Carbohydrate Polymers 99 (2014) 415–422

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Microwave superheated water and dilute alkali extraction of brewers' spent grain arabinoxylans and arabinoxylo-oligosaccharides

Elisabete Coelho*,[1], M. Angélica M. Rocha[1], Jorge A. Saraiva, Manuel A. Coimbra

QOPNA, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 20 February 2013
Received in revised form 30 August 2013
Accepted 3 September 2013
Available online 6 September 2013

Keywords:
Brewer's spent grain
Polysaccharides
Arabinoxylans
Feruloylated arabinoxylo-oligosaccharides
Microwave-assisted extraction
Prebiotics
Food ingredients
Barley

ABSTRACT

Microwave superheated water extractions (MWE) were performed to evaluate the feasibility of this technology for quantitative recovery of the arabinoxylans (AX) or arabinoxylo-oligosaccharides (AXOS) from brewers' spent grain (BSG). The AX + AXOS yield increased with the increase of the temperature in the range from 140 to 210 °C during 2 min. The higher temperatures promoted depolymerisation, debranching, and deesterification of the polysaccharides, with formation of brown products. The conditions that promote a compromise between the yield and the structure obtained, minimizing the thermal degradation of the fractions extracted by MWE are the following: (1) 140 °C, to remove the residual starch mixed with β-glucans; (2) Suspension of the residue left in water and treated at 180 °C; (3) suspension of the residue in 0.1 M KOH and treated at 180 °C. Using this sequential procedure, it was possible to extract 62% of BSG AX + AXOS, presenting degrees of polymerization ranging between 7 and 24 xylose residues, and a degree of phenolic acids esterification between 5 and 21%. The structural variability obtained by MWE allows defining specific types of compounds for different applications and uses depending on the extraction conditions used.

1. Introduction

During germination of the barley grain, cell walls, mainly of the endosperm, are enzymatically degraded. After filtration of the liquid extracted from the mashing, remaining unhydrolysed cell walls, which are derived mainly from the aleurone and some insoluble material present in the endosperm, are disposed of as the brewers' spent grain (BSG). The BSG is composed exclusively by the material from barley husks, with 75% moisture, having a high percentage of polysaccharides, namely, arabinoxylans (AX, 22–23%), cellulose (12–25%) and small amounts of starch (4%) and β-glucans (Aliyu & Bala, 2011; Kabel et al., 2002a). BSG represents up to 85% of the total residues from the brewing process and it amounts to about 20 kg/hL beer. It is estimated that Europe produces 3.5 million tonne per year of BSG (Jay et al., 2008). This by-product has been used for animal feed as a way to reduce the residue produced by these industries. The use of BSG as a source of polysaccharides with fibre properties and other biological activities that have been associated with AX and β-glucans, namely prebiotic activity (Broekaert et al., 2011; Crittenden et al., 2002), has a great potential. These polysaccharides are considered as dietary fibre due to their resistance to hydrolysis by the digestive tract enzymes. Additionally, they can present immunomodulatory (Van Craeyveld et al., 2008), antioxidant (Ohta, Semboku, Kuchii, Egashira, & Sanada, 1997) and anticarcinogenic effect (Broekaert et al., 2011), and reduce blood serum triglycerides and cholesterol levels (Broekaert et al., 2011).

BSG AX are composed by a backbone of β-(1 → 4)-linked xylose residues containing single units of arabinose as side chains, phenolic acids, such as ferulic and *p*-coumaric acids are esterified to arabinofuranosyl residues (Bartolomé, Santos, Jiménez, del Nozal, & Gómez-Cordovés, 2002; Mandalari et al., 2005). Feruloylated arabinoxylo-oligosaccharides (AXOS) are more potent antioxidants and radical scavengers than ferulic acid itself (Broekaert et al., 2011; Katapodis et al., 2003; Ohta et al., 1997). *In vitro*, feruloylated AXOS strongly inhibit copper mediated oxidation of low density lipoprotein (Katapodis et al., 2003). The antioxidant properties of feruloylated AXOS derived from wheat bran have recently been confirmed in an *in vivo* model of diabetic rats (Ou et al., 2007). In diabetic rats, feruloylated AXOS significantly increased total antioxidant capacity level, glutathione peroxidase, and superoxide dismutase activities, decreased blood glucose and malondialdehyde levels, and decreased xanthine oxidase activity in serum and liver. Feruloylated AXOS were, overall, more efficient in mitigating oxidative damage in diabetic rats than sodium ferulate and ascorbic acid. Furthermore, feruloylated AXOS showed greater antioxidant capacity *in vivo* than *in vitro* when compared with ascorbic acid (Ou et al., 2007). The ester linkage between ferulic acid and AXOS prevents its uptake until reaching the colon, where AXOS-inducible bacterial feruloyl esterases are present (Vardakou, Nueno Palop,

* Corresponding author. Tel.: +351 234 370 706; fax: +351 234 370 084.
E-mail address: ecoelho@ua.pt (E. Coelho).
[1] These authors contributed equally to the work.

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.003

Gasson, Narbad, & Christakopoulos, 2007). Feruloylated AXOS are therefore a special type of antioxidants whose active principle is released in the colon (Broekaert et al., 2011). Moreover, formulations combining AXOS with AX potentiate their prebiotic activity (Broekaert, Courtin, Damen, & Delcour, 2010).

Microwave-assisted extraction is a process that uses microwave energy to heat the solvent that is in contact with a sample in order to partition analytes from the sample matrix into the solvent. The main advantage of this technique is the ability to rapidly heat the sample solvent mixture. By using closed vessels the extraction can be performed at elevated temperatures accelerating the mass transfer of target compounds from the sample matrix. Typically, microwave-assisted extraction procedure takes 15–30 min and uses small solvent volumes, about 10 times smaller than volumes used by conventional extraction techniques (Eskilsson & Bjorklund, 2000). Recently, microwave superheated water extraction (MWE) of arabinogalactans and galactomannans from spent coffee grounds was proposed (Passos & Coimbra, 2013). The use of microwaves for AX extraction has also been proposed for some industry residues, such as barley husks (Roos, Persson, Krawczyk, Zacchi, & Stalbrand, 2009), corn and corn stover (Benkő et al., 2007; Pang et al., 2012; Rose & Inglett, 2010; Yoshida, Tsubaki, Teramoto, & Azuma, 2010), wheat straw (Janker-Obermeier, Sieber, Faulstich, & Schieder, 2012), and flax shives (Buranov & Mazza, 2010). These polymers can be obtained directly in water (Roos et al., 2009; Yoshida et al., 2010), dilute alkali and/or acid (Benkő et al., 2007; Janker-Obermeier et al., 2012) or in a hydroalcoholic solution (Buranov & Mazza, 2010). The aim of this work was to determine the yield and changes in the structure of AX that can be recovered from BSG when microwave technology is used to carry out the extraction using water at temperatures reaching 140–210 °C during 2 min. In addition a sequential extraction using water and diluted alkali solution was assayed.

