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Improved Efficiency of Brewer's Spent Grain Arabinoxylans by Ultrasound-Assisted Extraction

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Improved efficiency of brewer's spent grain arabinoxylans by ultrasound-assisted extraction

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ABSTRACT

Arabinoxylan (AX) rich extracts from brewer's spent grain (BSG) were produced by the application of ultrasound-assisted extraction (UAE) and conventional alkaline extraction (AKE). UAE and AKE were optimised for the production of the highest yield of ethanol insoluble material using response surface methodology (RSM). The efficiency of UAE was established by the significant reduction of time (7 h to 25 min) and energy when compared to AKE, to recover similar amounts of AX (60%) from BSG, leading to the production of starch-free AX-rich extracts.

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1. Introduction

Arabinoxylans (AX) are the main non-starch polysaccharide of cereal grains such as wheat, barley, oat, corn and rice. The AX are composed of β-D-(1 → 4)-linked-xylopyranosyl residues to which α-L-arabinofuranose units are linked as side chains, with some of them substituted with monomeric or dimeric ferulic acid residues (Fig. 1) [1]. Similar to other non-digestible carbohydrates, AX are of particular interest in the formulation of functional foods due to the health benefits associated with their consumption [2]. They have been classified as prebiotics because they (i) are not hydrolysed nor absorbed in the upper part of the gastrointestinal tract, (ii) maintain a good gastrointestinal environment and (iii) selectively stimulate the microflora that confer benefits upon the host wellbeing and health [3].

Brewer's spent grain (BSG) is the residue left after barley malting and separation of the wort (fermentation medium to produce beer) during the brewing process. BSG is the most abundant brewing by-product amounting to around 85% of total by-products generated by the brewing industry. Million of tonnes of BSG are produced annually across Europe and common applications are direct disposal in a landfill or use as an animal feed. BSG is a lignocellulosic material composed of AX (28%), cellulose (17%) and lignin (28%) [4,5]. As the germinated grain during brewing has already been submitted to a hot water extraction process, the BSG AX are mostly not extractable with water. Thus, chemical and/or enzymatic methods need to be used for their extraction. These include sequential extraction with mild and strong alkali solutions [6,7] and sequential extraction with alkali solution and a mixture of feruloyl esterases and glycoside hydrolases. This last extraction method allows for the recovery of phenolic acids and diferulate AX oligosaccharides [8]. Autohydrolysis of BSG, an environmentally friendly treatment carried out in a reactor with hot water or steam, promotes the recovery of several AX oligosaccharide (AXOS) mixtures [9]. More recently, a sequential extraction applying microwave superheated water and dilute alkali extraction of BSG AX was proposed, separating AX, AXOS, and feruloylated AXOS from the proteins and residual starch [10].

Ultrasound-assisted extraction (UAE) is a process that uses acoustic energy and solvents to extract target compounds from various plant matrices. The application of high-intensity ultrasound causes pressure fluctuations, which propagate through the material. These fluctuations give rise to microscopic bubbles that are highly unstable and collapse within a few milliseconds after their formation. In the wake of the collapse, high shear forces are applied to any material that is present in the vicinity of these cavitational bubbles. In addition to the mechanical shear forces, the temperature in the vicinity of the bubbles increases. The ultrasound pressure waves and resulting cavitation phenomena are able to break cell walls, promoting the release of the contents of the cell into the extraction medium [11,12].

UAE has already been used to obtain xylans from corn cobs [13,14], corn bran [15], wheat straw [16,17], buckwheat hulls [18], sugarcane bagasse [19], wheat bran [20] and almond shells

