Carbohydrate Polymers 105 (2014) 317–324

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Extraction by three processes of arabinoxylans from wheat bran and characterization of the fractions obtained

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ARTICLE INFO

Article history:
Received 24 October 2013
Received in revised form 27 January 2014
Accepted 29 January 2014
Available online 6 February 2014

Keywords:
Arabinoxylan
Wheat bran
Alkaline-extraction
Hydrothermal treatment
Endoxylanase

ABSTRACT

Arabinoxylans (AXs) were extracted from destarched wheat bran (DWB) according to different processes, with a view to their production at industrial scale. Two fractions (F3a and F3b, respectively, purified on 10 kDa and 100 kDa ultrafiltration membranes) were obtained with low yields by treating DWB with an endoxylanase and this process left a solid residue exhausted in enzyme-extractable AXs (EDWB). F1 and F2 AX fractions were obtained by treatment with sodium hydroxide of, respectively, DWB and EDWB. The fraction F4 resulted from a hydrothermal treatment of EDWB in a pressure reactor, followed by ethanol precipitation. The different AX fractions were characterized and compared for the composition in monosaccharides, for the contents in fats and in ferulic, phytic and uronic acids, for the molecular mass distribution and the degrees of methylation and acetylation. The alkaline extractions gave one deesterified AX population with molecular mass (MM) higher than 670 kDa and arabinose/xylose ratios (Ara/Xyl) around 1. The enzyme and thermal treatments yielded AXs with two main populations in size-exclusion chromatography (the largest one at 5–12.5 kDa and a second one at 140–160 kDa), having overall Ara/Xyl of, respectively, 0.7 and 0.5 for both processes. These data bring information about the influence of the process on the characteristics of AX fractions obtained from pretreated wheat bran. Here are also reported processes that enabled to recover enzyme-unextractable AXs from DWB, including an original and up-scalable hydrothermal extraction. Phytate contents of isolated AXs are described for the first time.

1. Introduction

Arabinoxylans (AXs) are major components of hemicellulosic fractions of cereals. They consist of a homopolymeric backbone chain of 1,4-linked-β-D-xylopyranose units, to which randomly branched *O*-acetyl-α-L-arabinofuranosyl are linked. In addition, the AXs are also substituted by α-D-glucopyranosyl uronic unit or its 4-*O*-methyl derivative in the position 2-O, and by ferulic and *p*-coumaric acids which are esterified to the C-5 of arabinosyl units (Peng, Peng, Xu, & Sun, 2012). Once destarched, the sugars from hemicellulose of wheat bran represent around 40% of the dry weight (Aguedo, Vanderghem, Goffin, Richel, & Paquot, 2013). AXs are valuable compounds and wheat bran may be a source of choice since it is a highly available and cheap agriculture by-product.

The commercial value of AXs stems mainly from their applications as food ingredients, but various other prospects have been arising in many industrial sectors, such as cosmetics, pharmaceutics, materials (notably in films formation), traditional or green chemistry, etc. (Deutschmann & Dekker, 2012). AXs can be used as a source of fiber, providing the known associated health benefits (Izydorczyk & Dexter, 2008; Zhou et al., 2010). Moreover, the prebiotic character of arabinoxylooligosaccharides (AXOS) has attracted a great deal of interest in the field of nutrition, both in scientific research and in food applications (Falck et al., 2013), or as feed additives (Geraylou et al., 2013). A prebiotic is a non-digestible and selectively fermented food ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that beneficially affects the host (Gibson, Probert, van Loo, Rastall, & Roberfroid, 2004). A second potential health benefit may result from the phenolic acids associated to AXs, and which are known to exhibit very strong antioxidant, free radical scavenging and anti-inflammatory activities (Rao & Muralikrishna, 2006). Ferulic acid associated with AXOS (linked or not) was demonstrated to have strong antioxidant activities (mainly when not bonded)

Abbreviations: AX, arabinoxylan; AXOS, arabinoxylooligosaccharides; Ara/Xyl, arabinose/xylose ratio; DWB, destarched wheat bran; EDWB, (enzymatically-)exhausted destarched wheat bran; Dw, dry weight; MM, weight-average molecular mass; MM_m, mass-average molecular mass; HPSEC, high performance size-exclusion chromatography.

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http://dx.doi.org/10.1016/j.carbpol.2014.01.096

(Snelders, Dornez, Delcour, & Courtin, 2013), moreover feruloylated AXOS decreased the oxidation of human low density lipoprotein, i.e. a potential role in preventing or reducing the progression of atherosclerosis (Katapodis et al., 2003).

Additionally, AXs or AXOS exhibit functional properties related to their molecular mass, which may affect the structure, the texture or the appearance of food products (Izydorczyk & Dexter, 2008).

Part of AXs in wheat bran can be dissolved in water (Escarnot, Aguedo, Agneessens, Wathelet, & Paquot, 2011), whereas a large portion remains not easily extractable from the cell-wall of wheat bran (Zhou et al., 2010). This is due to the fact that these heterogeneous polysaccharides form hydrogen bonds with cellulose, and are linked to lignin by covalent ether linkages (Ebringerová & Heinze, 2000).

Alkaline treatments can help to improve considerably solubilization of hemicellulose and various processes were reported (Bergmans, Beldman, Gruppen, & Voragen, 1996; Deutschmann & Dekker, 2012; García et al., 2013). Thus high molecular mass AXs were extracted from wheat bran with NaOH, with low to medium yields (Escarnot, Aguedo, & Paquot, 2011; Merali et al., 2013; Shiiba, Yamada, Hara, Okada, & Nagao, 1993; Zhou et al., 2010). Whereas acid treatments tend to depolymerize the AXs into monosaccharides and release phenolic compounds (Aguedo et al., 2013; Wallace et al., 1995), a process was reported for AXOS production under mild temperature and acidic conditions (Otieno & Ahring, 2012a).

