Extraction of ferulic acid and feruloylated arabinoxylo-oligosaccharides from wheat bran using pressurized hot water

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ABSTRACT

Pressurized water was tested as solvent for the hydrolysis and extraction of Ferulic acid (FA) and feruloylated arabinoxylooligosaccharides (F-AXOS) from destarched wheat bran (DWB). Results were dependent on the severity factor of the process (combination of temperature and time), obtaining the maximum extraction yields at 200°C and 3.5 min. 78% of the total FA was successfully extracted, being 17% in its free form and the rest, covalent ester-bounded to arabinoxylans. Under such conditions, 80% of the arabinoxylans are extracted with a degree of FA esterification of 1.34 g FA/100 g AXOS, and an average molecular weight of $1,3 \cdot 10^4$ Da. Pressurized Microwave Assisted Extraction was also studied to evaluate potential intensification of the process using microwaves. However, no significant differences were observed with the microwave heating. Residual solid after extraction was mainly composed by lignin and cellulose (56% and 21%, respectively) showing that hot compressed water technology can be integrated in the first steps of a biorefinery for the total valorization of the wheat bran.

1. Introduction

Ferulic acid (FA) is the main phenolic compound present in wheat bran and it has many potentials in food, cosmetic and health industries associated to many beneficial effects such as antioxidant, antithrombosis and anticancer activities, among others (Brouns et al., 2012) (Silva and Batista, 2017). In the cereal matrix, it is responsible for the crosslinking of the cell-wall components, such as arabinoxylans and proteins, and it is present in three forms: soluble free, soluble conjugated and insoluble bound, the latter being the most abundant (around 90% of FA is attached to arabinoxylans) (Acosta-Estrada et al., 2014) (Brouns et al., 2012). Benefits of recovering FA in its free form are associated to the valuable properties stated above. However, it has been reported that when it is recovered as part of the arabinoxylooligosaccharides (AXOS), the beneficial properties of these polysaccharides are enhanced. AXOS are the product of hydrolysis of the arabinoxylans (AX). They consist in a xylan backbone substituted with arabinose units on the 2-O- and/or 3-O-positions, some of which are esterified to ferulic acid. AX and AXOS have many beneficial health effects and they are well known as prebiotics and dietary fibers (Mendis and Simsek, 2014). When FA is esterified to AXOS (F-AXOS), their nutritional and physiological functions are combined.

Even synergistic effects between them have been reported, as in the case of the antioxidant potency (Ou and Sun, 2014). Molecular weight and degree of substitution have significant effects on the antioxidant and hypoglycemic activities (Chen et al., 2021).

Different methodologies for the recovery of bound phenolics have been reported. They include alkaline hydrolysis, enzymatic hydrolysis, microwave assisted extraction (MAE), ultrasound-assisted hydrolysis and pressurized liquid extraction (PLE), among others (Acosta-Estrada et al., 2014).

Alkali-based extraction causes the cleavage of the FA substitutions that are naturally available in cereal AX, and thus, it is an effective way to release phenolic acids and other related compounds from cereal grains (Fazary and Ju, 2007; Kim et al., 2006; Verma et al., 2009). However, alternative treatments to preserve functional and bioactive properties of F-AXOS, more selective and more environmentally friendly are desired. In this sense, the use of pressurized liquid extraction selecting water as solvent (PWE) constitutes a greener and efficient alternative to perform the fractionation of wheat bran (Pazo-Cepeda et al., 2019). Pressurized liquid extraction involves the use of a solvent above its boiling point, with the pressure necessary to maintain it in liquid state, which modifies its properties improving its extraction capability (Buranov and Mazza, 2009). Pourali et al. (2010) studied the

List of abbreviations

AX	ArabinoXylans
AXOS	ArabinoXylo-OligoSaccharides
DP	Degree of Polymerization
DWB	Destarched Wheat Bran
EFA	Esterified Ferulic Acid
FA	Ferulic Acid
FA	*trans*-Ferulic Acid
F-AXOS	Feruloylated Arabinoxylooligosaccharides
FFA	Free Ferulic Acid
HMF	5-(Hydroxymethyl)furfural
MAE	Microwave Assisted Extraction
PHWE	Pressurized Hot Water Extraction
PLE	Pressurized Liquid Extraction
PMWE	Pressurized Microwave-assisted Water Extraction
TFA	Total Ferulic Acid

production of phenolics from rice bran using pressurized water. They analyzed the free FA (and other phenolics) and the sugar content obtained (determined by a photometric method), reporting their optimum at about 200°C and 10 min (no extraction yields were reported). Alternatively, Ruthes et al. (2017) reported an extraction yield of 55.1% of the initial AX from defatted and destarched wheat bran, along with 40% of the initial FA esterified to them (at 160°C and 15 min). Yilmaz-Turan et al. (2020) obtained a maximum yield of 800 mg g^{-1} of total carbohydrate at 160°C and 60 min.

On the other hand, MAE has been recently used to intensify processes, either by reducing processing time and/or energy or solvent consumption (Alupului et al., 2009). Microwaves are electromagnetic waves, whose energy could be transformed rapidly into heat as consequence of the dipole rotation and ionic conduction in the solvent or sample. The main difference between microwave heating and conventional heating is that the former heats up the system from the interior towards the boundaries, resulting in a faster heating (Nuechter et al., 2003). Moreira et al. (2012) tested different solvents (NaOH, water, methanol acetonitrile and acetone) in the extraction of free FA from brewer's spent grain using MAE. Water at 150°C and 25 min was the second solvent giving the highest amount of FFA, after NaOH. Likewise, Coelho et al. (2014) tested the use of MAE with water for the recovery of valuable compounds from brewer spent grain, namely AXOS and F-AXOS. They claimed to have released 43% of the AX with a degree of esterification of 9.3% at 210°C and 2 min. On the other hand, Rose and Inglett (2010a, 2010b) reported similar optimal conditions for the extraction of AXOS from maize bran using MAE and water as solvent. They were able to extract up to 50% of initial AXOS along with around 8% and 60% of the initial FA in the free and esterified form, respectively, at the tested conditions of 180°C and 10 min or 200°C and 2 min.