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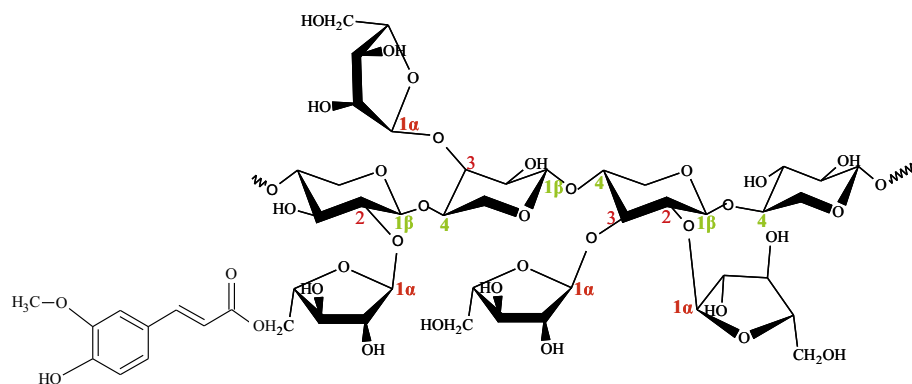
Fig. 1. Arabinoxylans structure.

[21]. The advantages reported are the substantial shortening of extraction time, solvent consumption, and extraction temperature, resulting in higher yields and purity of polysaccharides with no significant structural changes and no negative effects in their functional properties. However, high intensity ultrasound can break down polymers, which may negatively affect polysaccharides [22].

Due to the high demand of AX owing to their potential health benefits as dietary fibre and as prebiotics, in addition to the abundant availability of BSG as a source of AX, this work aims at examining the efficiency of ultrasound-assisted extraction in the production of AX-rich extracts in comparison to typical alkaline extraction procedures.

2. Material and methods

2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich (Wicklow, Ireland) except for sulphuric acid, acetone, dichloromethane and ethanol, which were purchased from Fisher scientific (Ballycoolin, Ireland).

2.2. Brewer's spent grain (BSG)

BSG was obtained from the micro distillery plant located at University College Cork, Cork (Ireland). The dried BSG was coarsely ground to 250 μm particle size and stored in polyethylene bags at −20 °C until further use.

2.3. Ultrasound-assisted extraction (UAE)

2.3.1. Ultrasound pre-washing treatment with water
Suspensions of BSG (2 g) and distilled water (50 mL) were processed at a constant frequency of 20 kHz using a 750 W ultrasonic processor (VC 750, Sonics and Materials Inc., Newtown, USA) using a 13 mm diameter solid probe. Different amplitudes (8%, 25%, 50%, 75%, 92% and 100%) combined with different times (3.3, 5, 7.5, 10 and 11.7 min) were applied with pulse durations of 5 s on and 5 s off. The ultrasound probe was submerged up to 25 mm in the sample. The residue was separated from the supernatant by centrifugation at 14,400 rpm for 20 min, and then resuspended in distilled water (50 mL) and autoclaved at 120 °C for different times (4.8, 15, 30, 45 and 55 min).

2.3.2. Ultrasound alkaline extraction
The autoclaved residue was suspended in an alkali solution (0.3, 1, 2, 3 and 3.7 M) and submitted to ultrasound treatment by combining different amplitudes (8%, 25%, 50%, 75%, 92% and 100%) and

times (3.3, 5, 7.5, 10 and 11.7 min) with pulse durations of 5 s on and 5 s off. The suspensions were then neutralised with HCl until pH 6–7 was reached and subsequently centrifuged. The supernatants were precipitated with 5 volumes of ethanol (96%) and the alcohol insoluble material was recovered as a powder; after centrifugation at 14,400 rpm for 10 min, solubilisation in distilled water, dialysis using a cellulose acetate membrane of 12 kDa cut off (Sigma, D9652) and freeze-drying. The scheme for the isolation of the polysaccharide fractions is shown in Fig. 2.

The ultrasonic intensity (UI) was determined using the following formula:

$$UI = 4P/\pi D^2 \qquad (1)$$

where P is the ultrasonic power (W) and D is the probe diameter (cm). Ultrasonic power was calculated using the following formula:

$$P = mC_p(dT/dt)_{t=0}\pi D^2 \qquad (2)$$

where m is the mass (g), C_p is the specific heat (H_2O – 4.187 J g^{-1}°C^{-1}; 2 M KOH – 0.93 J g^{-1}°C^{-1}) and (dT/dt) is the change in temperature over time (°C s^{-1}).