2. Experimental

2.1. Samples

The BSG used in this study was provided by Unicer-Bebidas S.A., Leça do Balio, Portugal, with 76% of moisture, being the dry material composed by carbohydrates (50%), proteins (30%), fats (8%), and ashes (5%).

2.2. Effect of temperature on the extraction of AX by microwave superheated water

BSG (10 g) was suspended in 60 mL distilled water in a 100 mL Teflon-coated vessel at a liquid/solid ratio of 6:1 and the pH was measured before the microwave irradiation. The slurry was then treated in a microwave oven (MicroSYNTH Labstation for Synthesis), using temperatures of 140, 170, 180, 190, 200, and 210 °C, during 2 min under continuous stirring. The temperature and pressure were controlled with a thermocouple immersed in the slurry.

After microwave irradiation, the pH was measured. The reactor was cooled at room temperature and centrifuged at 24,652 × *g* for 15 min at 1 °C and filtered to separate the water-soluble fractions from the cellulosic residues (CRs). The CRs were suspended in water, frozen, and freeze-dried. The MWE was performed in duplicate for each temperature.

Absolute ethanol was added to the water-soluble fractions to perform 70% (v/v) ethanol solutions, assuming additive volumes. These solutions were stirred for 2 h at 4 °C until formation of a precipitate and then were centrifuged to obtain the precipitate (Et70) that was removed from the supernatant solution (EtSn). To remove the ethanol completely, each precipitate and supernatant solution was dissolved in water, concentrated by rotary evaporation at 35 °C under reduced pressure, frozen, and then freeze-dried.

2.3. Sequential extraction of AX by microwave superheated aqueous solutions

Based on the temperature conditions studied, the BSG was submitted to a sequential extraction. BSG (10 g) was suspended in 60 mL distilled water using a liquid/solid ratio of 6:1. The microwave treatment was performed at 140 °C during 2 min under continuous stirring, reaching a pressure of 4 bar. The reactor was cooled at room temperature and the slurry was filtered. Absolute ethanol was added to the filtrate solution to reach 70% (v/v) ethanol and the material precipitated was recovered by centrifugation (140_Et70). The residue obtained was suspended in water using the same liquid/solid ratio used previously for the BSG extraction. The microwave treatment was performed at 180 °C during 2 min, reaching a pressure of 8 bar. The reactor was cooled at room temperature and the slurry was filtered. Absolute ethanol was added to the filtrate solution to reach 70% (v/v) ethanol and the material precipitated was separated from the supernatant by centrifugation, giving origin to fractions 180_Et70 and 180_EtSn, respectively. The residue obtained was re-suspended in an aqueous solution of 0.1 M KOH, using the same liquid/solid ratio and temperature. Under these MWE conditions a pressure of 27 bar was reached. The slurry was filtered and the residue was washed with water, frozen and freeze-died. The filtrate solution was neutralized until pH 5 with glacial acetic acid, leading to the formation of a precipitate, separated from the supernatant by centrifugation (180_KOH_pp). The material present in the supernatant was precipitated with ethanol 70% (v/v), allowing to obtain the 180_KOH_Et70 fraction (Fig. 1). Each precipitate and supernatant solution was dissolved in water, concentrated by rotary evaporation at 35 °C under reduced pressure, frozen, and then freeze-dried.

2.4. Neutral sugar analysis

Monosaccharides were released from cell wall polysaccharides by a pre-hydrolysis in 0.2 mL of 72% H_2SO_4 (w/w) for 3 h at room temperature followed by 2.5 h hydrolysis in 1 M H_2SO_4 at 100 °C (Selvendran, March, & Ring, 1979). For the hydrolysis of the cellulosic residue of the sequential extraction, a 2 M H_2SO_4 at 120 °C during 1 h was also used. Neutral sugars were analyzed as their alditol acetates by gas-chromatography-flame ionization detection (Blakeney, Harris, Henry, & Stone, 1983; Harris, Blakeney, Henry, & Stone, 1988). The hydrolysis of all fractions was performed in duplicate. Results used have less than 5% variability in the major component sugars.

2.5. Glycosidic-linkage composition of polysaccharide fractions

Glycosidic-linkage composition was determined by gas chromatography-quadrupole mass spectrometry (GC-qMS) of the partially methylated alditol acetates (Ciucanu & Kerek, 1984; Coelho, Rocha, & Coimbra, 2011; Harris, Henry, Blakeney, & Stone, 1984).

2.6. Determination of total phenolic compounds and antioxidant activity

Total phenolic composition was determined by the Folin–Ciocalteu colorimetric method (Coelho et al., 2011; Folin & Ciocalteu, 1927), and measured as gallic acid equivalents. The antioxidant activity was measured by scavenging of the ABTS