Therefore, the main objective of this work is the extraction of FFA and F-AXOS from wheat bran using pressurized water as a first step of a wheat bran biorefinery, trying to alter as little as possible the residual cellulose and lignin fractions in the solid for further valorization. The comparison of conventional heating in an autoclave (Pressurized Hot Water Extraction, PHWE) with microwave heating (Pressurized Microwave-assisted Water Extraction, PMWE) is performed to elucidate the role of microwaves in the intensification of the extraction procedure. The influence of process variables such as stirring rate, solid/liquid ratio, temperature and extraction time is studied. Besides FA and F-AXOS analysis, monomeric sugars and their main degradation products (HMF and furfural) were also quantified in the liquid product.

Up to the authors' knowledge, comparison of these two technologies for the recovery of FFA or F-AXOS from biomass to infer the role of the microwaves is not evident in the open literature since every work has

been performed under significantly different operational conditions or raw materials.

2. Materials and methods

2.1. Chemicals

All the chemicals, standards and reagents used in this study were supplied by Sigma-Aldrich (Merck Life Science S.L.U., Madrid, Spain), and they are analytical grade.

2.2. Raw material

Wheat bran was kindly provided by Emilio Esteban SA, a milling industry located nearby Valladolid (Spain). It was subjected to a destarching process, performed according to the enzymatic procedure: 20 g of wheat bran were suspended in 400 mL of phosphate buffer (pH 5–6) at 65 °C. After that, 1 mL of α-amylase (Fungamyl®800 L; Novozymes, Bagsvaerd, Denmark) was added and stirred for 1 h. Then, the solid was rinsed with distilled water, and finally freeze dried (LyoQuest; Telstar®, Tokyo, Japan) and milled in a ball mill (PM100, Retsch®, Haan, Germany) (Sánchez-Bastardo et al., 2017).

2.3. Characterization of the raw material

The final particle size distribution of the milled destarched wheat bran (DWB) was determined by light scattering (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, United Kingdom) coupled with a Sirocco dry powder feeder.

The DWB compositional analysis was performed according to the procedure described in a previous work (Sánchez-Bastardo et al., 2017), that is an adaptation of the Laboratory Analytical Procedure (LAP) for biomass analysis, provided by the National Renewable Energy Laboratory, NREL (Sluiter and Sluiter, 2010, pp. 1–10). The details of this procedure are described hereafter:

The moisture content was determined in a convective air-oven (Memmert UNP400, Memmert GmbH + Co.KG, Schwabach, Germany) at 105°C until constant weight (AACC Method 44–15.02), and the ash content by calcination in a muffle furnace (HD 230, Hobersal, Barcelona Spain) of the dried sample at 575°C for 24 h (AACC Method 08–01.01). The extractives were obtained after two consecutive Soxhlet extractions of the DWB, first with water and then with ethanol, both for 24 h. After the Soxhlet extractions, the remaining solid was dried at 65 °C and used to characterize the structural carbohydrate and lignin content. The procedure involves the addition of 3 mL of H_2SO_4 72% w/w to 300 mg of solid, incubated at 30 °C for 1 h and then diluted with 84 mL of milli-Q water. It was maintained at 121°C in an autoclave for 1 h and, after reaching room temperature (20°C), it was filtered under vacuum. The remaining solid was used to determine the acid insoluble lignin (after drying the filter at 105 °C) and the ashes (after calcination at 575 °C). One aliquot of the liquid was immediately used to determine the acid soluble lignin by using an UV–Visible spectrophotometer (UV-2450 Shimadzu Corp., Tokyo, Japan) at 280 nm and an extinction coefficient of 18.675 L g^{-1} cm^{-1} (Fukushima and Hatfield, 2004). Another one was analyzed by HPLC to determine the amount of monomeric sugars: arabinose, xylose and glucose (after adding calcium carbonate to neutralize the solution). The protein content was determined following a standardized Kjeldahl method (AACC Method 46–16.01) using a nitrogen to protein conversion factor of 5.7 applicable to wheat bran (Sánchez-Bastardo et al., 2017) and the starch content that remained after the destarching process was analyzed by using the kit "Total Starch Assay Kit (AA/AMG) - Method A" (Kit K-TSTA-50A, AOAC Method 996.11 AOAC, AACC Method 76–13.01, Megazyme International Ltd., Scotland, United Kingdom).

The total amount of FA in the raw material (FA$_{raw}$) was determined by an alkaline hydrolysis as described by Barberousse et al. (2009). For

that, 250 mg of DWB were treated with 15 mL of NaOH 2 M for 3 h, under a nitrogen atmosphere and in the absence of light. The reaction was stopped by adding HCl 37% w/w until pH < 2 (c.a. 3 mL). After 30 min, it was centrifuged, and 15 mL of this liquid were extracted 3 times with diethyl ether. The organic phase, containing the FA, was evaporated to dryness under vacuum in a rotary evaporator (Heizbed Hei-VAP, Heidolph Instruments GmbH & Co, Schwabach, Germany) and the resulting solid was re-suspended in 5 mL of methanol 50% in water for quantification of FA by HPLC.

2.4. Extraction procedures

Two extraction procedures were tested for the extraction of FA in its free form (FFA) and bound form (as F-AXOS) from destarched wheat bran: (i) pressurized microwave water extraction (PMWE) and (ii) pressurized hot water extraction (PHWE). The influence of solid/liquid ratio, temperature, time and agitation rate was studied.

Additionally, the simultaneous effect of temperature and time was tested by introducing the severity factor (Eq. (1)). This factor was introduced for the first time by Overend and Chornet (1987) and has been widely used as a measurement of the severity of thermal processes of lignocellulosic materials.

$$R_0 = t.exp\left(\frac{T-100}{14.75}\right) \tag{1}$$

being t time in minutes, T temperature in °C, 100°C a reference temperature and 14.75 an arbitrary constant related with activation energy.