2.4. Alkaline extraction (AKE)

BSG samples were also extracted using different concentrations (1, 2, 3, 4 and 5 M) of KOH solutions with 20 mM NaBH$_4$, in an incubator (Innova 42, Mason technology, Dublin, Ireland) over different times (1, 4, 7, 10 and 13 h) and temperatures (25, 35, 45, 55 and 65 °C). After each alkaline extraction the same steps described after ultrasound treatment using alkali solution (Section 2.3.2) were followed.

2.5. Sugar analysis

Neutral sugars were released by Saeman hydrolysis and analysed as their alditol acetates by gas chromatography [23,24] using a FISONS 8340 chromatograph with a split injector (split ratio 1:60) and a FID detector. A DB-225 column (Agilent J&W, USA; 30 m × 0.25 mm × 0.15 μm) was used. The injector and detector temperatures were 220 and 230 °C, respectively. The oven temperature program started at 200–220 °C at a rate of 40 °C per min and was held at 220 °C for 15 min, then increased up to 230 °C with a rate of 20 °C per min and was held at 230 °C for 1 min. The flow rate of the carrier gas (H$_2$) was set at 1 mL/min at 200 °C. Uronic acids (UA) were determined colorimetrically according to Coimbra et al. [23]. The hydrolysis of all samples was done in duplicate and each sample was injected twice.
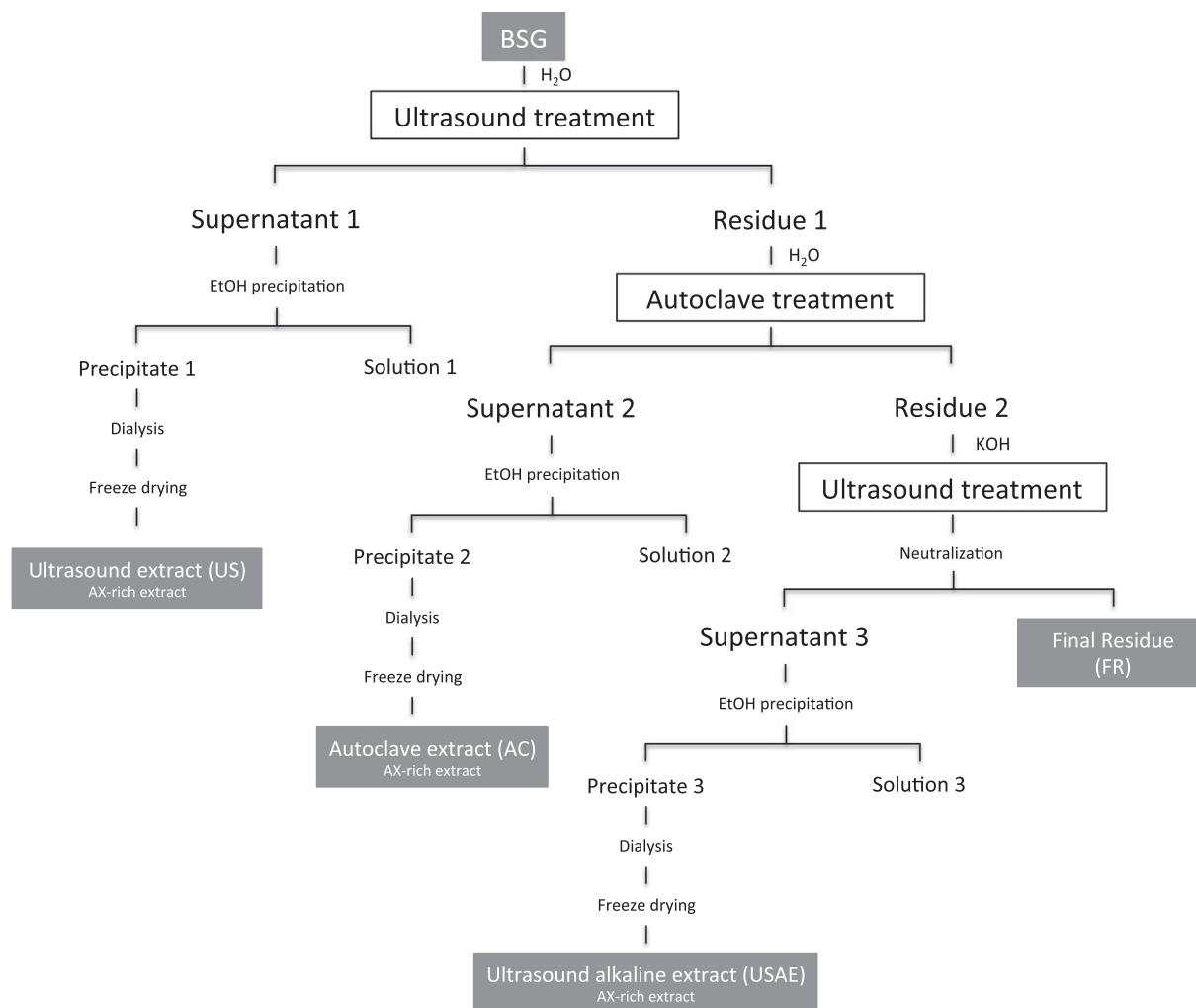
Fig. 2. Scheme for the isolation of alcohol insoluble material from BSG by ultrasound-assisted extraction (UAE).

