kg) and data were also statistically analyzed. In general, microwave consumption ranged 13.62–28.13 MJ/kg. No significant differences were found at lower severities, for instance at 3.90, however, pretreatments leading to hemicelluloses removal between 82 and 84 % implied significant differences between energy consumptions, with a confidence interval of 95 %, achieving values of 29.82 (200 °C for 30 min, $S_0 = 4.43$), 22.20 (215 °C for 5 min, $S_0 = 4.13$) and 20.28 MJ/kg (230 °C for 2 min, $S_0 = 4.25$), consuming up to 1.47-fold higher energy comparing temperatures of 200 and 230 °C. In addition, similar energy consumption may lead to significantly different values of hemicelluloses removal. For instance, a spent of energy of 22–23 MJ/kg may provide a percentage of hemicelluloses solubilization of 69.59 at 200 °C ($S_0 = 4.06$), 82.75 at 215 °C ($S_0 = 4.13$) and 93.79 at 230 °C ($S_0 = 4.57$).

On the other hand, the energy consumed to achieve the maximal xylooligosaccharides content in the liquor (see Fig. 1) was not significantly different ($p > 0.05$) between the pretreatments at 215 °C (2.5 min) and 230 °C (0.25 min), however they were significantly different ($p < 0.05$) comparing with the temperature of 200 °C (10 min). These results exemplify that the employment of higher temperatures for smaller reaction times may decrease the energy consumption during the processing of RW, as was also stated by other authors (del Río, Gullón, Pérez-Pérez, et al., 2021), and that similar energy consumptions would lead to different hemicelluloses removal when employing different temperatures. These findings are in line with the ones reported by del Río et al. (2022) when evaluated the energy consumption of microwave hydrothermal treatment of PW. In that study, 18.3 MJ/kg were necessary to obtain the highest amount of oligosaccharides (230 °C for 0.5 min).

4. Conclusions

Microwave-assisted autohydrolysis is a successful technology to recover high value-added compounds, namely oligosaccharides and antioxidant compounds, from *Robinia pseudoacacia* wood. Under the optimal conditions (230 °C, $S_0 = 3.79$), 79 % of the initial xylan was converted into xylooligosaccharides, leading to liquors containing up to 7.69 g XO/L, with a rich substitution pattern and limited formation of degradation compounds (0.04 g HMF/L and 0.08 g furfural/L). Besides, RW liquors also exhibited good antioxidant activity and HPLC-ESI-MS allowed to identify various metabolites with potential health benefits like *p*-cumaric acid, di-*O*-methylquercetin B or piceatannol. The FTIR spectra showed characteristic bands of oligosaccharides, and the thermogravimetric analysis revealed that the solubilized hemicelluloses were stable at high temperatures. This hydrothermal treatment also provided cellulose and lignin-rich solids that can be fractionated for use in different applications. Therefore, the results obtained in this work revealed that the assistance of the autohydrolysis by microwaves constitutes a suitable and eco-friendly pretreatment for the valorization of *Robinia pseudoacacia* wood within the framework of a biorefinery.

CRediT authorship contribution statement

Alba Pérez-Pérez: Conceptualization, Investigation, Methodology, Writing – review & editing. **Beatriz Gullón:** Project administration, Funding acquisition, Conceptualization, Investigation, Methodology, Writing – review & editing. **Álvaro Lobato-Rodríguez:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Gil Garrote:** Project administration, Funding acquisition, Writing – review & editing. **Pablo G. del Río:** Conceptualization, Investigation, Methodology, Writing – review & editing.

Table 4
Energy consumption after microwave-assisted autohydrolysis at 200 °C, 215 °C and 230 °C.

$T = 200$ °C		
t (min)	S_0	Energy consumption (MJ/kg)
0	2.90	13.62 ± 0.68^a
5	3.71	17.53 ± 0.56^c
8	3.90	20.80 ± 0.00^{gi}
10	3.98	21.24 ± 0.76^{hi}
12	4.06	23.34 ± 0.00^{jk}
15	4.14	24.58 ± 0.41^k
20	4.26	26.94 ± 0.61^l
30	4.43	29.82 ± 0.67^m

$T = 215$ °C		
t (min)	S_0	Energy consumption (MJ/kg)
0	3.27	15.57 ± 0.18^b
1.25	3.68	17.62 ± 0.92^{cd}
2.5	3.90	18.37 ± 0.34^{ce}
3	3.96	18.77 ± 0.34^{ce}
3.5	4.01	19.36 ± 0.41^{eg}
5	4.13	22.20 ± 0.67^{ij}