2.4.1. Pressurized microwave water extraction (PMWE)

PMWE experiments were performed in a Monowave 300 microwave (Anton Paar GmbH, Graz, Austria) with a maximum delivered power of 850 W and able to operate up to 30 bar and 300°C.

Preliminary assays performed by PMWE varying temperature (185–225 °C), time (1–6 min), solid/liquid ratio (20–80 g/L) and agitation rate (600–1200 rpm) suggested that neither the solid/liquid ratio nor the agitation rate, affected the extraction yield (Annex A, Table A1 and Figure A1). Consequently, 600 rpm and 50 g L^{-1} of DWB were selected as agitation rate and initial concentration, respectively, for all the subsequent extraction experiments.

In a typical experiment, 1 g of DWB was mixed with 20 mL of water and heated with microwaves radiation. A factorial design was carried out with temperatures of 185, 200 and 225 °C and extraction times of 1, 3.5 and 6 min. Time zero of the experiments was considered when the desired temperature was reached. This heating period was always below 1 min. Finally, when the desired extraction time was completed, the system was cooled down with air at room temperature.

2.4.2. Pressurized hot water extraction (PHWE)

PHWE experiments were performed in a 300 mL reactor coupled with a turbine stirrer and a ceramic band heater (EZE-SEAL Model, Autoclave Engineers®, Pennsylvania, USA). The system can operate up to 200 bar and 300 °C. The reactor was partially filled with nitrogen and heated up to the desired temperature. Then, a DWB suspension was introduced using a metering pump to avoid the heating period. Once the extraction time was completed, the reactor was refrigerated with cold water, by means of an internal coil and an external bath. Following the same line of data processing, a factorial design was carried out for temperatures in the range 160, 200 and 240°C and extraction times of 0, 3.5 and 7.5 min (0 min refers to the time when all the suspension is inside the vessel, which is followed by an immediate cool down).

2.5. Characterization of the liquid extract

2.5.1. Determination of ferulic acid

FA in its free form (FFA) was determined by HPLC diluting the sample in the same volume of methanol. On the other hand, the total FA in the liquid extracts (TFA) was obtained after the alkaline hydrolysis of 5 mL of sample with 5 mL of NaOH 4 M and the same separation procedure used for solids (described in Section 2.3). The esterified ferulic acid (EFA) was determined by the difference between TFA and FFA, and it refers to the bound FA whose main linkage is an ester bond.

FA extraction yield was expressed as percentage of the initial FA content in the matrix, according to the following equations (Eqs. (2)–(4)):

$$Y_{TFA}, \% = \frac{TFA}{FA_{raw}} \times 100 \tag{2}$$

$$Y_{FFA}, \% = \frac{FFA}{FA_{raw}} \times 100 \tag{3}$$

$$Y_{EFA}, \% = \frac{TFA - FFA}{FA_{raw}} \times 100 \tag{4}$$

where FA_{raw} represents the initial FA content in the matrix determined according to procedure described in section 2.3.

2.5.2. Determination of arabinoxylans

The total amount of AXOS was determined by the quantification of the monomers (arabinose and xylose) in the liquid extract after an acid hydrolysis. For that, 20 mL of the sample were treated with 0.8 mL of sulfuric acid 72% w/w, during 1 h in the autoclave at 121°C. The sample was neutralized using calcium carbonate and analyzed by HPLC.

Results in terms of the arabinoxylooligosaccharides (AXOS) were calculated as follows:

$$Y_{AXOS}, \% = \frac{(AX_T - AX_F)}{(Arabinose + Xylose)raw} \times 100 \tag{5}$$

$$AXOS, \frac{g}{100gDWB} = \frac{(AX_T - AX_F)}{initial\ mass\ of\ DWB\ in\ the\ experiment} \times 100 \tag{6}$$

where AX_T represents the sum of the total arabinose and xylose (after acid hydrolysis of the liquid extract), and AX_F is the sum of the free arabinose and xylose in the liquid extract. The subscript *raw* represents the initial content in the raw material.

On the other hand, the degree of polymerization of the AXOS and/or F-AXOS was determined by determining the molecular weight distribution of the solubilized material by Size Exclusion Chromatography (HPLC-SEC). The molecular weight distribution allows determining relatively the length of the polymer chains by linking it with the molecular weights of the monomers that form the chain. Therefore, according to the number of monomer units forming the polymer chain, a classification can be made in terms of dimers, trimers, oligomers, or polymers.

2.5.3. Determination of monomeric sugars and degradation products

Quantification of monomeric sugars (arabinose, xylose and glucose) and furfural and 5-HMF in the liquid extract was performed by direct injection of an aliquot into the HPLC system. The production of degradation products (furfural and 5-HMF) is expressed according to the following equations (Eqs. (5) and (6)):

$$Y_{furfural}, \% = \frac{Furfural\ in\ liquid\ extract}{(Arabinose + Xylose)raw} \times 100 \tag{7}$$

$$Y_{HMF}, \% = \frac{5 - HMF\ in\ liquid\ extract}{(Glucose)\ raw} \times 100 \tag{8}$$

where the subscript *raw* represents the content in the initial matrix.

2.6. HPLC procedures

2.6.1. Quantification of ferulic acid

Quantification of FA and other phenolic compounds was performed using a Cortecs T3 column (2.7 μm, 4.6 × 100 mm, Waters Corporation, Massachusetts, USA) in a gradient mode of 4% acetic acid in Milli-Q water (solvent A) and acetonitrile (solvent B) with total flow rate of 0.7 mL min^{-1} and 30°C. The gradient was set as follows: 0 min 95% A, 10 min 90% A, 15 min 85% A, 20 min 75% A, 35 min 95% A and 45 min 95% A. Detection was made at two different wavelengths 280 and 320 nm, using a PDA detector (PDA 2998 Waters Corporation), the method was adapted from Sacristán San Cristóbal et al., 2011.