Table 1
Experimental designs and corresponding response values for UAE of BSG AX.

	Ultrasound + Autoclave extracts (USAC)							Ultrasound Alkaline extract (USAE)						
	X_{USAC1}		X_{USAC2}		X_{USAC3}		Y	X_{USAE1}		X_{USAE2}		X_{USAE3}		Y
1	7.5	(0)	92	(2)	30	(0)	3.9	5.0	(−1)	75	(1)	3.0	(1)	19.5
2	11.7	(2)	50	(0)	30	(0)	3.0	7.5	(0)	92	(2)	2.0	(0)	20.7
3	7.5	(0)	50	(0)	5	(−2)	1.8	7.5	(0)	50	(0)	2.0	(0)	19.8
4	10.0	(1)	75	(1)	15	(−1)	3.4	7.5	(0)	8	(−2)	2.0	(0)	10.7
5	7.5	(0)	8	(−2)	30	(0)	1.9	10.0	(1)	75	(1)	3.0	(1)	18.1
6	5.0	(−1)	25	(−1)	15	(−1)	1.6	11.7	(2)	50	(0)	2.0	(0)	19.4
7	10.0	(1)	25	(−1)	45	(1)	2.3	7.5	(0)	50	(0)	3.7	(2)	20.1
8	10.0	(1)	75	(1)	45	(1)	3.4	10.0	(1)	25	(−1)	3.0	(1)	16.4
9	7.5	(0)	50	(0)	30	(0)	2.0	10.0	(1)	75	(1)	1.0	(−1)	17.6
10	7.5	(0)	50	(0)	55	(2)	2.5	5.0	(−1)	25	(−1)	3.0	(1)	16.1
11	7.5	(0)	50	(0)	30	(0)	2.0	5.0	(−1)	25	(−1)	1.0	(−1)	6.6
12	3.3	(−2)	50	(0)	30	(0)	1.8	5.0	(−1)	75	(1)	1.0	(−1)	16.5
13	5.0	(−1)	75	(1)	45	(1)	2.7	3.3	(−2)	50	(0)	2.0	(0)	15.9
14	10.0	(1)	25	(−1)	15	(−1)	2.1	7.5	(0)	50	(0)	0.3	(−2)	6.0
15	5.0	(−1)	75	(1)	15	(−1)	2.2	10.0	(1)	25	(−1)	1.0	(−1)	9.7
16	5.0	(−1)	25	(−1)	45	(1)	2.5	7.5	(0)	50	(0)	2.0	(0)	17.0

X_{USAC1} = ultrasound time (min); X_{USAC2} = ultrasound amplitude (%); X_{USAC3} = autoclave time (min); X_{USAE1} = ultrasound time (min); X_{USAE2} = ultrasound amplitude (%); X_{USAE3} = KOH concentration (M); Y = Yield (%).