$T = 230$ °C		
t (min)	S_0	Energy consumption (MJ/kg)
0	3.62	18.46 ± 0.18^{ce}
0.25	3.79	18.12 ± 0.19^{ce}
0.5	3.87	19.08 ± 0.14^{deg}
0.75	3.96	19.54 ± 0.00^{egh}
1	4.04	19.28 ± 0.33^{def}
2	4.25	20.28 ± 0.50^{fgh}
5	4.57	22.61 ± 0.56^j
7	4.71	24.65 ± 0.73^k
10	4.85	28.13 ± 0.63^l

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carbpol.2022.120364.

References

Agarwal, C., Hofmann, T., Visi-Rajczi, E., & Pásztory, Z. (2021). Low-frequency, green sonoextraction of antioxidants from tree barks of Hungarian woodlands for potential food applications. *Chemical Engineering and Processing - Process Intensification, 159*, Article 108221. https://doi.org/10.1016/j.cep.2020.108221

Aguilar-Reynosa, A., Romaní, A., Rodríguez-Jasso, R. M., Aguilar, C. N., Garrote, G., & Ruiz, H. A. (2017a). Comparison of microwave and conduction-convection heating autohydrolysis pretreatment for bioethanol production. *Bioresource Technology, 243*, 273–283. https://doi.org/10.1016/j.biortech.2017.06.096

Aguilar-Reynosa, A., Romaní, A., Rodríguez-Jasso, R. M., Aguilar, C. N., Garrote, G., & Ruiz, H. A. (2017b). Microwave heating processing as alternative of pretreatment in second-generation biorefinery: An overview. *Energy Conversion and Management, 136*, 50–65. https://doi.org/10.1016/j.enconman.2017.01.004

Ahorsu, R., Cintorrino, G., Medina, F., & Constantí, M. (2019). Microwave processes: A viable technology for obtaining xylose from walnut shell to produce lactic acid by Bacillus coagulans. *Journal of Cleaner Production, 231*, 1171–1181. https://doi.org/10.1016/j.jclepro.2019.05.289

Amorim, C., Silvério, S. C., Prather, K. L. J., & Rodrigues, L. R. (2019). From lignocellulosic residues to market: Production and commercial potential of xylooligosaccharides. *Biotechnology Advances, 37*(7), Article 107397. https://doi.org/10.1016/j.biotechadv.2019.05.003

Ankona, E., Multanen, V., Nisnevitch, M., Billig, M., & Anker, Y. (2021). Investigation of pyrolysis kinetics and gaseous compounds emitted during charcoal production from woods commonly used in the Eastern Mediterranean. *Biofuels, Bioproducts and Biorefining, 15*(3), 646–656. https://doi.org/10.1002/bbb.2188

Ballesteros, L. F., Ramirez, M. J., Orrego, C. E., Teixeira, J. A., & Mussatto, S. I. (2017). Optimization of autohydrolysis conditions to extract antioxidant phenolic compounds from spent coffee grounds. *Journal of Food Engineering, 199*, 1–8. https://doi.org/10.1016/j.jfoodeng.2016.11.014

Bhaumik, M., Choi, H. J., Seopela, M. P., McCrindle, R. I., & Maity, A. (2014). Highly effective removal of toxic Cr(VI) from wastewater using sulfuric acid-modified avocado seed. *Industrial and Engineering Chemistry Research, 53*(3), 1214–1224. https://doi.org/10.1021/ie402627d

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M. P., Albertini, M. C., & Piatti, E. (2006). Raw Millefiori honey is packed full of antioxidants. *Food Chemistry, 97*(2), 217–222. https://doi.org/10.1016/j.foodchem.2005.03.039

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*, 484–489.

Boskov, I. A., Savic Gajic, I. M., Savic, I. M., Spalovic, B. R., & Strbac, N. D. (2021). Black locust flowers: Antioxidant extraction kinetics, reducing capacity, mineral composition, and antioxidant activity. *Chemical Engineering Communications, 209*(9), 1182–1190. https://doi.org/10.1080/00986445.2021.1949304

Chandel, A. K., Garlapati, V. K., Jeevan Kumar, S. P., Hans, M., Singh, A. K., & Kumar, S. (2020). The role of renewable chemicals and biofuels in building a bioeconomy. *Biofuels, Bioproducts and Biorefining, 14*(4), 830–844. https://doi.org/10.1002/bbb.2104