AXs can also be extracted by using xylanolytic enzymes (Benamrouche, Crônier, Debeire, & Chabbert, 2002; Escarnot, Aguedo, & Paquot, 2012; Zhou et al., 2010). Endo-1,4-β-xylanases randomly cleave the 1,4 backbone of the xylan chain, releasing fractionated AXs and/or AXOS of various sizes (Collins, Gerday, & Feller, 2005; Moers, Celus, Brijs, Courtin, & Delcour, 2005).

Hydrothermal methods have also been used to extract hemicellulose, for instance by heating in reactors (Rose & Inglett, 2010), with pressurized water on wood (Leppänen et al., 2011), by using microwave heating or steam explosion in aqueous conditions (Peng et al., 2012) or in ethanol (Hongzhang & Liying, 2007). However hydrothermal or thermo-chemical processes may be associated with formation of undesirable byproducts such as furfural, and lower oligosaccharide yields, two main disadvantages (Otieno & Ahring, 2012b).

According to the extraction method used, the hemicellulose fractions obtained can present different compositions and consequently their properties may differ. For example AXs from barley husks isolated by different methods led to variable film forming properties (Höije, Gröndahl, Tømmeraas, & Gatenholm, 2005).

In this work, AX fractions were obtained from pretreated wheat bran using three kinds of processes: an alkaline extraction, an enzyme treatment and a hydrothermal process. Then the samples were thoroughly characterized, which enabled to evaluate in what extent their composition can be impacted by the isolation procedures. The processes described here were conceived to be directly transposable on an industrial scale.

2. Experimental

2.1. Materials

Food grade wheat bran was provided by Meneba Taelman (Bossuit, Belgium) to Cosucra Groupe Warcoing S.A. (Warcoing, Belgium). There, the bran was destarched by mixing for 10 min with water at 95 °C in a ratio of 1/10 (w/w). The resulting suspension was separated by filtration and further washed with an equal amount of water. The destarched wheat bran (DWB) was then dried at 100 °C for 24 h. This and the following extractions were done at a semi-pilot scale.

2.2. Extractions of arabinoxylan (AX)

2.2.1. Extraction of AX by enzymatic treatment

A suspension of DWB in water (9% dry matter) was submitted to a hydrolysis at 55 °C by an endoxylanase from *Bacillus subtilis* (Beldem – a division of Puratos NV, Groot Bijgaarden, Belgium) during 24 h under stirring. The residual bran exhausted in enzyme-extractable AXs (EDWB) was separated by vacuum filtration and further washed with demineralized water. The filtrate and the washing water were mixed and purified by ultrafiltration on 10 kDa or on 100 kDa membranes, to yield, respectively, the fractions named F3a and F3b.

2.2.2. Extraction of AX with sodium hydroxide

100 g of DWB or of EDWB was mixed with 900 mL of 0.44 M NaOH (pH 12.5). The suspension was kept under stirring for 15 h at 80 °C. The supernatant was recovered by centrifugation (4400 rpm, 15 min). Further purification was done by ultrafiltration on 100 kDa fiber membranes. The initial volume of retentate was decreased by a factor of 2.5. Diafiltration water was added back to the initial volume and then the volume was reduced by a factor of 2.5. This was repeated twice. The fractions obtained from DWB and EDWB were, respectively, named F1 and F2.

2.2.3. Extraction of AX by hydrothermal treatment

The EDWB underwent a hydrothermal treatment in order to recover the remaining AX fraction. 250 g of the solid residue was mixed with 5 L of distilled water and the pH was adjusted to 5 with H_2SO_4 1 M. The treatment was done at 180 °C during 3 min (temperature increase of 5 °C/min), in a stainless steel pressure reactor (7.5 L, Parr Series 4552 HT, Parr Instrument Co, Moline, IL, USA), equipped with a 6-blades impeller and a controller system (model 4848 Modular Controller, Parr Instrument Co, Moline, IL, USA). Once cooled, the medium was filtered on 100 μm nylon mesh and then the solid residue was rinsed with 1 L of distilled water. The whole liquid (around 5 L) was evaporated to 700 mL with a rotary evaporator (Rotavapor®, Büchi Labortecknik AG, Flawil, Switzerland). Then 4 volumes of ethanol were added in order to precipitate the polysaccharides and it was left overnight. It was then filtered on 25 μm nylon mesh and rinsed with 1 L of a 80% ethanol solution. The solid was then freeze-dried and finally it was ground with a laboratory mill (IKA Labortechnik, Staufen, Germany) to yield a homogeneous AX fraction. This sample was named F4.

2.3. AX characterization

2.3.1. Proximate analyses

Dry weight (Dw) was determined by drying samples at 106 °C to constant weights (AOAC, 1995). Total ash content (525 °C for 3 h, expressed as percent of Dw) and proteins (Kjeldahl procedure, a factor of 6.25 was used for conversion of nitrogen to crude protein) were analyzed according to AOAC methods (AOAC, 1995).

2.3.2. Total monosaccharides composition

Non-cellulosic total monosaccharides composition was determined according to the method of Englyst and Cummings (1984). The samples were hydrolyzed in triplicate with 1 M H_2SO_4 (3 h at 100 °C); total monosaccharides (rhamnose, arabinose, xylose, mannose, glucose and galactose) were reduced with $NaBH_4$ and acetylated by anhydrous acetic acid (10 min at room temperature). 2-Deoxy-glucose (Sigma–Aldrich, Bornem, Belgium) was used as internal standard. The acetylated alditols were extracted with dichloromethane before being analyzed on a Hewlett-Packard Agilent 6890 series gas chromatograph equipped with a high-performance capillary column, HP1-methylsiloxane

(30 m × 0.32 mm, 0.25 μm film thickness) (Scientific Glass Engineering, Melbourne, Australia). Helium was the carrier gas with a flow rate of 1.6 mL/min. The injection temperature was 290 °C, and the temperature program was 1 min at 120 °C, followed by a linear increase in 4 min to 220 °C and then in 35 min to 290 °C which was maintained for 4 min. Compounds were detected using a flame ionization detector at 320 °C. Standard monosaccharides (purity >99.5%, Sigma Chemical Co., St Louis, MO, USA) were used for the determination of response factors. The monosaccharide content was expressed as polysaccharide mass, in percent of Dw.