2.6. Methylation analysis

Glycosidic-linkage composition was determined by gas chromatography–quadrupole mass spectrometry (GC–qMS) of the partially methylated alditol acetates [25–27]. Briefly, the polysaccharides were methylated with 80 µL of methyl iodide and the mixture was allowed to react for 20 min under stirring. The permethylated polysaccharides were hydrolysed with 0.5 mL of 2 M

Table 2
Regression coefficients and analysis of variance of uncoded units for UAE yield.

Coefficient	USAC		USAE	
	Estimate	p-Value	Estimate	p-Value
$\beta 0$	0.3617		−23.490	
$\beta 1$	0.1038	0.0004	1.926	0.1806
$\beta 2$	−0.0257	0.0000	0.460	0.0005
$\beta 3$	0.0794	0.0038	16.120	0.0003
$\beta 11$	−0.0013	0.8812	−0.051	0.5688
$\beta 12$	0.0032	0.0139	−0.007	0.4462
$\beta 13$	−0.0040	0.0420	−0.265	0.2873
$\beta 22$	0.0003	0.0209	−0.002	0.1047
$\beta 23$	−0.0002	0.2453	−0.063	0.0313
$\beta 33$	−0.0004	0.1238	−1.944	0.0102
R^2	97.4		95.4	

USAC – ultrasound + autoclave extract; USAE – ultrasound alkaline extract.

trifluoroacetic acid (1 h at 121 °C) and dried by centrifugal evaporation. The reduction of monosaccharides was performed for 1 h at 30 °C with 20 mg of sodium borodeuteride in 300 μL of 2 M NH$_3$. The acetylation was performed with 3 mL of acetic anhydride using 450 μL 1-methylimidazole as catalyst, for 30 min at 30 °C. The partially methylated alditol acetates were dissolved in 50–100 μL of acetone and 0.2 μL were injected and analysed by GC–qMS on an Agilent Technologies 6890N Network gas chromatograph,

equipped with a 30 m × 0.25 mm (i.d.), 0.1 μm film thickness DB-1 fused silica capillary column (J&W Scientific Inc., CA, USA) connected to an Agilent 5973 quadrupole mass selective detector. The oven temperature was programmed as follows: 50–140 °C at 8 °C/min (hold 5 min at 140 °C), to 150 °C at 0.5 °C/min and then to 280 °C at 40 °C/min (hold 1 min at 280 °C). The helium carrier gas had a flow rate of 1.7 mL/min and the column head pressure was 2.8 psi. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV scanning in the range of 40–500 m/z, in a full scan acquisition mode. Identification was achieved by comparing with the standard mass spectra and with other spectra available at the laboratory made database.

2.7. Determination of arabinoxylans (AX)

The arabinoxylans content in the final extracts were calculated from the sum of total arabinose and total xylose quantified by methylation analysis, after correction for the presence of arabinogalactans (AG), assuming an arabinose to galactose ratio of 0.7 [28].