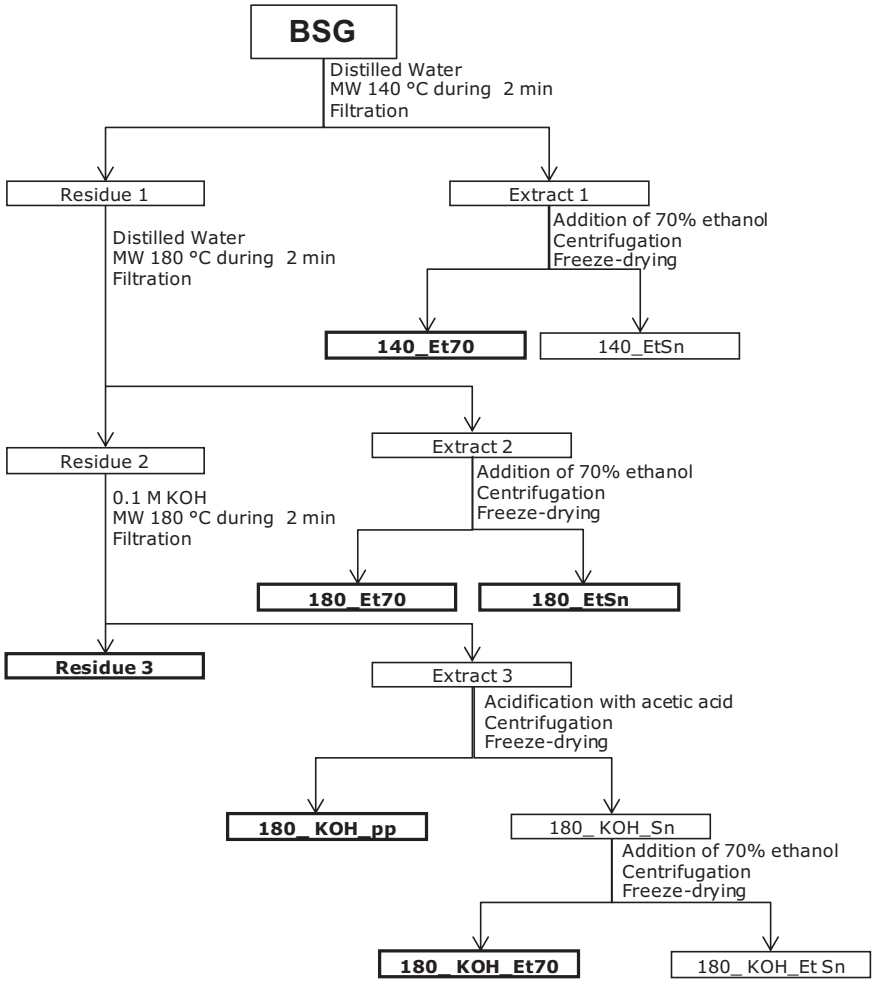

Fig. 1. Scheme of the AX and AXOS recovery from BSG by sequential MWE.

radical cation (Re et al., 1999). The results were expressed as trolox equivalents.

2.7. Determination of the presence of starch

In order to evaluate the presence of starch in the fractions obtained by the sequential MWE, the samples were treated with α-amylase from *Bacillus subtilis* (62 U/mg) at 37 °C overnight. During the enzymatic assay were used as positive control amylase, and as negative control cellulose. The sugars released by enzymatic hydrolysis were analyzed as described in Section 2.4.

3. Results and discussion

3.1. Influence of temperature on yield and sugar composition of the microwave extracted material from the BSG

The microwave superheated water extraction (MWE) was applied to BSG using the following temperatures: 140, 170, 180, 190, 200, and 210 °C. After MWE, the water-soluble fractions were precipitated with 70% (v/v) of ethanol, obtaining the Et70 fractions that were separated from the supernatant solutions (EtSn). Table 1 shows the results of the yield of soluble solids extracted from BSG for the different temperatures applied, *e.g.* calculated as the percentage of the mass of the fraction obtained in relation to the mass applied in the reactor for extraction. The amount of material extracted with hot water, on BSG dry basis, increased with the increase of temperature. The yield in the aggregate of fractions Et70 and EtSn recovered at 140 °C was 2%, increasing to 8% and 10% at 170 °C and 180 °C, respectively. Taking into consideration the mass not solubilized (residue, Table 1), it is possible to infer that the use of these temperatures allowed a recovery of the totality of the BSG compounds. However, although the yields of extraction increased to 17, 23, and 29% when the treatments were performed at 190, 200, and 210 °C, respectively, recovery of the BSG compounds was only 90%, showing the occurrence mass loss of the BSG components at these higher temperatures. These results suggest that the components of BSG were more thermolabile than those extracted from spent coffee ground, as at the temperature of 200 °C no mass loss was noticed (Passos & Coimbra, 2013).

3.1.1. Arabinoxylan recovery

The Et70 fractions recovered at all temperatures were richer in sugars, ranging between 64.6 and 84.2%, than the EtSn fractions, which were composed by 40.6–49.0% of sugars. Although for Et70 a tendency to obtain fractions richer in sugars with the increase of the temperature of extraction, no tendency was observed for EtSn. This higher extraction yields of sugars at higher temperatures, seems to result in residues with lower content of sugars. Concerning the residue left upon extraction, the relative amount of xylose increased with the increment of the temperature. Based on the amount of xylose in the fractions and their yield, the amount of xylose recovered in the Et70 fractions was estimated to range from 0.34% for 140 °C to 21.0% for 210 °C, whereas for EtSn the amount of

Table 1
Effect of temperature on extraction yields and sugar composition of the fractions obtained from brewers' spent grain by microwave superheated water extraction.

Fraction	Temperature	Yield (%)	% AX or AXOS (w/w)	mol %							Total sugars (mg/g)
				Rha	Fuc	Ara	Xyl	Man	Gal	Glc[a]	
Et70	140	1	0.4	0.6	0.1	9	10	1	3	76	646
	170	4	4	0.4	0.1	19	29	1	3	48	762
	180	5	9	0.4	0.1	21	37	1	3	37	826
	190	8	13	0.4	0.1	21	38	1	3	37	802
	200	9	16	0.2	0.0	19	45	1	3	31	808
	210	10	17	0.2	0.0	15	48	1	3	33	842
EtSn	140	1	0.3	1.0	0.1	10	5	2	1	80	487
	170	4	3	1.2	0.2	29	13	2	3	52	471
	180	5	5	1.1	0.4	40	22	2	4	32	406
	190	9	10	0.8	0.3	39	25	1	4	30	438
	200	14	19	0.8	0.2	41	36	1	4	17	476
	210	19	26	0.6	0.3	34	46	1	4	15	490
Residue	140	98	88	0.6	0.1	21	39	1	3	36 (47)	455
	170	95	92	0.3	0.0	19	39	1	3	38 (42)	494
	180	92	78	0.0	0.0	20	37	1	3	38 (47)	444
	190	79	65	0.2	0.1	18	37	2	3	41 (54)	445
	200	67	43	0.2	0.1	12	35	2	2	49 (39)	412
	210	61	29	0.2	0.0	9	29	2	2	58 (46)	385

[a] Numbers within parenthesis report the % of glucose released without pre-hydrolysis.

xylose ranged from 0.15 to 24.4%. The relative amount of arabinose increased with the increase of temperature applied until 180 °C. The further increase of temperature did not cause increment of the relative recovery of arabinose and at 210 °C a decrease of arabinose in both Et70 and EtSn fractions was observed (Table 1). The relative amount of arabinose was much higher in EtSn than in Et70 fractions.