2.3.3. Ferulic acid content

Phenolic compounds (expressed as ferulic acid) were quantified as described previously (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). Briefly, they were analyzed by HPLC after extraction and saponification. The solid samples were saponified in NaOH 2 M overnight under nitrogen in the dark, at room temperature. After acidification to pH 2 with HCl 2 M, the phenols were extracted by ethyl acetate and this phase was evaporated under nitrogen. The residue was dissolved in methanol/H_2O (1:1, v/v) and this phase was analyzed by HPLC coupled to a photodiode array detector (Waters 2690 and 996, respectively, Waters Co., Zellik, Belgium), on a C_{18}-reversed phase column Zorbax 300SB-C18 (150 mm × 4.6 mm ID, 3.5 μm particle size, Agilent Technologies Belgium S.A./N.V., Diegem, Belgium). Elution (1 mL/min) was carried out at 35 °C with 75% of a 0.05% trifluoroacetic acid (TFA) solution in deionized water, and 25% of a 0.05% TFA solution in acetonitrile. Phenolic compounds were detected at 320 nm. Ferulic acid (Sigma–Aldrich, Bornem, Belgium) was used as an external standard.

2.3.4. Galacturonic and glucuronic acids content

Both uronic acids were first released by hydrolyzing 50 mg of each sample in 7 mL of 2 M TFA during 4 h in a heating chamber at 105 °C. Once cooled down, the solutions were neutralized with NaOH 50%, before being completed to 100 mL with distilled water. A volume was then filtered through a 0.45 μm syringe filter and poured into 2 mL vials. The uronic acids were detected by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) equipped with a Carbopac PA-100 column and a PA-100 precolumn (the whole from Dionex, Sunnyvale, USA). Calibration curves were done with commercial galacturonic and glucuronic acids (Sigma–Aldrich, Bornem, Belgium).

2.3.5. Fats content

The total fat content was determined according to Folch method. 5 g of dry sample were extracted with 50 mL of $CHCl_3$/methanol (2:1, v/v), overnight under stirring. The whole was filtrated on a pleated paper filter, and washed with the same mixture. The liquid was put in separating funnel and 20 mL of NaCl 0.58% was added, stirred and left to settle. The $CHCl_3$ phase was recovered and was filtered on $MgSO_4$ in a previously weighted Büchi flask. It was dry evaporated at 35 °C and left under vacuum for 15 min, before the flask was weighted. The main fatty acids were esterified with BF_3-methanol and then identified and quantified by gas chromatography using a Supelco® 37 component FAME mix (Sigma–Aldrich, Bornem, Belgium).

2.3.6. Phytates content

The determination of phytates was done by a method adapted from Latta and Eskin (1980). All the glassware used was thoroughly cleaned with 2.5 M hydrochloric acid. Total phytates from milled homogeneous samples were extracted by HCl 0.65 M at 5% (w/v), during 1 h. After centrifugation (8000 rpm, 25 min), the supernatant was recovered. Two to 10 mL (depending on the presumed phytate content) were transferred with a volumetric pipette into a volumetric flask and filled to 50 mL with deionized water: this constituted the extract solution to be eluted on a column. The column was made from a 10-mL plastic straight pipette, clogged with a mineral wool plug, and containing 0.63 g of ion exchange resin Dowex® 1X8, 200–400 mesh (Acros Organics, Geel, Belgium) in deionized water, then re-conditioned with 10 mL of NaCl 0.7 M and rinsed with deionized water until complete removal of Cl^- ions (checked through reaction with $AgNO_3$). A volume of 10 mL of the extract solution, charged with a volumetric pipette, was eluted on the column and then rinsed with 10 mL of deionized water followed by 15 mL of NaCl 0.1 M to remove inorganic phosphate. Total phytates were then eluted with 15 mL of NaCl 0.7 M and this eluate was recovered in a 25 mL volumetric flask. Wade reagent (5 mL of a 500-mL solution in deionized water containing 0.1875 g of $FeCl_3 \cdot 6H_2O$ and 1.875 g sulfosalicylic acid) was immediately added with a volumetric pipette and the volume was filled to 25 mL with deionized water, before being centrifuged (2000 rpm, 12 min). Two mL of the supernatant was gently withdrawn and its absorbance at 500 nm was measured. Phytates were quantified with the aid of a calibration curve obtained with phytic acid sodium salt (from rice, Sigma–Aldrich, Bornem, Belgium).

2.3.7. Molecular mass distribution

Apparent molecular mass (MM) distributions were assessed by size-exclusion high performance liquid chromatography (HPSEC), performed with a Waters 2690 Alliance chromatograph coupled to a refractometer Waters 2410. The column used was TSK-Gel GMPWxl (300 mm × 7.8 mm I.D.) (Tosoh, Tokyo, Japan). The column temperature was maintained at 30 °C and the refractometer was held at 40 °C. The mobile phase was an aqueous solution of 50 mmol/L $NaNO_3$ and 0.05% NaN_3 and the flow rate was 0.7 mL/min. Samples solutions in distilled water (2 mg/mL) were filtered through 0.45 μm syringe filters prior to injection of 100 μL. A calibration curve was established using dextran standards with mean peak molecular masses (Mp) of 1080, 4440, 9890, 21,400, 43,500, 66,700, 123,600, 196,300, 276,500, 401,300 Da (Sigma–Aldrich, Bornem, Belgium). The data were processed using Millennium®32 software (Waters Co., Zellik, Belgium).