Dávila, I., Gordobil, O., Labidi, J., & Gullón, P. (2016). Assessment of suitability of vine shoots for hemicellulosic oligosaccharides production through aqueous processing. *Bioresource Technology, 211*, 636–644. https://doi.org/10.1016/j.biortech.2016.03.153

Dávila, I., Gullón, B., Alonso, J. L., Labidi, J., & Gullón, P. (2019). Vine shoots as new source for the manufacture of prebiotic oligosaccharides. *Carbohydrate Polymers, 207*, 34–43. https://doi.org/10.1016/j.carbpol.2018.11.065

Dávila, I., Gullón, P., & Labidi, J. (2021). Influence of the heating mechanism during the aqueous processing of vine shoots for the obtaining of hemicellulosic oligosaccharides. *Waste Management, 120*, 146–155. https://doi.org/10.1016/j.wasman.2020.11.014

Del Castillo-Llamosas, A., Rodríguez-Martínez, B., del Río, P. G., Eibes, G., Garrote, G., & Gullón, B. (2021). Hydrothermal treatment of avocado peel waste for the simultaneous recovery of oligosaccharides and antioxidant phenolics. *Bioresource Technology, 342*, Article 125981. https://doi.org/10.1016/j.biortech.2021.125981

del Río, P. G., Domínguez, V. D., Domínguez, E., Gullón, P., Gullón, B., Garrote, G., & Romaní, A. (2020). Comparative study of biorefinery processes for the valorization of fast-growing Paulownia wood. *Bioresource Technology, 314*, Article 123722. https://doi.org/10.1016/j.biortech.2020.123722

del Río, P. G., Gullón, B., Pérez-Pérez, A., Romaní, A., & Garrote, G. (2021). Microwave hydrothermal processing of the invasive macroalgae Sargassum muticum within a green biorefinery scheme. *Bioresource Technology, 340*, Article 125733. https://doi.org/10.1016/j.biortech.2021.125733

del Río, P. G., Gullón, B., Romaní, A., & Garrote, G. (2021). Fast-growing Paulownia wood fractionation by microwave-assisted hydrothermal treatment: A kinetic assessment. *Bioresource Technology, 338*, Article 125535. https://doi.org/10.1016/j.biortech.2021.125535

del Río, P. G., Pérez-Pérez, A., Garrote, G., & Gullón, B. (2022). Manufacturing of hemicellulosic oligosaccharides from fast-growing Paulownia wood via autohydrolysis: Microwave versus conventional heating. *Industrial Crops and Products, 187*, Article 115313. https://doi.org/10.1016/j.indcrop.2022.115313

Đurović, S., Nikolić, B., Luković, N., Jovanović, J., Stefanović, A., Šekuljica, N., Mijin, D., & Knežević-Jugović, Z. (2018). The impact of high-power ultrasound and microwave on the phenolic acid profile and antioxidant activity of the extract from yellow soybean seeds. *Industrial Crops and Products, 122*, 223–231. https://doi.org/10.1016/j.indcrop.2018.05.078

Ganeshpurkar, A., & Saluja, A. K. (2018). Protective effect of catechin on humoral and cell mediated immunity in rat model. *International Immunopharmacology, 54*, 261–266. https://doi.org/10.1016/j.intimp.2017.11.022

Gullón, B., Eibes, G., Dávila, I., Moreira, M. T., Labidi, J., & Gullón, P. (2018). Hydrothermal treatment of chestnut shells (Castanea sativa) to produce oligosaccharides and antioxidant compounds. *Carbohydrate Polymers, 192*, 75–83. https://doi.org/10.1016/j.carbpol.2018.03.051

Gullón, B., Eibes, G., Moreira, M. T., Dávila, I., Labidi, J., & Gullón, P. (2017). Antioxidant and antimicrobial activities of extracts obtained from the refining of autohydrolysis liquors of vine shoots. *Industrial Crops and Products, 107*, 105–113. https://doi.org/10.1016/j.indcrop.2017.05.034

Gullón, B., Gullón, P., Tavaria, F., Pintado, M., Gomes, A. M., Alonso, J. L., & Parajó, J. C. (2014). Structural features and assessment of prebiotic activity of refined arabinoxylooligosaccharides from wheat bran. *Journal of Functional Foods, 6*(1), 438–449. https://doi.org/10.1016/j.jff.2013.11.010

Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: Functional food applications and legislation. *Journal of Applied Phycology, 23*(3), 543–597. https://doi.org/10.1007/s10811-010-9632-5