2.6.2. Quantification of sugars and degradation products

Sugar quantification was performed by using a Supelcogel™ Pb, 6% Cross Linked HPLC column and milli-Q water as mobile phase with a flow rate of 0.5 mL min^{-1} and 85°C, coupled with an IR detector (Waters 2414; Waters Corporation). This column is sensible to Ca^{2+} cations, thus, samples after the acid hydrolysis and the addition of carbonate, were mixed with an ion exchange resin (Dowex Monosphere MR-450 UPW, Sigma-Aldrich) for 20 min and filtered (Sánchez-Bastardo et al., 2017).

Sugar degradation products (furfural and HMF) were quantified by HPLC equipped with an UV–Vis detector (Waters 2489; Waters Corporation) at 254 nm, using the column Sugar SH 1011 (Shodex column; Showa Denko America, Inc., New York, USA), with H$_2$SO$_4$ 0.01 N as mobile phase at 0.8 mL min^{-1} and 50°C (Sánchez-Bastardo et al., 2017).

2.6.3. Molecular weight distribution of solubilized material

The molecular weight distribution of the solubilized material was determined by Size Exclusion Chromatography (HPLC-SEC). The system consists of a SB-804 HQ column (Shodex column; Showa Denko America, Inc.,) coupled with an IR detector (Waters 2414; Waters Corporation) that employs (NaNO$_3$ 0.1 M + NaN$_3$ 0.02% in milli-Q water) as mobile phase at a flow rate of 0.5 mL·min^{-1} at 35 °C (Sánchez-Bastardo et al., 2017).

A set of eight pullulan standards (P-82 Shodex Standard kit; Showa Denko America, Inc., New York, USA) ranged between 6.1 and 642 kDa of average molecular weight (M$_w$), were dissolved in milli-Q water and used to obtain the corresponding calibration curve. Chromatograms were processed by means of the Breeze Software (Waters Corporation). The molecular weight was given in terms of the weight average (M$_w$) and the number average (M$_n$); likewise, the polydispersity index (M$_w$/M$_n$) was calculated.

3. Results and discussion

3.1. Characterization of the raw material

Destarched wheat bran (DWB) is the raw material used in all the experiments with a mean particle size of 74.9 ± 2.5 μm and an initial moisture content of 2.8 ± 0.0%. A complete characterization of the DWB was performed in triplicates and showed in Table 1. The total alkali-extractable ferulic acid was 5157 ± 66 mg/kg dry DWB. The initial starch content in the Wheat Bran is 19.0 ± 1% and the enzymatic destarching process had an efficiency close to 97%.

3.2. Effect of operational variables on FA extraction

Operational variables must be studied and optimized to maximize FA extraction yield. In that sense, the effects of temperature and time in PMWE and PHWE were analyzed by means of two factorial designs (Annex B, Table B1 and Table B2, respectively). Fig. 1 shows the main results in terms of TFA extraction yield obtained for PMWE and PHWE

Table 1
Chemical composition of destarched wheat bran (DWB).

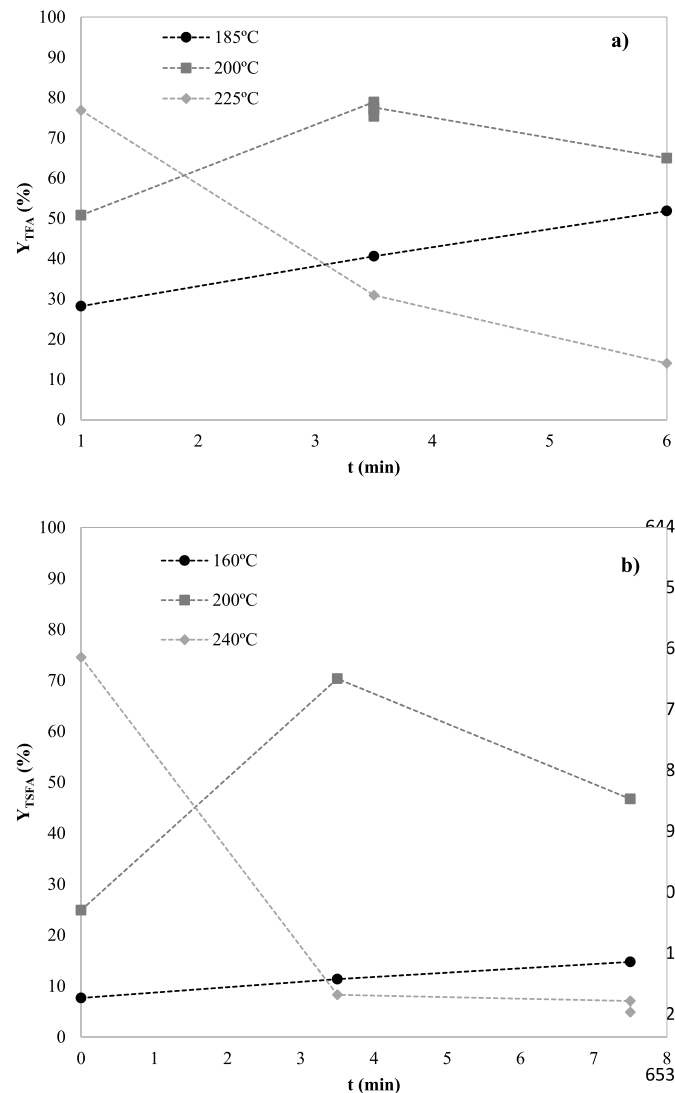| g/100 g dry DWB	
Extractives	5.5 ± 0.0
Carbohydrate content:	11.9 ± 1.0
Gluc	
Ara	11.3 ± 0.5
Xyl	19.5 ± 1.2
A/X	0.6
Proteins	16.3 ± 1.1
Total Lignin	28.5 ± 1.1
Starch	0.5 ± 0.0
Ash	2.5 ± 0.1
FA	0.5 ± 0.0

Fig. 1. Effect of temperature and time in the Total Ferulic Acid (TFA) extraction yield by a) PMWE and b) PHWE.

processes. Similar trends have been obtained for both techniques. At the lowest tested temperature, the amount of extracted FA increased directly with time. When the extraction temperature reached 200 °C, a maximum FA extraction at 3.5 min was obtained, since longer times lead to the thermal degradation of FA. Furthermore, at the highest tested temperature, TFA extraction yield decreased with time for both extraction processes and the thermal degradation was observed since the beginning.