2.3.8. Degrees of methylation and acetylation

The method was that described by Voragen, Schols, and Pilnik (1986). Briefly, after saponification (2 h at 4 °C in isopropanol/NaOH 0.2 M), methanol and acetic acid were quantified through HPLC (Waters 2690 Alliance chromatograph), equipped with an Aminex HPX-87H column (300 mm × 7.8 mm) (Bio-Rad Laboratories N.V., Nazareth Eke, Belgium) coupled to a refractometer (detector at 40 °C) (Waters 2410). The eluent was an isocratic H_2SO_4 5 mM solution at 0.6 mL/min and at 30 °C. Succinic acid was used as the internal standard.

3. Results and discussion

3.1. Extraction of AX

DWB contained 41% total neutral non-cellulosic sugars, with only 5.4% glucose of which a small part came from residual starch evidencing the efficiency of the destarching, since the present native wheat bran contained 20–22% of starch (not shown). Arabinose and xylose amounted to 34.3% and the arabinose/xylose ratio (Ara/Xyl) of this bran sample was 0.54 (Table 1). The treatment of DWB with an endoxylanase left a solid residue exhausted in enzyme-extractable AXs (EDWB) which dry weight represented 75% of the DWB. EDWB had an Ara/Xyl ratio of 0.83, higher than DWB, and was composed of 41.4% of total saccharides, with arabinose and xylose representing 37.6% of the dry weight (Table 1).

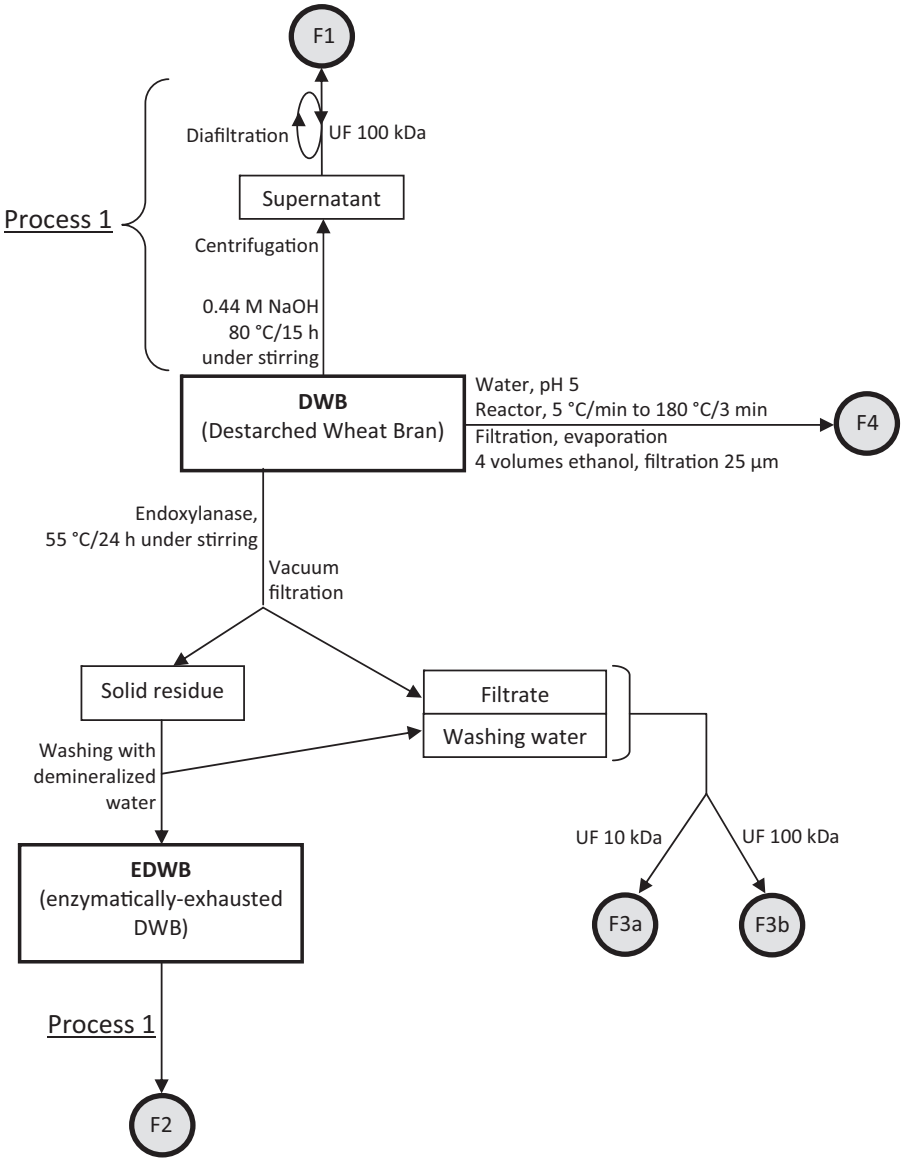
Table 1
Yield of recovering and total non-cellulosic neutral saccharides (as % of Dw) of the samples and arabinose/xylose ratios (Ara/Xyl). Values ± SD with $n = 3$ independent samples.