Jiang, H., Chen, Q., Ge, J., & Zhang, Y. (2014). Efficient extraction and characterization of polymeric hemicelluloses from hybrid poplar. *Carbohydrate Polymers, 101*(1), 1005–1012. https://doi.org/10.1016/j.carbpol.2013.10.030

Kamperidou, V., Terzopoulou, P., & Barboutis, I. (2021). Marginal lands providing tree-crop biomass as feedstock for solid biofuels. *Biofuels, Bioproducts and Biorefining, 15*(5), 1395–1405. https://doi.org/10.1002/bbb.2235

Kurian, J. K., Gariepy, Y., Orsat, V., & Vijaya Raghavan, G. S. (2015). Comparison of steam-assisted versus microwave-assisted treatments for the fractionation of sweet sorghum bagasse. *Bioresources and Bioprocessing, 2*(1), 1–16. https://doi.org/10.1186/s40643-015-0059-3

Martín-Sampedro, R., Santos, J. I., Eugenio, M. E., Wicklein, B., Jiménez-López, L., & Ibarra, D. (2019). Chemical and thermal analysis of lignin streams from Robinia pseudoacacia L. generated during organosolv and acid hydrolysis pre-treatments and subsequent enzymatic hydrolysis. *International Journal of Biological Macromolecules, 140*, 311–322. https://doi.org/10.1016/j.ijbiomac.2019.08.029

Monteiro Espíndola, K. M., Guimarães Ferreira, R., Mosquera Narvaez, L. E., Rocha Silva Rosario, A. C., Machado Da Silva, A. H., Bispo Silva, A. G., Oliveira Vieira, A. P., & Chagas Monteiro, M. (2019). Chemical and pharmacological aspects of caffeic acid and its activity in hepatocarcinoma. *Frontiers in Oncology, 9*, 541. https://doi.org/10.3389/fonc.2019.00541

Morales, A., Hernández-Ramos, F., Sillero, L., Fernández-Marín, R., Dávila, I., Gullón, P., Erdocia, X., & Labidi, J. (2020). Multiproduct biorefinery based on almond shells: Impact of the delignification stage on the manufacture of valuable products. *Bioresource Technology, 315*, Article 123896. https://doi.org/10.1016/j.biortech.2020.123896

Pinales-Márquez, C. D., Rodríguez-Jasso, R. M., Araújo, R. G., Loredo-Treviño, A., Nabarlatz, D., Gullón, B., & Ruiz, H. A. (2021). Circular bioeconomy and integrated biorefinery in the production of xylooligosaccharides from lignocellulosic biomass: A review. *Industrial Crops and Products, 162*, Article 113274. https://doi.org/10.1016/j.indcrop.2021.113274

Pino, M. S., Rodríguez-Jasso, R. M., Michelin, M., & Ruiz, H. A. (2019). Enhancement and modeling of enzymatic hydrolysis on cellulose from agave bagasse hydrothermally pretreated in a horizontal bioreactor. *Carbohydrate Polymers, 211*, 349–359. https://doi.org/10.1016/j.carbpol.2019.01.111

Rajesh Banu, J., Preethi, Kavitha, S., Tyagi, V. K., Gunasekaran, M., Karthikeyan, O. P., & Kumar, G. (2021). Lignocellulosic biomass based biorefinery: A successful platform towards circular bioeconomy. *Fuel, 302*, Article 121086. https://doi.org/10.1016/j.fuel.2021.121086

Remón, J., Santomauro, F., Chuck, C. J., Matharu, A. S., & Clark, J. H. (2018). Production of fermentable species by microwave-assisted hydrothermal treatment of biomass carbohydrates: Reactivity and fermentability assessments. *Green Chemistry, 20*(19), 4507–4520. https://doi.org/10.1039/c8gc02182a

Rico, X., Gullón, B., Alonso, J. L., Parajó, J. C., & Yáñez, R. (2018). Valorization of peanut shells: Manufacture of bioactive oligosaccharides. *Carbohydrate Polymers, 183*, 21–28. https://doi.org/10.1016/j.carbpol.2017.11.009

Rigual, V., Santos, T. M., Domínguez, J. C., Alonso, M. V., Oliet, M., & Rodríguez, F. (2018). Evaluation of hardwood and softwood fractionation using autohydrolysis and ionic liquid microwave pretreatment. *Biomass and Bioenergy, 117*, 190–197. https://doi.org/10.1016/j.biombioe.2018.07.014