2.8. Determination of protein

The protein in the extracts was determined by using the Bio-Rad protein assay kit (Bio-Rad, USA) based on the method of Bradford.
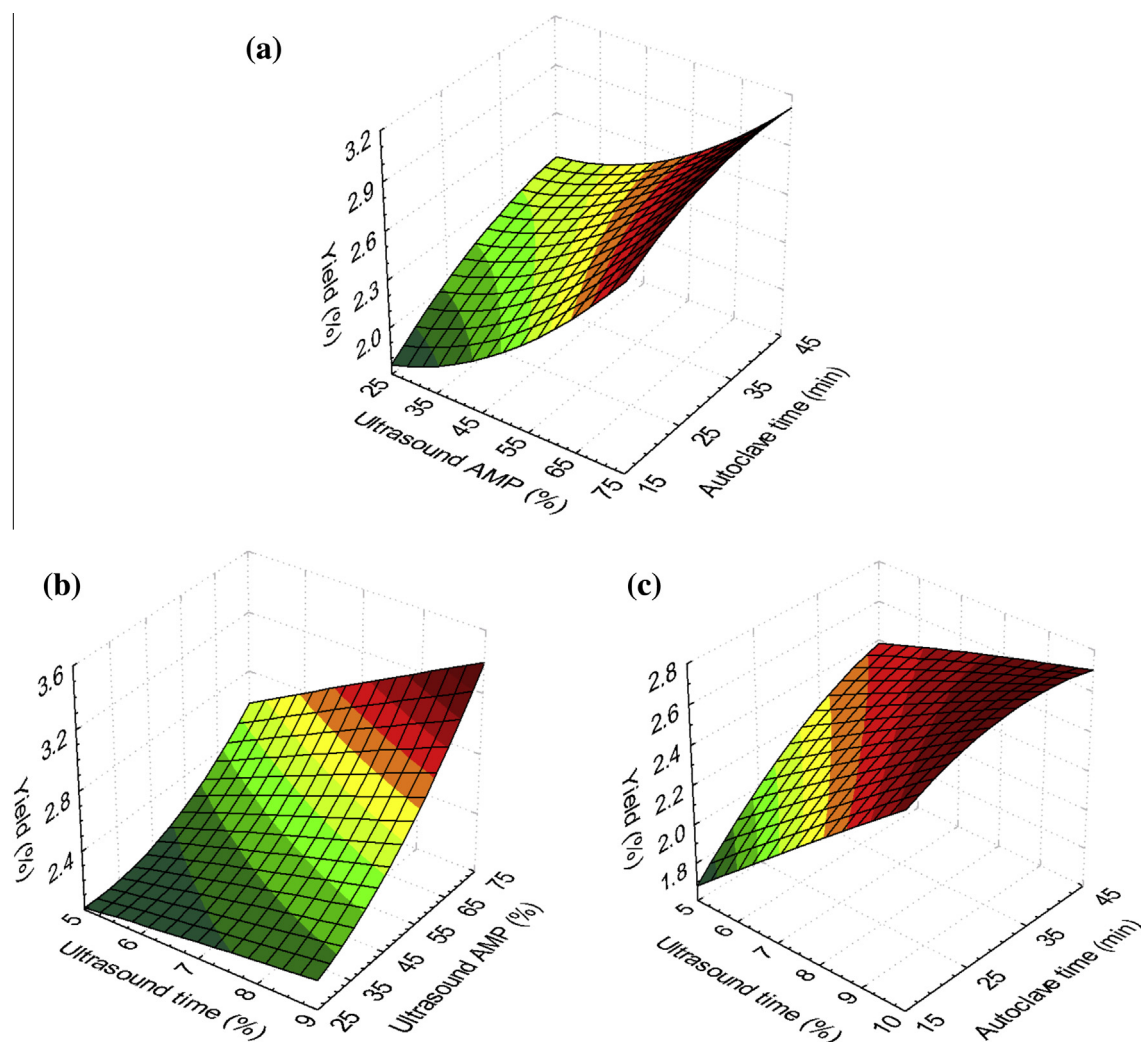
Fig. 3. Estimated response surfaces for the effect of (a) ultrasound amplitude (AMP) and autoclave extraction time, (b) ultrasound extraction time and ultrasound amplitude and (c) ultrasound extraction time and autoclave extraction time on the yield of ethanol insoluble material (USAC) obtained.

Table 3
Sugar analysis after acid hydrolysis of BSG and the extracts obtained from UAE and AKE and the respective final residues.

	Yield (%)	Mol (%)						Total sugar (mg/g)
		Ara	Xyl	Man	Gal	Glc	UA	
BSG	–	15	34	1	2	38	7	442
UAE								
US	2.2	26	29	–	3	27	15	437
AC	1.1	14	16	–	3	44	23	398
USAE	20.3	25	65	–	2	–	8	474
FR $_{UAE}$	–	6	19	–	–	41	24	449
AKE								
AE	20.3	26	49	–	2	9	13	763
FR$_{AKE}$	–	9	25	–	–	66	0	475

UAE – ultrasound-assisted extraction; US – ultrasound extract; AC – autoclave extract; USAE – ultrasound alkaline extract; AKE – alkaline extraction; AE – alkaline extract; FR – final residue.