Samples	Yield (% Dw)	Total saccharides (% Dw)								Ara/Xyl
		Rhamnose	Arabinose	Xylose	Mannose	Glucose	Galactose	Total	Others[a]	
DWB	100.0[b]	0.1 ± 0.0	12.0 ± 0.4	22.3 ± 0.4	0.2 ± 0.0	5.4 ± 0.2	1.1 ± 0.0	41.0 ± 1.1	0	0.54
EDWB	75.0[b]	0.1 ± 0.0	17.1 ± 0.3	20.5 ± 0.1	0.2 ± 0.0	2.1 ± 0.0	1.4 ± 0.2	41.4 ± 0.7	0	0.83
F1	20.8[b]	0.1 ± 0.0	27.8 ± 0.7	29.7 ± 1.3	0.1 ± 0.0	2.9 ± 0.2	2.0 ± 0.1	62.8 ± 2.4	2.6 ± 0.3	0.94
F2	25.7[c]	0.1 ± 0.0	28.7 ± 0.5	27.3 ± 0.4	0.1 ± 0.0	0.5 ± 0.1	1.9 ± 0.0	58.6 ± 1.0	2.5 ± 0.1	1.05
F3a	4.3[b]	0.3 ± 0.1	27.8 ± 0.9	41.1 ± 0.8	0.2 ± 0.0	6.1 ± 0.0	2.4 ± 0.0	77.8 ± 1.9	2.7 ± 0.3	0.68
F3b	4.2[b]	0.5 ± 0.0	29.1 ± 0.5	40.1 ± 0.6	0.3 ± 0.1	5.3 ± 0.3	3.2 ± 0.1	78.3 ± 1.6	2.7 ± 0.2	0.73
F4	19.5[c]	0.2 ± 0.0	19.5 ± 0.3	37.2 ± 0.4	0.2 ± 0.0	3.5 ± 0.1	1.9 ± 0.0	62.5 ± 0.8	1.9 ± 0.1	0.52

[a] Estimated from the unknown peaks on GC spectra that were further identified as sugar derivatives by GC–MS analysis. An arbitrary response factor of 1 was used.
[b] Dry weight in kg obtained from 100 kg DWB.
[c] Dry weight in kg obtained from 100 kg EDWB.

The total sugar contents of DWB and EDWB were similar due to an extraction of non-saccharidic matter occurring during the enzymatic process; however the higher Ara/Xyl ratio in EDWB indicated that the endoxylanase released preferentially sparsely substituted AXs.

DWB and EDWB were then used to produce AX fractions according to different processes, as described in paragraph 2.2 and summarized on Scheme 1. As already mentioned, DWB was processed with a commercial endoxylanase from *B. subtilis* and this was done according to optimized conditions in order to solubilize a maximum of the AX content. The AXs were then purified by ultrafiltration into fractions F3a and F3b by using, respectively, 10 or 100 kDa membranes (Scheme 1) so that AXs with populations of rather oligomeric or polymeric sizes, respectively, could be



Scheme 1. The multi-step processes leading to the different AX fractions (F1–F4).

obtained. Both isolated F3 fractions represented around 4% of the dry weight of DWB, which is a low yield value (Table 1). EDWB still had a substantial AX content (41.4% of total sugars) which could no more be significantly accessed by different enzymes, as broadly tested by us (not shown). So, alkaline and hydrothermal treatments were applied. An extraction in NaOH medium (pH 12.5) was performed on EDWB, and also directly on DWB, yielding after ultrafiltration (with a 100 kDa membrane), respectively, F2 and F1, two fractions to be compared. By this process, the recovered fractions F1 and F2 represented, respectively, 20.8 and 25.7% of the bran weight (Table 1).

The thermal treatment was conducted on EDWB at 180 °C during 3 min and pH 5, according to conditions previously optimized by us in order to maximize the AX recovery and minimize the free monosaccharides (not shown). The AXs were then precipitated by adding ethanol, as reported by García et al. (2013). Thus, F4 represented 19.5% of the weight of EDWB. The thermal treatment was also applied to DWB, but it only resulted in an increase of free saccharides when compared to the fraction obtained from EDWB, so the results were not presented here.

The dried and powdered AX fractions are presented in Fig. 1. As can be evaluated from the image, the process used affected the color of the products, i.e. the hydrothermal treatment resulted in the darkest sample (F4), the alkaline extractions led to slightly less brownish products (F1 and F2), whereas the enzyme gave lighter yellowish powders (F3a and b). High temperatures or alkaline pH led to the formation of sugar-degradation compounds bringing brownish/reddish tones; however small amounts of lignin and phenolics may also be extracted and contribute to a darkening of the products (Bergmans et al., 1996; Carvalheiro, Duarte, & Gírio, 2008). The use of an enzyme limited the formation of side-products and yellowish fractions were thus obtained.

3.2. Characterization of AX fractions

The dry weights determined at the time of the characterization of the five powders are given in Table 2. Whatever the process, the protein content of the samples was between 6.3 and 7.8% of the Dw. In contrast, the process used had an impact on the ash contents, since the extractions with sodium hydroxide gave the final products with the highest content in salts, slightly above that of the thermal treatment, whereas the enzymatic process yielded the lowest contents of 1% (Table 2). The composition in total monosaccharides (expressed as equivalent polysaccharides) of the five products is presented in Table 1. The highest total contents in non-cellulosic saccharides of the AX fractions were reached by the enzyme treatment (78%), the other processes led to products with around 60% total polymerized saccharides. Two or three unknown peaks appeared on GC spectra, some of which were further identified by mass spectrometry analyses as sugar derivatives (not shown). For information, their amount was estimated between 1.9 and 2.7% of the Dw using an arbitrary theoretical response factor in GC equal to 1 ("others" in Table 1).

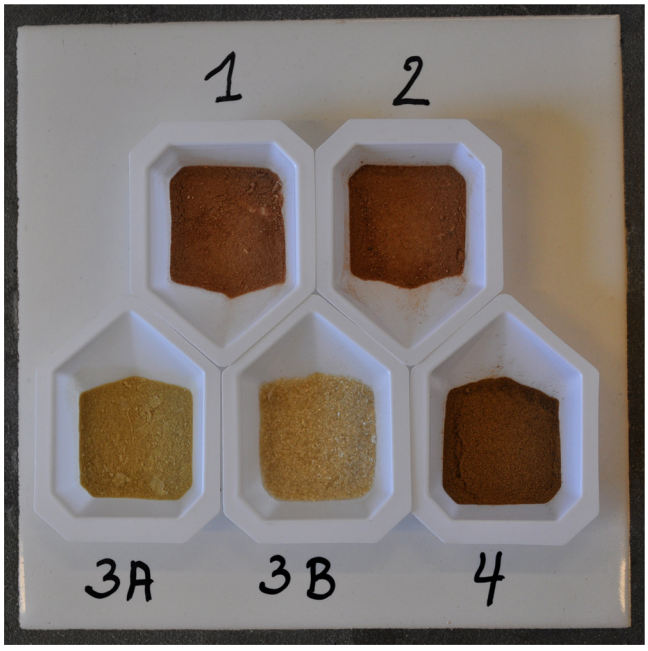


Fig. 1. Image of the different AX fractions obtained.