The amount of xylose + arabinose extracted increased with the increase of temperature, however, the lower temperatures (140, 170, and 180 °C) extracted only small amounts of these sugars (0.69, 6.9, and 13.3%, respectively). As observed for the amount of material extracted, the best extraction conditions of xylose + arabinose were also achieved using higher temperatures (190, 200, and 210 °C), yielding 22.6, 34.5, and 43.4%, respectively. Nevertheless, the increase of the yield when higher temperatures are used caused a loss of xylose + arabinose content (Fig. 2). The temperatures until 180 °C showed a recovery of 92%, considering all the three fractions (Et70 + EtSn + residue), but from 190 °C to 210 °C the recovery decreased from 88% to 72%, respectively. At 210 °C, 28% of the total xylose + arabinose content was lost, probably due to the thermal degradation. A compromise between temperature and xylose + arabinose yield should be achieved.

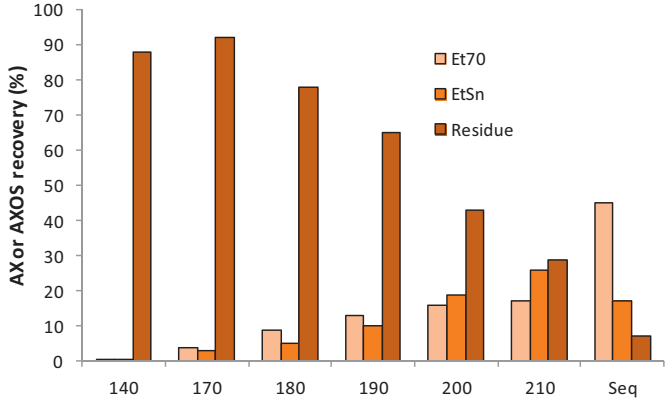

Fig. 2. AX or AXOS recovery from BSG, for 140, 170, 180, 200, and 210 °C MWE and for sequential MWE.

The pH of the BSG suspensions was measurement before and after the MWE. The initial pH value, before the treatment, was 5.93. Values of the final pH of the aqueous extracts ranged from 5.64 to 4.81. It was observed that the pH values decreased gradually with the increase of the temperature of MWE, which may contribute also for the higher amount of material extracted at higher temperatures. This pH decrease and the release of insoluble material into solution may be due to the occurrence of autohydrolysis of the insoluble BSG material upon microwave treatment (Benkő et al., 2007). The internal superheating of the cell water content, by absorbing the microwave radiation, should enable the cell disruption, allowing the solvent to access the polysaccharides (Kratchanova, Pavlova, & Panchev, 2004). The pH decrease can be due to the formation of acids "*in situ*" by the hydrolysis of esters, namely those of AX with ferulic and acetic acids or by the thermal degradation of arabinose residues that can be converted into formic and levulinic acids (Carvalheiro, Esteves, Parajó, Pereira, & Gírio, 2004).

3.1.2. Structural analysis of AX

In order to evaluate the structure of the arabinoxylans (AX) and derived oligosaccharides (AXOS) obtained for the different temperatures, a methylation analysis was performed to all Et70 and EtSn fractions (Table 2). The main linkage observed in Et70 fractions was (1 → 4)-Xyl, with exception of the sample treated with 140 °C, where the main linkage found was (1 → 4)-Glc, confirming the extraction of glucans at this lower temperature. The relative amount of (1 → 4)-Xyl in relation to total xylose recovered in the fraction increased gradually with the temperature range applied, from 51% to 80%. Arabinose occurred mainly as terminally linked for all temperatures (78–90% in relation to total arabinose). The content of arabinose decreased in relation to the xylose content with the increase of the temperature. This decrease is also in accordance with the relative decrease of the xylose branched residues, allowing to infer the debranching effect of temperature. These observations are in accordance with the temperature effect reported to occur in arabinoxylans (Benkő et al., 2007; Carvalheiro et al., 2004), as well as in arabinogalactans and galactomannans (Passos & Coimbra, 2013).

Concerning the EtSn fractions, the relative amount of (1 → 4)-Xyl was almost constant for the different fractions, varying from 50 to 74% in relation to the total xylose. However, the amount of branched xylose residues decreased in relation to the amount

Table 2
Extraction yields and sugar composition of the fractions obtain from brewers' spent grain with sequential extraction by microwave superheated water extraction.

	Yield (%)	% AX or AXOS (w/w)	mol %							Total sugars (mg/g)
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc[a]	
BSG	–	–	0.3	0.1	22	36	1	3	37 (38)	522
140_Et70	5	1	0.3	0.0	5	6	4	1	84	814
180_Et70	11	22	0.3	0.0	25	46	1	3	25	782
180_EtSn	10	13	0.9	0.2	55	33	1	4	7	408
180_KOH_pp	14	4	0.7	0.1	29	53	1	4	12	87
180_KOH_Et70	8	22	0.1	0.0	33	61	1	3	2	831
Residue	19	7	0.9	0.0	3	21	4	1	74 (42)	669
Residue[b]	19	7	0.5	0.0	3	21	4	1	72 (42)	799

[a] Numbers within parenthesis report the % of glucose released without pre-hydrolysis.
[b] Residue hydrolysed with 2 M H_2SO_4.

of total xylose. Also, the amount of arabinose tend to decrease in comparison to the total amount of xylose, allowing to infer that the compounds obtained in EtSn are less branched as the temperature increased, as observed for the Et70.