The optimal extraction conditions obtained were identical for both extraction methodologies (200°C and 3.5 min) and therefore, three replicas of this optimum were performed. Under these conditions, the extraction yields of total, free and esterified FA were quite similar with both techniques. By PMWE, $76.1 \pm 0.5\%$ of the initial FA in the DWB passed to the liquid phase, being $82.9 \pm 0.4\%$ of the extracted FA in the bound form (EFA) and the remaining $17.1 \pm 0.4\%$ in the free form (FFA). On the other hand, with PHWE it was possible to extract $77.6 \pm 1.5\%$ of the total FA available in the DWB matrix, from which $83.1 \pm 0.2\%$ corresponded to EFA, and the rest ($16.9 \pm 0.2\%$) to FFA.

Since the trends of the extracted FA varied greatly with time accordingly to the temperature considered, the study of the extraction performance was made in terms of the severity factor (Eq. (1)). By using this factor, the simultaneous influence of temperature and time can be considered. In Fig. 2, the extraction yields of the TFA and FFA obtained with both technologies were plot as function of the $log R_0$. Both parameters presented a Gaussian-type behavior. An absolute maximum at $log R_0$ 3.7 was obtained for TFA extraction, while higher severity factors contribute to decrease the total amount of FA obtained (associated to the thermal decomposition of FA). However, the amount of FFA continue increasing until $log R_0$ 4.0, which indicates that the bound FA is firstly released to its free form and then, degraded.

As a result, the extraction conditions that produced a $log R_0$ 3.7 (200°C and 3.5 min) were selected as the optimum, being independent of the use of microwaves or conventional heating. In this regard, it must be said that previous authors working with PHWE or PMWE did not use the severity factor in their discussion (Rose and Inglett, 2010a, 2010b; Ruthes et al., 2017), but their optimum conditions lead to similar severity factors ($log R_0 \sim 3.6–3.7$). Moreover, the extraction yields obtained in the present work are higher than those in the literature: Rose and Inglett (2010a, 2010b) and Ruthes et al. (2017) reported 30 and 40% of EFA extraction yield, respectively using wheat bran as raw material; while regarding FFA, Rose and Inglett (2010a, 2010b) reported 8% of FFA extraction yield from maize bran. On another hand, in a previous work, Pazo-Cepeda et al. (2019) reported a slightly higher FFA extraction yield from wheat bran: 14.8%, however, they did not report the amount of EFA obtained.

3.3. Effect of operational variables on arabinoxylooligosaccharides extraction

The co-extraction of AXOS was also quantified in the two factorial designs. Fig. 3-A presents the amount of extracted AXOS by PMWE and PHWE, as function of the severity factor. The obtained trend is similar to
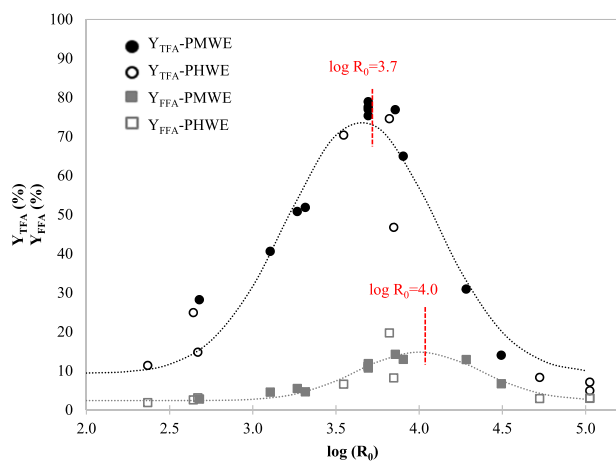
Fig. 2. Release of total (TFA) and free (FFA) ferulic acid using PMWE and PHWE, as function of the severity factor.
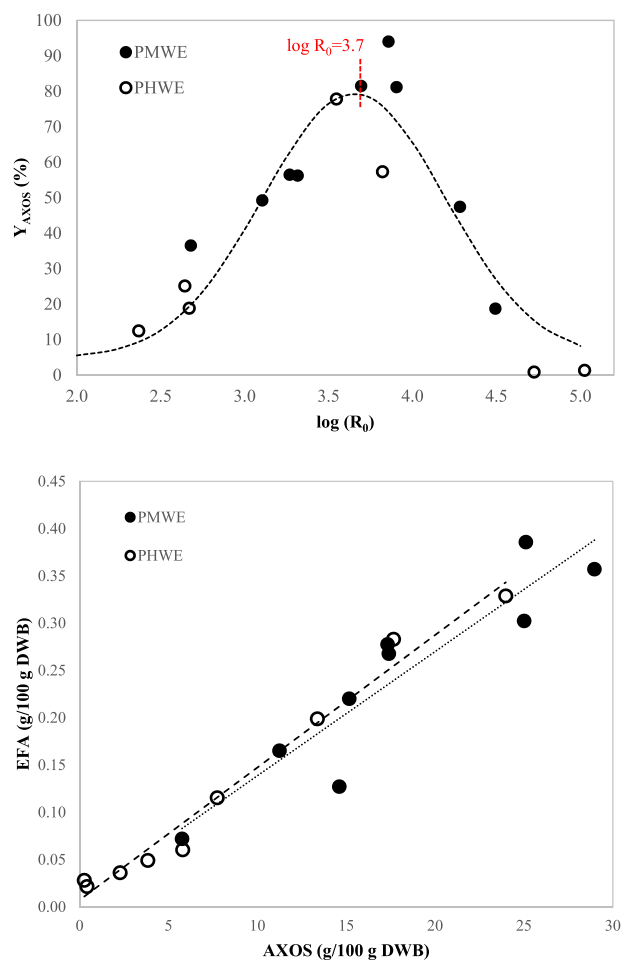
Fig. 3. Analysis of the F-AXOS extraction: A) Co-extraction of AXOS as function of the severity factor and B) Relation between the extracted bound FA (EFA) and the amount of AXOS, using PMWE and PHWE.