Table 2
Proximate analyses of the AX fractions: dry weight (Dw), proteins and ash contents (% of Dw). Values ± SD with $n = 3$ independent samples.

Samples	Dw (%)	Proteins (% Dw)	Ash (% Dw)
F1	92.51 ± 0.05	7.13 ± 0.01	5.67 ± 0.28
F2	91.82 ± 0.24	7.76 ± 0.01	4.17 ± 0.48
F3a	96.12 ± 0.14	7.67 ± 0.10	1.01 ± 0.18
F3b	96.33 ± 0.03	6.34 ± 0.01	0.99 ± 0.12
F4	90.74 ± 0.23	7.03 ± 0.04	3.80 ± 0.08

The AX fractions had variable Ara/Xyl ratios, around 0.7 for the enzymatically produced fractions, 0.5 for the one obtained thermally and 1.0 for the samples resulting from the alkaline treatments. These values showed in what extent the process used can impact the characteristics of the isolated AXs. Thermal treatments release rather high amounts of free arabinose from bran, depending on the pH (Aguedo et al., 2013), which consequently here led to AX with a low Ara/Xyl. Alkali-extracted AXs contained the same percentages of arabinose as the enzyme-extracted AXs, however they contained less xylose, resulting in Ara/Xyl around 1. Ara/Xyl ratios of the AX fractions can be compared with those of the native AX in DWB and EDWB (0.6 and 0.8, respectively) (Table 1). The alkaline conditions seem to decrease the total xylose, possibly by releasing it from AX, whereas arabinose is rather freed under acid conditions (Aguedo et al., 2013). Nevertheless the degradation of xylan under alkaline conditions was reported to be a slow reaction proceeding only from the reducing end-group (Aspinall, Greenwood, & Sturgeon, 1961). The fractions obtained from DWB (F1, F3a and b) contained more glucose than the AXs alkali-extracted from EDWB (F2), which could indicate that a glucan or xyloglucan fraction of DWB is extractable by the xylanase. However F4, obtained from EDWB through the hydrothermal process, contained a higher amount of glucose than F2; two hypotheses arise to possibly explain this: the treatment with NaOH degraded the main part of glucose, or the thermal process released small amounts of the cellulose fraction of EDWB.

The patterns obtained by SEC chromatography for the AX fractions are presented in Fig. 2. On those outlines, gray vertical lines indicate the position of the peaks for dextran standards with their mass average MM (MM_m). The alkaline fractions F1 and F2 were composed of a unique wide population which peak values were higher than that of a dextran standard with a MM_m of 670 kDa. So, the extraction with sodium hydroxide from DWB or from EDWB gave similar AX populations with high MM (degree of polymerization higher than 4000). In contrast, the profiles from enzyme-extracted fractions (F3a and b) presented two main populations: one major (around 80%) with a maximum very close to that of a dextran with a MM_m of 5 and 12.5 kDa, respectively, for F3a and F3b, and a second one (around 20% of the total) with a peak around a dextran MM_m of 140 kDa for both samples, but representing a higher proportion in F3b. The curve of F3b presented also a shoulder around 1.2 kDa, i.e. oligomers with an average