The branching degree calculated as the ratio of (2,4-Xyl + 3,4-Xyl + 2*2,3,4-Xyl)/total xylose of the fractions Et70 and EtSn decrease with the increase of the temperature (Table 2 and Fig. 3). Fig. 3 shows a linear decreasing ($y = -44.315x + 30.747$, $r^2 = 0.894$) of the ratio with the amount of AX recovered. The debranching of the AX promotes the increasing of arabinose in solution, converting it into furfural and caramelisation products (Carvalheiro et al., 2004). The amount of arabinose by itself does not justify all branching points present in the xylan backbone. Concerning the Et70 fractions, the ratio of Ara/(2,4-Xyl + 3,4-Xyl + 2*2,3,4-Xyl) ranges between 0.5 and 0.6. This range of ratios were also achieved for arabinoxylans present in barley residue (left after removal of water extractable polysaccharides) (Izydorczyk, Macri, & MacGregor, 1998). Wheat bran arabinoxylans also showed a similar ratio when in native form. However, when treated with TFA, they decreased to a ratio of 0.2 due to the lability of arabinose in acidic media. In these experiments, the presence of galactose and glucuronic acid attached to the heteroxylan backbone was suggested (Brillouet, Joseleau, Utille, & Lelièvre, 1982). Taking into account the contribution of terminal galactose and glucuronic acid, the ratio of the terminal to the branched residues [(t-Ara + t-Gal + t-GlcA)/(2,4-Xyl + 3,4-Xyl + 2*2,3,4-Xyl)] is 1.0. Accordingly, the presence of uronic acids has been also reported to occur in BSG (5–6 mol%), mainly in the water soluble solids from the alcohol insoluble residue, which accounted for 8 mol% (Kabel et al., 2002a), as well as in fractions retained in anion exchange chromatography (Kabel, Schols, & Voragen, 2002b). These authors reported that the acid fractions were more difficult to degrade by endoxylanase, presenting characteristics like those of highly branched glucuronoarabinoxylan-like material (Kabel et al., 2002b). As the BSG used in this work presented 7 mol% of uronic acids and terminally linked galactose, it is possible that these structures contain similar structural features as those observed previously by these authors.

The amount of (1 → 5)-linked arabinose, when compared with the amount of total arabinose, was higher in all EtSn fractions. These residues may result from the ester-linked ferulic and *p*-coumaric acids (Bartolomé et al., 2002; Chanliaud, Saulnier, & Thibault, 1995; Mandalari et al., 2005). These residues decreased with the increase in temperature for all fractions, from 44% at 140 °C to 9% at 210 °C for the EtSn fractions, and from 9% to 2%, respectively, for the Et70 fractions. The temperature increment also promotes the desterification of phenolic acids from the arabinosyl residues. Fig. 4 shows that EtSn fractions present a decrease in the ratio 5-Araf/Ara_{total} from 44.4 to 30.5 at temperatures between 140 °C and 180 °C, to 16.2 at 190 °C, and to 9.3 at 210 °C. The same behaviour was observed for the Et70 but the ratio 5-Araf/Ara_{total} was smaller than those observed for the EtSn fractions, ranging between 9.2 and 0.8, for 140 °C and 190 °C, respectively. The presence of higher contents of esterified phenolic acids should justify their solubility in the ethanol solutions.

The analysis of the relative amount of total xylose divided by the amount of terminally linked xylose offers a way to determine the degree of polymerization (DP) of the AX (DP ≥ 10) and AXOS (1 < DP < 10) (Table 2). The Et70 fractions rich in arabinoxylans were mainly composed by a backbone of 28 to 40 xylose residues, with exception of fraction obtained at 210 °C that gave short chains with an average degree of polymerization of 16. The EtSn fractions were AXOS with an average DP between 8 and 18.

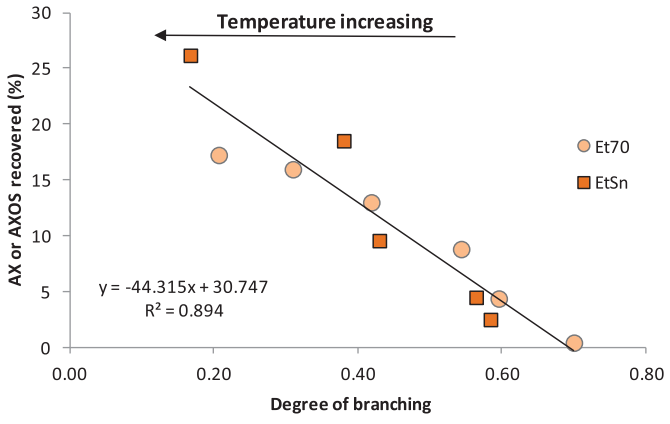

Fig. 3. AX or AXOS recovery from BSG plotted in function of its degree of branching.

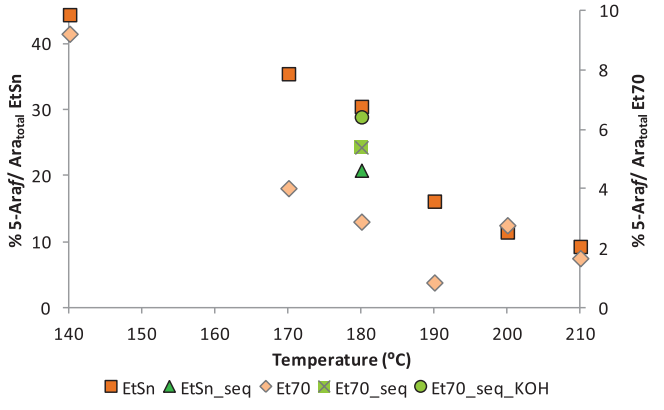

Fig. 4. Relative abundance of 5-Araf (linkage to the phenolic acids) in the fractions obtained by MWE as function of the temperature used.

Table 3
Glycosyl linkage composition (mol %) of the fractions obtain from brewers' spent grain by microwave superheated water extraction.