that of TFA, showing the maximum extraction yield at $log R_0$ 3.7. Under such conditions, 81.5 ± 2.6 and $79.3 \pm 3.0\%$ of the initial AX content was extracted by PMWE and PHWE, respectively. These percentages correspond to an extraction of 25.1 ± 0.8 and 24.4 ± 0.9 g of AXOS·100 g^{-1} DWB.

Since the optimum conditions for the extraction of TFA matched with that of AXOS ($log R_0$ 3.7), it seems possible to link the amount of extracted EFA with the amount of AXOS, to determine the amount of F-AXOS. Therefore, Fig. 3-B shows the amount of extracted EFA (g per 100 g of DWB) as a function of the extracted AXOS, at the different tested conditions. It can be observed that, independently of the heating method, the amount of EFA extracted increases linearly with the amount of co-extracted AXOS, revealing that the FA is bound to the AXOS in a ratio of $\sim 1.34 \pm 0.13$ g EFA/100 g AXOS. The resulting amount of F-AXOS (quantified as the sum of EFA and AXOS) at the optimum extraction conditions of 200°C and 3.5 min ($log R_0$ 3.7) accounted 24.4 ± 0.8 and 24.3 ± 0.9 g F-AXOS·100 g^{-1} DWB with PMWE and PHWE, respectively.

In order to explain the obtained results, the nature of the AXOS must be considered. AXOS are short chains of xylose units, linked by β-(1–4) glycosidic linkages, with α-arabinose substituents on the O-2 and/or O-3 positions (Saulnier et al., 2007), that can be esterified to FA. The energy required for the cleavage of a glycosidic bond is smaller than that of an ester bond, according to literature estimations (Fig. 4): the former is in the range -7.1 kJ/mol and -15.5 kJ/mol (Guvench et al., 2009), while
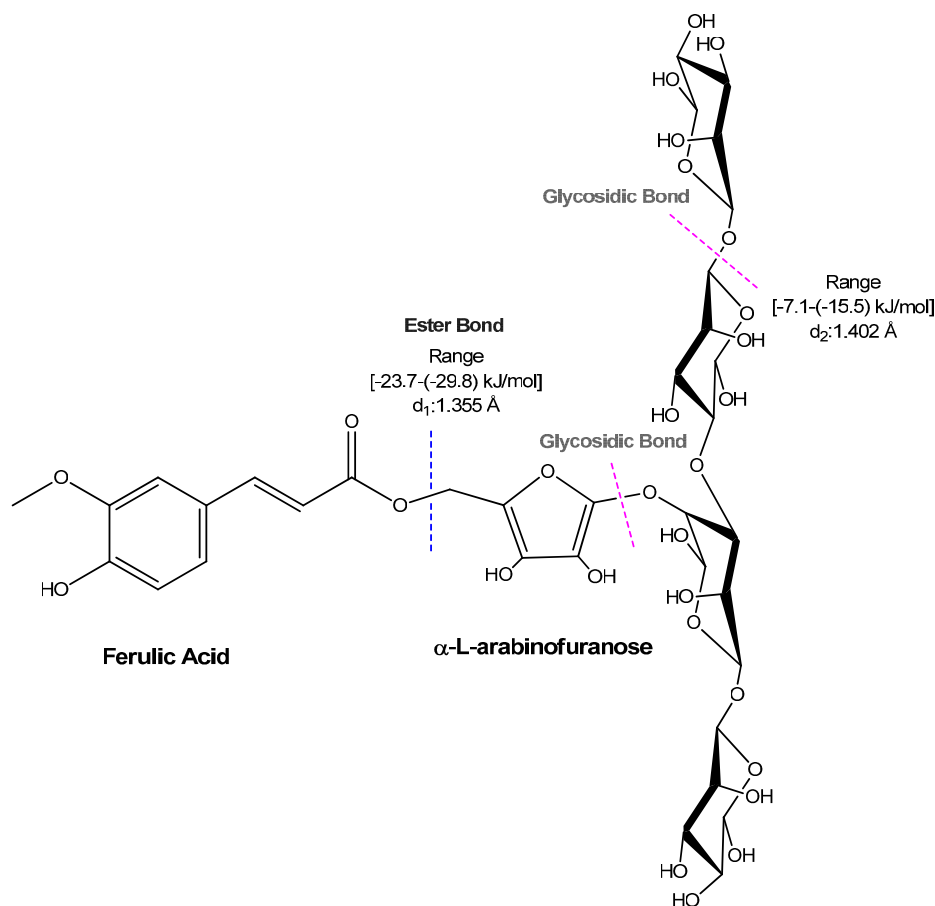
Fig. 4. Scheme of FA linked to arabinoxylans.

the latter ranged between −23.7 and −29.8 kJ/mol for arabinose esters (Hunt et al., 2017). Consequently, enhanced energy conditions are required to break the ester bonds, and therefore, it could explain why under the tested conditions, FA is still obtained in the bound form, esterified to the AXOS. However, it should be considered that other parameters such as pH can also influence the cleavage mechanism.