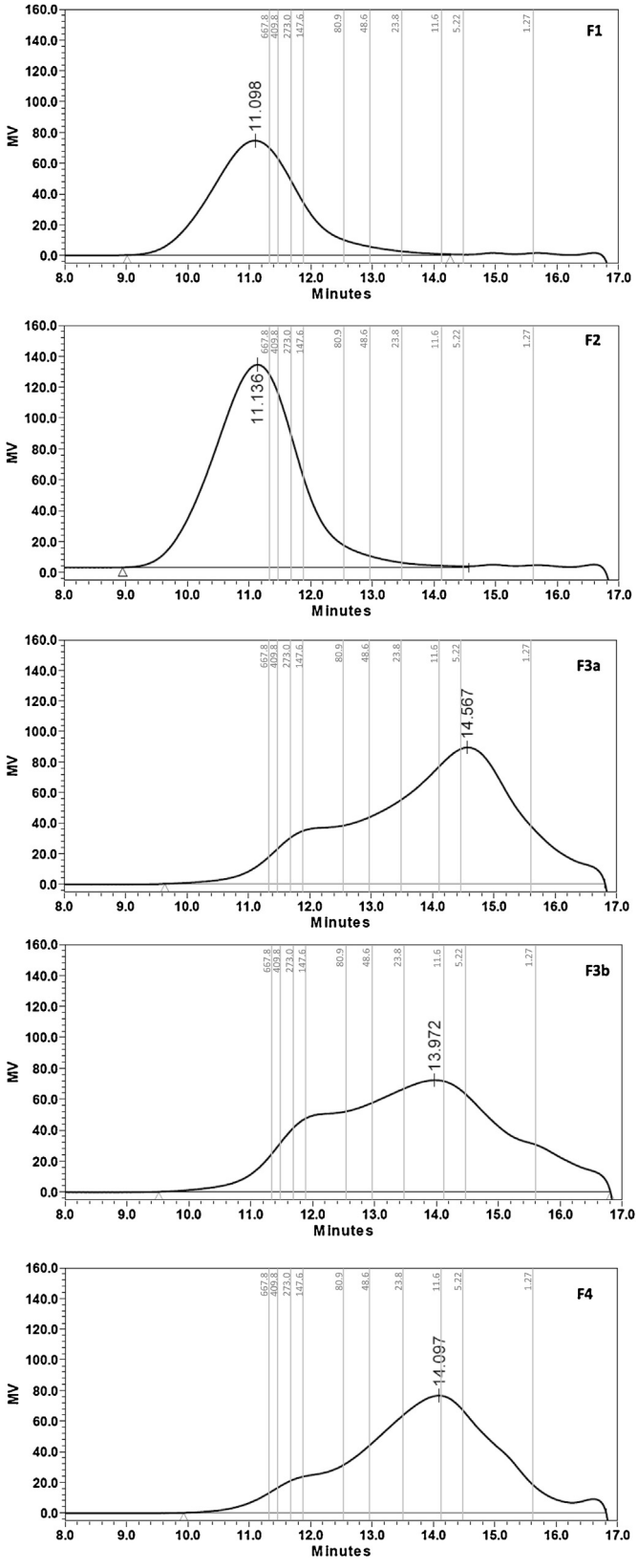


Fig. 2. HPSEC profiles (RI response) of the different AX fractions (names are indicated at the top right of each figure); the vertical gray lines indicate the peak positions of dextran standards with their values of mass average MM (MM_m) in kDa. Each profile is representative of three independent analyses.

degree of polymerization of 9–10. F3a and b were isolated using ultrafiltration membranes of 10 and 100 kDa, respectively; the consequences are a fraction F3b with a greater proportion of high MM AXs and a main population with more than twice the value of MM than that of F3a. The profiles of both F3 fractions show that the isolated AXs were poorly degradable by an endoxylanase, which is a consequence of their complex structure with mono- and di-substitutions of xylose by arabinose or even dimeric arabinose (Schooneveld-Bergmans, Beldman, & Voragen, 1999). The profile for F4 resembled that of F3a, i.e. two main polysaccharide populations having dextran-equivalent MM_m of 12 and 160 kDa, with additionally a slight shoulder around 2.6 kDa (Fig. 2). It is worth noting that the AXs with the highest MM (F1 and F2) had the highest Ara/Xyl ratios, whereas the fraction with the lowest amount of high MM AXs (F4) had the lowest Ara/Xyl ratio (Table 2). Using ethanol precipitation enabled to obtain a fraction with the lesser content in high MM_m compounds (F4), even less than using an ultrafiltration membrane of 10 kDa (F3a). Using ethanol in an industrial process may be problematic, however if it could enable to get higher-added value products, then its use may be considered, particularly if the ethanol can be recycled. Otherwise, an optimization of the "size" and the yields of the products obtained by membrane-separation at an industrial scale should be envisaged.

The total fat content of the fractions was determined (Table 3). Xylanase and hydrothermal treatments yielded practically defatted AX fractions while the extraction with sodium hydroxide led to the solubilization of hydrophobic compounds that ended up in the isolated fractions, representing 3–4% of the Dw. Some fatty acids were identified by mass spectrometry, the main ones being, in decreasing order, oleate, palmitate, linoleate and myristoleate (C14:1) (Table 3). Quite commonly when dealing with fragmentation of bran (to obtain polysaccharides or fibers for example), the reported processes include a cumbersome defatting step, such as a Soxhlet extraction with *n*-hexane for 6 h (Bergmans et al., 1996). The enzymatic and hydrothermal processes used here to isolate AXs show that they need no defatting step. In contrast alkaline extraction would need a previous defatting in case the presence of 3–4% fats in AXs is problematic.

The osyl units laterally substituting the xylose chains of AXs are acetylated and methylated to varying degrees (Peng et al., 2012). The most acetylated product was the one obtained by the thermal process, then came the enzyme-extracted AXs and the lowest acetylation was observed for the fractions from alkaline treatment. The presence of methyl groups was detected only in F4 and F3b (Table 4), which suggests that in the case of F3b, methylation occurs on some high MM AXs not present in F3a (Fig. 2) as a consequence of the different membrane cut-off used to obtain both fractions. NaOH has a non-specific de-esterifying action, and it led also to AXs containing no ferulic acid. In contrast, the samples from the enzyme treatment contained around 0.9% (of Dw) ferulate. F4 was almost 4 times less feruloylated (Table 4). Ferulic acid, a major bound phenolic acid, is ester linked to AXs and influences their physicochemical properties. Enzymatically produced feruloyl-oligosaccharides from AX have antioxidant activity (Katapodis et al., 2003; Rao & Muralikrishna, 2006) although it was recently demonstrated that this antioxidant activity decreases for ferulate bound to AXOS or cross-linked through dehydrodiferulate bond (Snelders et al., 2013). The thermal treatment released part of ferulic acid, as previously described with barley bran under steam explosion treatment releasing phenolic compounds (Gong, Huang, & Zhang, 2012).

Galacturonic and glucuronic acids were quantified by HPAEC (Table 4). The fractions alkali-extracted (F1 and F2) seemed to contain slightly less galacturonate than the other fractions, whereas the fractions enzyme-extracted (F3a and F3b) clearly had

Table 3
Total fats extracted through Folch method (as % of Dw) and the main fatty acid species identified (expressed as % of the Dw of the fats extract). Values ± SD with $n = 3$ independent samples.

Samples	Total fats (% Dw)	Main fatty acids detected (% of Dw of the total fats extracted)						
		C14:1	C15:1	Palmitate	Stearate	Oleate	Linoleate	C20:1
F1	3.37 ± 0.26	9.3 ± 0.8	3.1 ± 0.3	17.8 ± 1.5	1.6 ± 0.1	33.7 ± 3.3	11.3 ± 3.8	3.5 ± 0.3
F2	4.33 ± 0.34	7.9 ± 0.6	3.5 ± 0.3	20.3 ± 0.0	1.2 ± 0.0	26.0 ± 0.5	9.3 ± 2.6	2.6 ± 0.0
F3a	0.12 ± 0.02							
F3b	0 ± 0.0							
F4	0.08 ± 0.01							

Table 4
Methanol and acetic acid released from the samples, and contents in ferulic, galacturonic and glucuronic acids (as % of Dw). Values ± SD with $n = 3$ independent samples.