Glycosidic linkage	Temperature optimization											
	Et70						EtSn					
	140 °C	170 °C	180 °C	190 °C	200 °C	210 °C	140 °C	170 °C	180 °C	190 °C	200 °C	210 °C
T-Ara*f*	5.3	13.2	12.0	10.6	8.5	4.7	0.9	4.9	8.1	8.2	7.2	5.2
2-Ara*f*	0.2	0.5	0.6	0.5	1.5	0.6	–	0.2	0.6	0.8	1.5	1.9
3-Ara*f*	0.4	0.6	0.8	0.6	0.5	0.5	0.1	0.8	2.1	1.8	2.1	1.7
5-Ara*f*	0.6	0.6	0.4	0.1	0.3	0.1	0.8	3.3	4.7	2.1	1.4	0.9
Total	6.5	14.9	13.8	11.8	10.8	6.0	1.8	9.3	15.4	13.0	12.2	9.7
T-Xyl*p*	0.6	1.4	1.9	2.0	1.4	3.5	0.4	1.2	3.0	3.5	5.4	6.8
4-Xyl*p*	8.1	27.7	31.0	36.1	41.1	45.0	2.1	10.8	21.8	35.2	41.3	52.3
2,4-Xyl*p*	1.0	4.1	4.9	4.9	4.4	2.9	–	1.7	4.2	5.7	6.1	4.9
3,4-Xyl*p*	2.4	8.2	8.3	7.1	6.1	3.1	0.5	4.8	8.8	9.5	9.5	7.0
2,3,4-Xyl*p*	3.9	8.8	8.1	5.7	3.5	1.9	–	3.0	5.8	5.1	5.0	–
Total	16.0	50.2	54.1	55.9	56.5	47.4	3.1	21.4	43.6	59.1	67.3	71.1
T-Gal	0.7	0.3	0.5	0.3	0.2	1.7	0.1	0.3	0.8	0.6	1.3	1.3
6-Gal	0.1	0.2	–	–	–	–	0.4	–	–	–	–	–
3-Gal	–	–	–	–	–	–	–	–	–	–	–	–
Total	0.8	0.5	0.5	0.3	0.2	1.7	0.5	0.3	0.8	0.6	1.3	1.3
T-Glc	6.8	2.6	2.4	1.6	1.6	3.1	43.1	28.1	14.7	7.5	5.1	3.7
3-Glc	1.3	0.6	0.3	0.3	0.2	0.3	0.3	–	–	–	–	–
4-Glc	65.7	30.1	28.3	29.5	30.3	32.2	51.2	40.9	25.5	19.8	14.0	14.2
6-Glc	–	–	–	–	–	–	–	–	–	–	–	–
4,6-Glc	2.3	0.9	0.4	0.6	0.3	0.4	–	–	–	–	–	–
3,4-Glc	0.4	0.1	–	–	–	–	–	–	–	–	–	–
3,6-Glc	0.2	0.1	–	–	–	–	–	–	–	–	–	–
Glucitol	0.1	–	–	–	–	–	–	–	–	–	–	–
Total	76.7	34.4	31.4	32.0	32.4	36.0	94.6	69.0	40.2	27.3	19.1	17.9
(2,4-Xyl + 3,4-Xyl + 2*2,3,4-Xyl)/Xyl	0.70	0.60	0.54	0.42	0.31	0.21	0.16	0.58	0.56	0.43	0.38	0.17
AX or AXOS DP (%)	27	36	28	28	40	14	8	18	15	17	12	10

Glycosidic linkage	Sequential extraction		
	Et70 180 °C	EtSn 180 °C	KOH Et70 180 °C
T-Ara*f*	14.7	21.4	15.5
2-Ara*f*	0.8	1.8	2.5
3-Ara*f*	1.0	3.0	3.1
5-Ara*f*	0.9	6.9	1.5
Total	17.4	33.1	22.7
T-Xyl*p*	2.4	9.2	9.5
4-Xyl*p*	34.5	28.8	37.7
2,4-Xyl*p*	4.7	6.4	6.1
3,4-Xyl*p*	9.6	10.5	15.4
2,3,4- Xyl*p*	6.6	5.3	6.7
Total	57.8	60.2	75.4
T-Gal	0.5	1.7	1.3
6-Gal	0.3	–	–
3-Gal	0.1	–	–
Total	0.9	1.7	1.3
T-Glc	1.4	2.0	0.1
3-Glc	0.5	–	–
4-Glc	21.2	3.0	0.6
6-Glc	0.1	–	–
4,6-Glc	0.6	–	–
3,4-Glc	–	–	–
3,6-Glc	–	–	–
Glucitol	–	–	–
Total	23.9	5.0	0.7
(2,4-Xyl + 3,4-Xyl + 2*2,3,4-Xyl)/Xyl	0.48	0.46	0.46
AX or AXOS DP (%)	24	7	8

3.1.3. Structural analysis of glucans

The AX recovery was always accompanied by the presence of glucose (Tables 1 and 2). For the temperature of 140 °C, glucose was the main sugar present in both fractions, Et70 and EtSn, with 76% and 80% respectively. According to the glycosidic linkage analysis (Table 2), the main glucose linkage was (1 → 4)-Glc, followed by terminal- and (1 → 4,6)-linked glucose. The relative amount of (1 → 4)-Glc in relation to the total glucose was 86–94% and 54–79% for Et70 and EtSn fractions, respectively. The amount of terminal glucose was comprised between 5–9% for Et70 fractions and 21–46% for EtSn, and (1 → 4,6)-Glc was only present in Et70 fractions (1–3%). These results suggest that the BSG sample contained residual starch, recovered in all fractions, mainly at 140 °C. The occurrence of (1 → 3)-Glc is diagnostic of the presence of β-glucans in the Et70 fractions.

3.2. Sequential microwave superheated water extraction

In order to obtain starch free AX fractions, a sequential extraction was performed, first with 140 °C to remove the majority of the residual starch. For the AX recovery, a compromise between the yield attained with the temperature used and the maintenance of

the ester linkage of phenolic acids was tried. The temperature of 180 °C seems to be adequate to obtain AXOS esterified with phenolic acids in EtSn without an extensive debranching of AX chains. However, at this temperature condition the yields obtained were not the highest ones (Fig. 2). In order to increase the yield of AX and AXOS preserving its chemical structure, a sequential MWE was designed (Fig. 1).

The BSG was initially irradiated at 140 °C with water in order to remove the residual starch and β-glucans. The fraction Et70 recovered at 140 °C showed 81% of sugars, representing glucose 84 mol%. A small amount of xylose (6 mol%) was also recovered. The residue obtained was extracted with water at 180 °C, allowing to obtain 22% of the AX present in BSG. In this fraction, xylose represented 46 mol% of total sugars and arabinose 25 mol%. The AX recovered had a DP of 26 and a ratio (2,4-Xyl + 3,4-Xyl + 2*2,3,4-Xyl)/total xylose of 0.48, which was similar to those obtained with the single treatment at 180 °C (Table 2). However, in this fraction glucose still accounted for 25 mol%. The high abundance of (1 → 4)- and (1 → 4,6)-linked glucose allows to infer that it resulted from residual starch. To evaluate the amount of starch present, fraction 180_Et70 was treated with an α-amylase, allowing to release 87% of the glucose present.