It is necessary to determine the molecular weight of the obtained AXOS/F-AXOS to elucidate their final application. On the one hand, short chain AXOS are classified as possible prebiotics, which include non-digestible oligosaccharides that selectively stimulate the growth of beneficial bacteria (bifidobacteria). On the other hand, AXOS of longer chains are likely to ferment more slowly, therefore, they supply carbohydrates to a disease-prone region of the gut (Moura et al., 2008; Rastall and Maitin, 2002; Rose and Inglett, 2010a, 2010b). Alternatively, high molecular weight AXOS and F-AXOS can be used as bio-based materials, due to their biocompatibility, biodegradability, and barrier properties to oxygen (Ruthes et al., 2017). Consequently, a complete characterization of the liquid extract requires the study of the molecular weight distribution (MWD). Fig. 5 presents the MWD of the extracts obtained by PMWE and PHWE at the optimum conditions of 200 °C and 3.5 min. The so obtained curves resulted in a wide range of molecular weights and with a similar pattern between the two techniques. Following the classification proposed by Rose and Inglett (2010a, 2010b), the molecular weight value of 1338 Da has been highlighted in Fig. 5, since the AXOS with higher molecular weights of 1338 Da present a degree of polymerization (DP) higher than 10 sugar monomeric units and therefore, they can be considered as polysaccharides. Nevertheless, it must be highlighted that the shown molecular weight could be influenced by
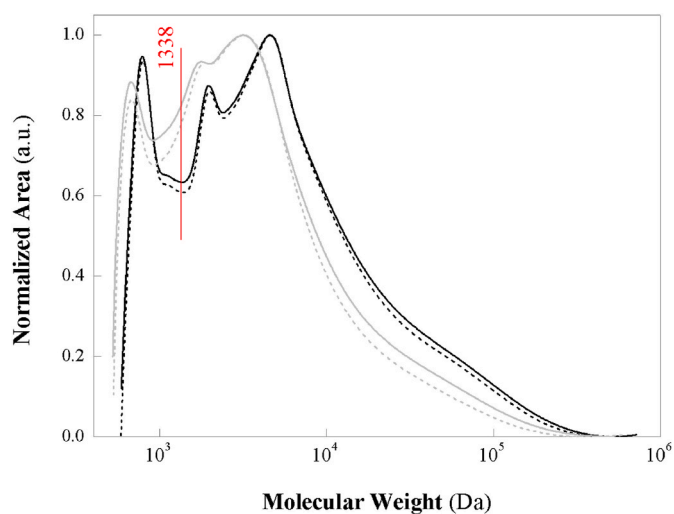

Fig. 5. Distribution of molecular weights obtained by means of size-exclusion chromatography of the liquid extract. Extraction conditions: 200 °C and 3.5 min using PMWE (grey) and PHWE (black). Dashed line: duplicate extraction. 1338 Da indicates DP > 10.

other extracted compounds such as proteins.

The AXOS/F-AXOS obtained with PHWE at the optimum conditions (200°C and 3.5 min) present higher values of the average molecular mass, both, in number (M_n) and in weight (M_w), 2477 Da and 12,990

respectively, in comparison with that obtained with PMWE (M_n1967 Da and M_w8214 Da). However, it is important to note that the value of the polydispersity (M_w/M_n) obtained with microwaves is lower (4.18) than the obtained with the autoclave (5.30), which means a greater homogeneity of the molecular weights of the obtained AXOS.

In comparison to other authors, the extraction yields here obtained are comparable and even higher than those previously reported in the literature. Ruthes et al. (2017) reported an extraction yield of 55.1% of the initial AX, along with 40% of the initial FA esterified to them, using defatted and destarched wheat bran as raw material and PHWE at 160°C and 15 min ($log\ R_0 \sim 3$). The lower severity factor applied by these authors explain the lower extraction yields, however, their F-AXOS presented a higher molecular weight since the molecular weight of the AXOS decrease with the severity factor. On the other hand, Rose and Inglett (2010a, 2010b) proposed a two-stage hydrothermal processing using microwave heating to obtain F-AXOS from wheat bran. They were able to recover up to 70% of the initial AXOS with a 30% of the initial ferulic acid at 200-210°C followed by immediate cool down. In addition, Rose and Inglett (2010a, 2010b) obtained similar results when using PMWE in non-extra marinated and partially deproteinized corn bran. They were able to extract up to 50% of initial AXOS along with around 8% and 60% of the initial FA in the free and esterified form, respectively, at the tested conditions of 180°C and 10 min or 200°C and 2 min. The severity factor reported by these authors agree with the obtained in this work ($log\ R_0 \sim 3.6$–3.7), however, results were not discussed in these terms.

3.4. Monosaccharides, furfural and HMF production

Arabinoxylans are extracted as AXOS, but they can continue to be reduced to the simplest units, such as monomeric sugars (xylose, arabinose and glucose) and/or further degraded to furfural or HMF. Therefore, in this section, the total amount of sugars, and their main degradation products (furfural and 5-HMF), are analyzed.

Following the same line of result discussion, the influence of severity factor in the yields of total C5 sugars (arabinose and xylose) and furfural is presented in Fig. 6-A, whereas the evolution of glucose and 5-HMF (C6) is presented in Fig. 6-B. In both cases, the sugar fraction shows a similar trend to that observed for FA extraction. They increase till a maximum of $log\ R_0 \sim 3.6$–3.7, and beyond this value of severity factor the content of C5 sugars diminishes by their degradation into furfural, and the glucose, into 5-HMF. This indicates that values of $log\ R_0 > 3.7$ are not recommended since they produce the thermal degradation not only of the FA, but also of the sugar fraction.

Table 2 summarizes the obtained values for the total amount of sugars (as oligomers and monomers) and their degradation products after the extraction with PMWE and PHWE at the optimal conditions of 200 °C and 3.5 min ($log\ R_0$3.7). Most of the released sugars were arabinose and xylose. When the fractionation was carried out with conventional heating, 91.2%, 88.7% and 38.7% of the initial arabinose, xylose and glucose were recovered, respectively. The amount of monomeric xylose and glucose in the extract were almost negligible, these two molecules were part of the AXOS. However, 35.9% of the total arabinose was identified as monomer (3.7 ± 0.8 g/100 g DWB) in the extract and the rest was constituent of the AXOS. Similar results were obtained by PMWE: the arabinose, xylose and glucose extraction yields accounted 94.7%, 94.3% and 36.1%, respectively; expressed as total sugar recovered, while 36.5% of the total arabinose was quantified as monomeric arabinose (3.9 ± 1.3 g/100 g DWB) in the extract.