Samples	% Dw				
	Methyl groups	Acetates	Ferulates	Galacturonates	Glucuronates
F1	0	0.19 ± 0.05	<0.001	0.26 ± 0.02	0.41 ± 0.05
F2	0	0.08 ± 0.00	<0.001	0.20 ± 0.03	0.40 ± 0.04
F3a	0	0.28 ± 0.06	0.87 ± 0.02	0.30 ± 0.02	0.16 ± 0.02
F3b	0.20 ± 0.06	0.57 ± 0.09	0.89 ± 0.00	0.36 ± 0.08	0.11 ± 0.01
F4	0.12 ± 0.03	0.64 ± 0.13	0.23 ± 0.00	0.31 ± 0.04	0.49 ± 0.10

less glucuronate than the others. This could be due to a selective extraction by the enzyme of AXs with low glucuronate contents or to a side enzyme activity releasing part of the glucuronic acid. Here the total contents in uronic acids (from 0.46 to 0.80% of Dw) were much lower than values up to 3.5% reported by Bergmans et al. (1996) in fractions alkali-extracted from wheat bran, however these authors used the *m*-hydroxydiphenyl colorimetric assay for total uronic acids determination, which is a different approach.

The content in phytic acid of each fraction was measured by the UV–vis method described above. Phytic acid concentration is commonly ranging from 40–60 mg/g in wheat brans (Dost & Tokul, 2006; García-Estepa, Guerra-Hernández, & García-Villanova, 1999). There are differing opinions with regard to its nutritional role. Indeed, phytic acid is a chelator of several minerals of nutritional importance, decreasing their bioavailability and possibly leading to dietary deficiencies. It is moreover reported to negatively affect protein and lipid metabolism (Kumar, Sinha, Makkar, & Becker, 2010). However, these antinutritional properties are questioned by some researchers (Levrat-Verny et al., 1999) and it seems that deleterious effects are more likely to occur in case of an imbalance diet rich in cereal and poor in meat and dairy products. Besides, phytic acid can be a source of phosphate and it is thought to be anticarcinogenic thanks to antioxidant properties (Graf & Eaton, 1990; Kumar et al., 2010). Total phytates were found here to be 10.6 mg/g for F4 (thermal treatment), 7.9 and 3.0 mg/g, respectively, for F1 and F2, whereas F3a and b (enzyme process) had the lowest contents (0.5 and 0.7 mg/g, respectively) (Table 5). The hydrothermal treatment at 180 °C did not release phytic acid or at least less than in the other processes. The difference between F1 and F2 showed that the AX fraction extracted from EDWB contained less phytates than the fraction alkali-extracted from DWB. So the AX remaining in EDWB seem to contain less phytates than the total fraction of DWB, however the AX obtained from DWB by the action of a xylanase had low contents. This may be explained by an enzymatic activity within the used commercial xylanase and releasing the main part of phytates; this "phytase" activity (more precisely myo-inositol hexakisphosphate phosphohydrolase) could not come from the DWB, since the process for the production of DWB contained a step in hot water deactivating enzyme activities. In all cases, the amounts of phytic acid detected here in AXs were much lower than the values usually reported for wheat bran (Dost & Tokul, 2006; García-Estepa et al., 1999). In case AX fractions with the lowest phytate content would be intended, enzymes should be involved in the process.

Table 5
Total phytates content of the samples (in mg/g Dw). Values from duplicate analyses.

Samples	Total phytates (mg/g)
F1	7.9 ± 0.6
F2	3.0 ± 0.2
F3a	0.5 ± 0.0
F3b	0.7 ± 0.0
F4	10.6 ± 1.7

4. Conclusions

Here were described up-scalable processes that enable to recover AXs from DWB and notably enzyme-unextractable AXs. The hydrothermal extraction is an original method that appears to be quick and efficient. It was shown in what extent the process impacted the AX fractions isolated from pre-treated wheat bran and notably their content in arabinose and xylose.

Alkaline treatments led to one population of AXs with high MM, with high Ara/Xyl ratios, with 3–4% fats, non-esterified neither by acetyl or methyl groups nor by ferulic acid, containing 0.65% uronic acids and around 0.5% total phytates.

Although the use of enzymes is much in line with a green chemistry perspective, here processing DWB with an endoxylanase led to low extraction yields of AXs; the isolated light-colored fractions were composed of polymer populations with medium MM, with Ara/Xyl ratios slightly higher than native AXs in DWB, with practically no fats, with acetyl and feruroyl groups, a total of 0.5% uronic acids and ten times less phytates.

The hydrothermal processing of EDWB gave an AX fraction composed of broad populations with medium MM, a low Ara/Xyl ratio, containing virtually no fats, esterified with acetate and in a lesser extent with ferulate and methanol, a total of 0.8% uronic acids and 1% phytates. In all cases the fractions contained around 7% proteins.

To our knowledge, this was the first determination of phytates in hemicellulosic fractions. Depending on the process used and on the bran pretreatment, AX fractions with different compositions and properties could be obtained. The most profitable processes here seem to be the alkaline and the thermal extractions to obtain, respectively, high MM and medium MM AXs. Considering the isolation method, both ultrafiltration and ethanol-precipitation were efficient, so the choice should be based on financial and environmental costs evaluations. A combination of processes could also be undertaken in order to maximize the recovery of AXs from bran.

Acknowledgments

The authors gratefully acknowledge Mrs. Chemotti for the technical assistance. This study was financially supported by the Walloon Region through BARCELONE project (number SPW 6511).

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