The supernatants obtained (180_EtSn) showed 41% of sugars, where the main sugar was arabinose with 55 mol%, followed by xylose with 33 mol%. According to Table 2, arabinose occurred mainly as terminally linked (65%), which is slightly higher than the amount of branched xylose residues. These results suggest the presence of branched structures together with arabinose oligosaccharides resulting from thermal deglycosilation. According to Table 2, the DP of these AXOS was 7, which is in accordance with their high solubility in ethanol solutions. Furthermore, this fraction presented a higher amount of 5-Ara, accounting for 21 mol% of total arabinose, possibly arising from esterification of phenolic acids such as ferulic and *p*-coumaric acids to arabinofuranosyl residues (Bartolomé et al., 2002; Mandalari et al., 2005). This fraction presented a total amount of phenolic compounds of 0.34 μM/mg in equivalents of gallic acid, and an antioxidant activity of 208 μM/mg in equivalents of trolox. Feruloyl arabinoxylotrisaccharides are reported to contain higher antioxidant activity than ferulic acid (Katapodis et al., 2003).

The residue 2 was treated with an alkaline solution of KOH 0.1 M at 180 °C, and the supernatant was neutralized. The precipitate formed accounted for 14% of the initial mass (Table 3) and was poor in sugars (9%). On the contrary, the supernatant obtained after neutralization and precipitated in 70% (v/v) ethanol (180_KOH_Et70), comprising 22% of the AX present in BSG, was composed by 83% of sugars, with 61 mol% of xylose and 33 mol% of arabinose, and a small amount of glucose (2 mol%). This glucosidic material was resistant to α-amylase hydrolysis. This fraction showed a DP of 8, and a ratio (2,4-Xyl + 3,4-Xyl + 2*2,3,4-Xyl)/total xylose of 0.46. With this mild alkali extraction, the AX obtained were almost oligosaccharides instead of the polymers obtained in the Et70 with the water extraction (DP of 24). The ratio (2,4-Xyl + 3,4-Xyl + 2*2,3,4-Xyl)/total xylose was similar to the other fractions obtained by the sequential MWE (Table 2). Due to the different steps applied in the sequential microwave superheated water extraction, it was possible to enrich fractions of AX (180_Et70), feruloylated AXOS (180_EtSn), and AXOS (180_KOH_Et70) from proteins (180_KOH_pp) and starch (140_Et70).

The final residue accounted for 21 mol% of xylose together with glucose (72%) and minor amounts of mannose, arabinose and galactose. The amount of glucose released without pre-hydrolysis is used to estimate the acid hydrolysis resistant cellulosic material, was 42%, which showed that a relevant part of the cellulose was partially degraded by MWE originating smaller polymers. However, this residue contained also acid hydrolysis resistant material, as a 2 M sulphuric acid hydrolysis was able to release higher amount of sugars (83%) than a 1 M sulphuric acid hydrolysis (67%). These results showed that the final residue was mostly composed by carbohydrates, almost free from protein, which provides a material that can be used in several applications, namely as insoluble dietary fibre, paper industry or, at least, used for burn.

The advantage of the sequential extraction using MWE showed an AX yield (Et70) higher than the yield of MWE batch extractions (Fig. 2). Also, depolymerization was minimized because the EtSn yield, corresponding to the AXOS fraction, was lower than that obtained using 210 °C, which was the condition with higher polysaccharide yield. Moreover, the residue contained only 7% of AX, meaning that the AXs present in BSG were extensively extracted. The sequential extraction using MWE allowed to extract 62% of BSG AX + AXOS, presenting different structural features, and a final residue almost absent of AX.

4. Conclusion

This work is the first investigation on isolation of arabinoxylans (AX) and arabinoxylo-oligosaccharides (AXOS) by microwave-assisted extraction from brewers' spent grain (BSG). The AX + AXOS yield increased with the increase of the temperature in the range from 140 to 210 °C, during 2 min. The best conditions in terms of yield of extraction were achieved at 210 °C, allowing to obtain 43% of BSG AX + AXOS. As the higher temperatures promote depolymerisation, debranching, deesterification, and formation of brown products, a lower temperature was tested, at 180 °C during 2 min, in a sequential extraction of three steps. This allowed to extract 62% of BSG AX + AXOS, presenting different structural features, and a final residue rich in cellulose. Due to the different steps applied in the sequential microwave superheated water extraction it is possible to enrich fractions in AX, AXOS, and feruloylated AXOS from the proteins and residual starch. The compounds formed are highly soluble in water and even in ethanol solutions, furthermore, presenting antioxidant activity, allowing their use in aqueous and alcoholic matrices.

The results of this work open several opportunities for future valuation of the BSG under study as potential source of nutraceuticals with potential prebiotic effects and with antioxidant activity.

Acknowledgements

The authors gratefully acknowledge Unicer Bebidas S.A. for providing the brewers' spent grain, Prof. Diana Pinto from the University of Aveiro for microwave facilities and FCT for the financial support of the Research Unit 62/94-QOPNA (project PEst-C/QUI/UI0062/2013) and the post doctoral grant SFRH/BPD/70589/2010 of Elisabete Coelho.

References

Aliyu, S., & Bala, M. (2011). Brewer's spent grain: A review of its potentials and applications. *African Journal of Biotechnology*, *10*(3), 324–331.

Bartolomé, B., Santos, M., Jiménez, J. J., del Nozal, M. J., & Gómez-Cordovés, C. (2002). Pentoses and hydroxycinnamic acids in brewer's spent grain. *Journal of Cereal Science*, *36*(1), 51–58.

Benkő, Z., Andersson, A., Szengyel, Z., Gáspár, M., Réczey, K., & Stålbrand, H. (2007). Heat extraction of corn fiber hemicellulose. *Applied and Biochemistry Biotechnology*, *136–140*, 253–265.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research*, *113*(2), 291–299.

Brillouet, J. M., Joseleau, J. P., Utille, J. P., & Lelièvre, D. (1982). Isolation, purification, and characterization of a complex heteroxylan from industrial wheat bran. *Journal of Agricultural and Food Chemistry*, *30*(3), 488–495.

Broekaert, W., Courtin, C., Damen, B., & Delcour, J. (2010). *Processed food product, used for stimulating the butyrate production in the large intestine, comprises water-unextractable arabinoxylans and arabinoxylan-oligosaccharides*. Fugeia Nv: Univ Katholieke Leuven.

Broekaert, W. F., Courtin, C. M., Verbeke, K., Van de Wiele, T., Verstraete, W., & Delcour, J. A. (2011). Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Critical Reviews in Food Science and Nutrition*, *51*(2), 178–194.

Buranov, A. U., & Mazza, G. (2010). Extraction and characterization of hemicelluloses from flax shives by different methods. *Carbohydrate Polymers*, *79*(1), 17–25.