3.5. Characterization of the residual solid

The fractionation of FFA and F-AXOS as described in the previous sections can be planned as a valuable first step of a biorefinery, based on wheat bran. Therefore, to verify the capability of using the residual solid in further valorization steps, its composition was studied (Table 3). The
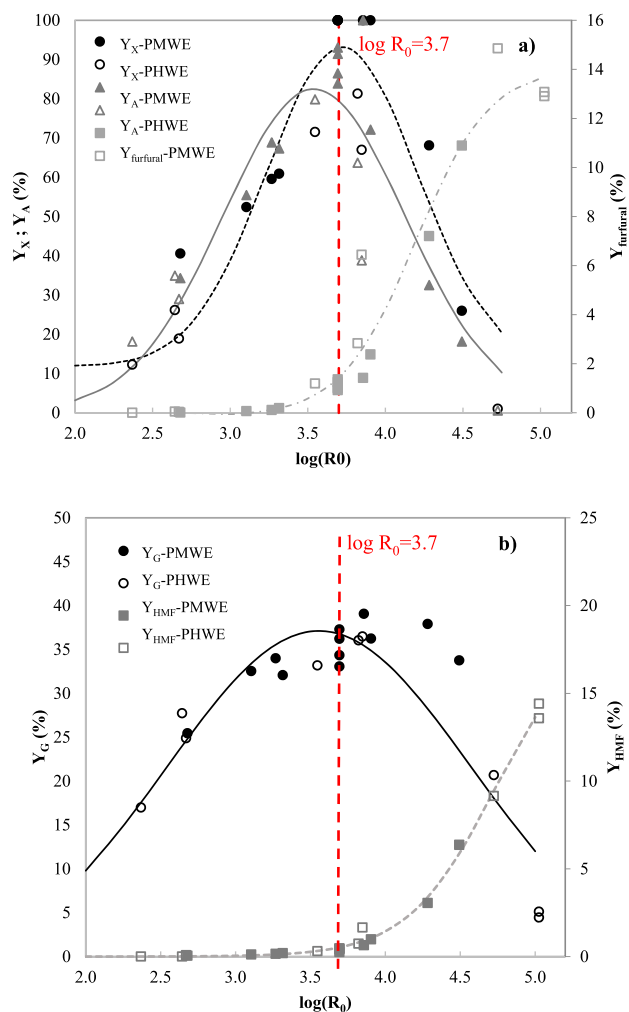
Fig. 6. Extraction yields of (A) total xylose (Y_x), arabinose (Y_A) and furfural ($Y_{furfural}$); and (B) total glucose (Y_G) and HMF (Y_{HMF}) versus log (R_0), by PMWE and PHWE methodologies.

Table 2
Characterization of the sugar fraction and its degradation products after the extraction at 200°C and 3.5 min by PMWE and PHWE. Solid/liquid ratio 50 g·L^{-1} and agitation rate 600 rpm.

	PMWE (g·100g^{-1} DWB)	PHWE (g·100g^{-1} DWB)
Arabinose	10.7 ± 0.8	10.3 ± 2.6
Free-Arabinose	3.9 ± 1.3	3.7 ± 0.8
Xylose	18.4 ± 1.2	17.3 ± 0.7
Furfural	0.35 ± 0.06	0.25 ± 0.05
Glucose	4.3 ± 1.2	4.6 ± 0.3
HMF	0.05 ± 0.01	0.05 ± 0.01

mass of residual solid after the extraction under optimum conditions (PHWE, 200°C and 3.5 min) represents a $48.6 \pm 2.0\%$ of the initial DWB. Glucose derived from the cellulose and lignin are the main constituents of the residual solid: 21% and 56%, respectively, since these fractions are usually extracted at harder conditions, using supercritical water (Cocero et al., 2018). Contrary, the hemicellulose content (represented by the amount of arabinose and xylose) highly decreased, because they were obtained in the liquid extract. On the other hand, part of the proteins was extracted, but they still constitute an 11% of the residual solid. It should be noticed that the nitrogen to protein conversion factor applied was the same as for wheat bran, despite it could have slightly

Table 3
Characterization of the residual solid after the extraction at 200°C and 3.5 min by PHWE. Residual solid 48.6 ± 2.0 g·100 g^{-1} DWB.

	g/100 g residue
Ash	1.5 ± 0.2
Proteins[a]	10.9 ± 0.8
Total lignin	55.7 ± 3.1
Glucose	20.9 ± 0.3
Arabinose	3.0 ± 0.4
Xylose	7.6 ± 0.5
Ferulic acid	0.1 ± 0.0
Total	99.7 ± 5.3

[a] Calculated using a nitrogen to protein conversion factor of 5.7 (as in destarched wheat bran).

varied. Moreover, the residual solid contains traces of the FA that was not extracted during the extraction procedure (around 20% of the initial FA present in the biomass).

These results indicate that the procedure proposed for the FA and F-AXOS extraction, let a solid completely available for its treatment in subsequent steps of the biorefinery, adding more value to the wheat bran.

4. Conclusions

The use of pressurized water is a suitable process as a first step in an integrated biorefinery, avoiding the severity of alkaline or acid extraction and preserving the solid residue for its use in subsequent stages. The maximum extraction yields for FA (~78%) and AXOS (~80% initial AX) were obtained at 200°C and 3.5 min ($log R_0$ 3.7). Degradation of FA and sugar fraction takes place beyond this severity factor. No significant effect in terms of extraction yields were obtained with PMWE. The remained solid is rich in lignin and cellulose, which makes it susceptible for further valorization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2021.101374.

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