Table 4
Methylation analysis of the extracts obtained by UAE and AKE.

Linkage	Ultrasound assisted extraction			Alkaline extraction
	US	AC	USAE	AE
t-Ara*f*	32.2	22.6	24.8	26.4
2-Ara*f*	0.5	0.3	1.8	1.4
3-Ara*f*	0.8	0.8	3.5	2.8
5-Ara*f*	1.0	0.8	1.1	1.4
3,5-Ara*f*	–	–	0.1	–
t-Xyl*p*	1.6	1.4	5.4	4.6
3-Xyl*p*	t	–	0.2	–
4-Xyl*p*	19.9	18.2	30.3	31.9
2,4-Xyl*p*	3.2	3.6	12.8	8.3
3,4-Xyl*p*	4.5	2.8	5.7	4.0
2,3,4-Xyl*p*	9.8	4.6	10.7	6.7
t-Man*p*	t	0.1	–	t
2-Man*p*	0.4	0.2	–	0.2
6-Man*p*	–	–	–	t
2,6-Man*p*	–	–	–	t
t-Gal*p*	0.4	0.3	1.4	0.9
3-Gal*p*	0.7	0.5	0.2	t
4-Gal*p*	0.2	0.1	0.2	0.2
6-Gal*p*	0.5	–	0.2	t
3,6-Gal*p*	0.3	–	0.2	t
t-Glc*p*	2.3	1.8	0.2	0.9
3-Glc*p*	1.3	0.4	–	0.7
4-Glc*p*	18.6	41.0	0.8	8.5
6-Glc*p*	0.3	0.2	t	0.1
4,6-Glc*p*	1.1	0.3	–	0.3
Glucitol	0.3	–	0.4	0.1

US – ultrasound extract; AC – autoclave extract; USAE – ultrasound alkaline extract; AE – alkaline extract; t-traces (Mol% < 0.1).

2.9. Response surface methodology (RSM)

A central composite rotatable design was used for the pre-washing treatment of UAE to investigate the effects of three independent variables: ultrasound extraction time (X_1), ultrasound amplitude (X_2) and autoclave extraction time (X_3) on the yield of ethanol insoluble material obtained. For the alkaline extraction in UAE the same design was used to investigate the effects of three independent variables: ultrasound extraction time (X_1), ultrasound amplitude (X_2) and alkali solution concentration (X_3), on the yield of ethanol insoluble material obtained. In AKE another central composite rotatable design was used to investigate the effects of four independent variables: sample/solvent (w/v) ratio (X_1), alkali solution concentration (X_2), temperature (X_3) and extraction time (X_4), on the yield of ethanol insoluble material obtained. Results from preliminary trials were used to select suitable values for the independent variables. A second order polynomial Eq. (3) for the

dependent variables was established to fit the experimental data. An analysis of variance (ANOVA) was carried out using STAT-GRAPHICS (Centurion XV.II 2.006) to determine the significance of the variables:

$$Y = \beta 0 + \beta 1 X_1 + \beta 2 X_2 + \beta 3 X_3 \cdots + \beta 11 X_1^2 + \beta 22 X_2^2$$
$$+ \beta 33 X_3^2 \cdots + \beta 12 X_1 X_2 + \beta 13 X_1 X_3 \cdots \quad (3)$$

where $X_1, X_2, X_3 \ldots X_1^2, X_2^2 \ldots X_1 X_2 \ldots$ are the independent variables with their linear, quadratic and interactive models, and $\beta 0$, $\beta 1$, $\beta 2 \ldots \ldots \beta 12$ are the regression coefficients of responses.

Response surface plots were designed using STATISTICA software (version 5.1, StatSoft Inc., Tulsa, USA).