Carvalheiro, F., Esteves, M. P., Parajó, J. C., Pereira, H., & Gírio, F. M. (2004). Production of oligosaccharides by autohydrolysis of brewery's spent grain. *Bioresource Technology*, *91*(1), 93–100.

Chanliaud, E., Saulnier, L., & Thibault, J. F. (1995). Alkaline extraction and characterisation of heteroxylans from maize bran. *Journal of Cereal Science*, *21*(2), 195–203.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, *131*(2), 209–217.

Coelho, E., Rocha, S. M., & Coimbra, M. A. (2011). Foamability and foam stability of molecular reconstituted model sparkling wines. *Journal of Agricultural and Food Chemistry*, *59*(16), 8770–8778.

Crittenden, R., Karppinen, S., Ojanen, S., Tenkanen, M., Fagerström, R., Mättö, J., et al. (2002). In vitro fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. *Journal of the Science of Food and Agriculture*, *82*(8), 781–789.

Eskilsson, C. S., & Bjorklund, E. (2000). Analytical-scale microwave-assisted extraction. *Journal of Chromatography A*, *902*(1), 227–250.

Folin, O., & Ciocalteu, V. (1927). On tyrosine and tryptophane determinations in proteins. *Journal of Biological Chemistry*, *73*(2), 627–650.

Harris, P. J., Blakeney, A. B., Henry, R. J., & Stone, B. A. (1988). Gas-chromatographic determination of the monosaccharide composition of plant-cell wall preparations. *Journal of the Association of Official Analytical Chemists*, *71*(2), 272–275.

Harris, P. J., Henry, R. J., Blakeney, A. B., & Stone, B. A. (1984). An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydrate Research*, *127*(1), 59–73.

Izydorczyk, M. S., Macri, L. J., & MacGregor, A. W. (1998). Structure and physicochemical properties of barley non-starch polysaccharides – II. Alkali-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers*, *35*(3–4), 259–269.

Janker-Obermeier, I., Sieber, V., Faulstich, M., & Schieder, D. (2012). Solubilization of hemicellulose and lignin from wheat straw through microwave-assisted alkali treatment. *Industrial Crops and Products*, *39*(0), 198–203.

Jay, A. J., Parker, M. L., Faulks, R., Husband, F., Wilde, P., Smith, A. C., et al. (2008). A systematic micro-dissection of brewers' spent grain. *Journal of Cereal Science*, *47*(2), 357–364.

Kabel, M. A., Carvalheiro, F., Garrote, G., Avgerinos, E., Koukios, E., Parajó, J. C., et al. (2002). Hydrothermally treated xylan rich by-products yield different classes of xylo-oligosaccharides. *Carbohydrate Polymers*, *50*(1), 47–56.

Kabel, M. A., Schols, H. A., & Voragen, A. G. J. (2002). Complex xylo-oligosaccharides identified from hydrothermally treated *Eucalyptus* wood and brewery's spent grain. *Carbohydrate Polymers*, *50*(2), 191–200.

Katapodis, P., Vardakou, M., Kalogeris, E., Kekos, D., Macris, B. J., & Christakopoulos, P. (2003). Enzymic production of a feruloylated oligosaccharide with antioxidant activity from wheat flour arabinoxylan. *European Journal of Nutrition*, *42*(1), 55–60.

Kratchanova, M., Pavlova, E., & Panchev, I. (2004). The effect of microwave heating of fresh orange peels on the fruit tissue and quality of extracted pectin. *Carbohydrate Polymers*, *56*(2), 181–185.

Mandalari, G., Faulds, C. B., Sancho, A. I., Saija, A., Bisignano, G., LoCurto, R., et al. (2005). Fractionation and characterisation of arabinoxylans from brewers' spent grain and wheat bran. *Journal of Cereal Science*, *42*(2), 205–212.

Ohta, T., Semboku, N., Kuchii, A., Egashira, Y., & Sanada, H. (1997). Antioxidant activity of corn bran cell-wall fragments in the LDL oxidation system. *Journal of Agricultural and Food Chemistry*, *45*(5), 1644–1648.

Ou, S.-Y., Jackson, G. M., Jiao, X., Chen, J., Wu, J.-Z., & Huang, X.-S. (2007). Protection against oxidative stress in diabetic rats by wheat bran feruloyl oligosaccharides. *Journal of Agricultural and Food Chemistry*, *55*(8), 3191–3195.

Pang, F., Xue, S., Yu, S., Zhang, C., Li, B., & Kang, Y. (2012). Effects of microwave power and microwave irradiation time on pretreatment efficiency and characteristics of corn stover using combination of steam explosion and microwave irradiation (SE–MI) pretreatment. *Bioresource Technology*, *118*(0), 111–119.

Passos, C. P., & Coimbra, M. A. (2013). Microwave superheated water extraction of polysaccharides from spent coffee grounds. *Carbohydrate Polymers*, *94*(1), 626–633.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231–1237.

Roos, A. A., Persson, T., Krawczyk, H., Zacchi, G., & Stalbrand, H. (2009). Extraction of water-soluble hemicelluloses from barley husks. *Bioresource Technology*, *100*(2), 763–769.

Rose, D. J., & Inglett, G. E. (2010). Production of feruloylated arabinoxylo-oligosaccharides from maize (*Zea mays*) bran by microwave-assisted autohydrolysis. *Food Chemistry*, *119*(4), 1613–1618.

Selvendran, R. R., March, J. F., & Ring, S. G. (1979). Determination of aldoses and uronic-acid content of vegetable fiber. *Analytical Biochemistry*, *96*(2), 282–292.

Van Craeyveld, V., Swennen, K., Dornez, E., Van de Wiele, T., Marzorati, M., Verstraete, W., et al. (2008). Structurally different wheat-derived arabinoxylooligosaccharides have different prebiotic and fermentation properties in rats. *Journal of Nutrition*, *138*(12), 2348–2355.

Vardakou, M., Nueno Palop, C., Gasson, M., Narbad, A., & Christakopoulos, P. (2007). In vitro three-stage continuous fermentation of wheat arabinoxylan fractions and induction of hydrolase activity by the gut microflora. *International Journal of Biological Macromolecules*, *41*(5), 584–589.

Yoshida, T., Tsubaki, S., Teramoto, Y., & Azuma, J.-I. (2010). Optimization of microwave-assisted extraction of carbohydrates from industrial waste of corn starch production using response surface methodology. *Bioresource Technology*, *101*(20), 7820–7826.