Rivas, S., Rigual, V., Domínguez, J. C., Alonso, M. V., Oliet, M., Parajó, J. C., & Rodriguez, F. (2020). A biorefinery strategy for the manufacture and characterization of oligosaccharides and antioxidants from poplar hemicelluloses. *Food and Bioproducts Processing, 123*, 398–408. https://doi.org/10.1016/j.fbp.2020.07.018

Sagar, N. A., Pareek, S., Sharma, S., Yahia, E. M., & Lobo, M. G. (2018). Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. *Comprehensive Reviews in Food Science and Food Safety, 17*(3), 512–531. https://doi.org/10.1111/1541-4337.12330

Santos, T. M., Alonso, M. V., Oliet, M., Domínguez, J. C., Rigual, V., & Rodriguez, F. (2018). Effect of autohydrolysis on Pinus radiata wood for hemicellulose extraction. *Carbohydrate Polymers, 194*, 285–293. https://doi.org/10.1016/j.carbpol.2018.04.010

Seo, M. J., Lee, Y. J., Hwang, J. H., Kim, K. J., & Lee, B. Y. (2015). The inhibitory effects of quercetin on obesity and obesity-induced inflammation by regulation of MAPK signaling. *Journal of Nutritional Biochemistry, 26*(11), 1308–1316. https://doi.org/10.1016/j.jnutbio.2015.06.005

Sergent, T., Kohnen, S., Jourez, B., Beauve, C., Schneider, Y. J., & Vincke, C. (2014). Characterization of black locust (Robinia pseudoacacia L.) heartwood extractives: Identification of resveratrol and piceatannol. *Wood Science and Technology, 48*(5), 1005–1017. https://doi.org/10.1007/s00226-014-0656-x

Sillero, L., Morales, A., Fernández-Marín, R., Hernández-Ramos, F., Davila, I., Erdocia, X., & Labidi, J. (2021). Study of different extraction methods of bioactive molecules from different tree species. *Chemical Engineering Transactions, 86*, 31–36. https://doi.org/10.3303/CET2186006

Sillero, L., Prado, R., Andrés, M. A., & Labidi, J. (2019). Characterisation of bark of six species from mixed Atlantic forest. *Industrial Crops and Products, 137*, 276–284. https://doi.org/10.1016/j.indcrop.2019.05.033

Singleton, V. L., & Rossi, J. A. (1965). Colorimetric of total phenolics with phosphomolybdic—Phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Straker, K. C., Quinn, L. D., Voigt, T. B., Lee, D. K., & Kling, G. J. (2015). Black locust as a bioenergy feedstock: A review. *Bioenergy Research, 8*(3), 1117–1135. https://doi.org/10.1007/s12155-015-9597-y

Tajik, N., Tajik, M., Mack, I., & Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: A comprehensive review of the literature. *European Journal of Nutrition, 56*(7), 2215–2244. https://doi.org/10.1007/s00394-017-1379-1

Vangeel, T., Neiva, D. M., Quilhó, T., Costa, R. A., Sousa, V., Sels, B. F., & Pereira, H. (2021). Tree bark characterization envisioning an integrated use in a biorefinery. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-021-01362-8. In Press.

Wagner, A. O., Lackner, N., Mutschlechner, M., Prem, E. M., Markt, R., & Illmer, P. (2018). Biological pretreatment strategies for second-generation lignocellulosic resources to enhance biogas production. *Energies, 11*(7), 1–14. https://doi.org/10.3390/en11071797

Wang, Z. W., Zhu, M. Q., Li, M. F., Wang, J. Q., Wei, Q., & Sun, R. C. (2016). Comprehensive evaluation of the liquid fraction during the hydrothermal treatment of rapeseed straw. *Biotechnology for Biofuels, 9*(1), 1–16. https://doi.org/10.1186/s13068-016-0552-8

Yang, B., & Wyman, C. E. (2008). Pretreatment: The key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioproducts and Biorefining, 2*(1), 26–40. https://doi.org/10.1002/bbb.49

Zhang, B., Zhang, Y., Li, H., Deng, Z., & Tsao, R. (2020). A review on insoluble-bound phenolics in plant-based food matrix and their contribution to human health with future perspectives. *Trends in Food Science and Technology, 105*, 347–362. https://doi.org/10.1016/j.tifs.2020.09.029

Zhao, H., Mikkonen, K. S., Kilpeläinen, P. O., & Lehtonen, M. I. (2020). Spruce galactoglucomannan-stabilized emulsions enhance bioaccessibility of bioactive compounds. *Foods, 9*(5), 672. https://doi.org/10.3390/foods9050672