3. Results and discussion

3.1. Optimisation of UAE of arabinoxylan-rich extracts from BSG by RSM

The procedure for UAE consisted of an ultrasound pre-washing treatment with water followed by an alkali extraction of the residue left (Fig. 2). Preliminary trials (data not shown) were conducted to estimate the optimum ratio of mass of sample to volume of solvent (w (g)/v (mL)) to be used in the experiments. The optimum w/v ratio was achieved at 0.04, allowing a good performance of the equipment for 5 min by using the highest ultrasound amplitude (100%). After the ultrasound treatment with water, an autoclave procedure was applied as a complementary washing treatment for the residue. Following the pre-washing treatment using ultrasound and autoclave, an extraction of the residue left was performed with ultrasound using an alkali solution. This procedure was optimised by RSM for the yield of ethanol insoluble material and the experimental designs and respective response values are presented in Table 1.

3.1.1. Ultrasound pre-washing treatment with water

The ultrasound pre-washing treatment experimental design (USAC) optimised the ultrasound treatment time (X_1), ultrasound amplitude (X_2) and autoclave time (X_3), in the ranges of 5–10 min, 25–75% and 15–45 min, respectively (Table 1). A regression analysis was carried out to fit mathematical models to the experimental data and the regression coefficients for the uncoded variables are shown in Table 2. The model fitted to the USAC experimental design explains 97.4% of the yield variability and the p-values of regression and ANOVA analysis showed that six elements were significantly affecting the yield at 95% confidence level. The linear factor ultrasound amplitude ($\beta 2$) was the main variable affecting the yield ($p \leqslant 0.05$), followed by ultrasound time ($\beta 1$) and autoclave time ($\beta 3$). The interaction between ultrasound time

and amplitude (β12) was the fourth element affecting the yield, followed by the quadratic factor of amplitude (β22) and the interaction between ultrasound time and autoclave time (β13).

The estimate response surfaces based on the experimental data (Fig. 3) shows that increasing ultrasound time (X_1), ultrasound amplitude (X_2) and autoclave time (X_3) increases the yield. Autoclave time increased yield at low ultrasound amplitudes but had no effect on the yield when high ultrasound amplitudes were applied (Fig. 3a). Increasing ultrasound time increased the yield and the increment was higher when high ultrasound amplitudes were used (Fig. 3b). Also, the increase in ultrasound time decreased the influence of autoclaving time, indicating that when higher ultrasound times were applied, lower autoclaving times were needed (Fig. 3c).

The combination of an ultrasound treatment for 12 min at 92% amplitude and 23 min of autoclave treatment, predicted by the regression Eq. (3) fitted to the data, resulted in an optimum yield value of 4.8%. In order to make use of all potentialities of the equipment, the amplitude was set at the maximum (100%). The combining factors to achieve the maximum yield, using the regression equation, were 5 and 15 min of ultrasound and autoclave treatment, respectively. At this amplitude setting the extraction time decreased and the maximum predicted yield was 3.1%. The experimental value obtained was 3.1 ± 0.3% ($n = 16$). The ultrasound intensity average as calculated by Eqs. (1) and (2) at this optimised point, was 14 W cm^{-2}.

Sugar analysis (Table 3) of these optimised extracts revealed that the US extract (2% of the BSG biomass) was composed of 44% polysaccharides, and the AC extract (1% of the BSG biomass) was composed of 40% polysaccharides. The major sugars identified were xylose, glucose, arabinose and uronic acids. Methylation analysis (Table 4) revealed the presence of high molar percentage of 4-Glc$p$, especially in AC extracts, that could be diagnostic of starch. In addition to the presence of AX, inferred by the high molar percentage of 4-Xyl$p$ corresponding to the xylan backbone of AX, and t-Ara$f$ accounting to the arabinofuranose AX side chain residues. These results suggest that the pre-washing treatment allows the removal of the BSG residual starch both by ultrasound and autoclave treatments. A first treatment with water and ultrasound was also reported as a refining step for the removal of contaminated starch and proteins [15] and to lower the amount of associated lignin to the xylans [19] before the alkaline extraction of xylans.

Despite AX already having been extracted from the germinated grain by the hot water extraction during the production of the wort, a portion of AX and starch is still trapped within the BSG
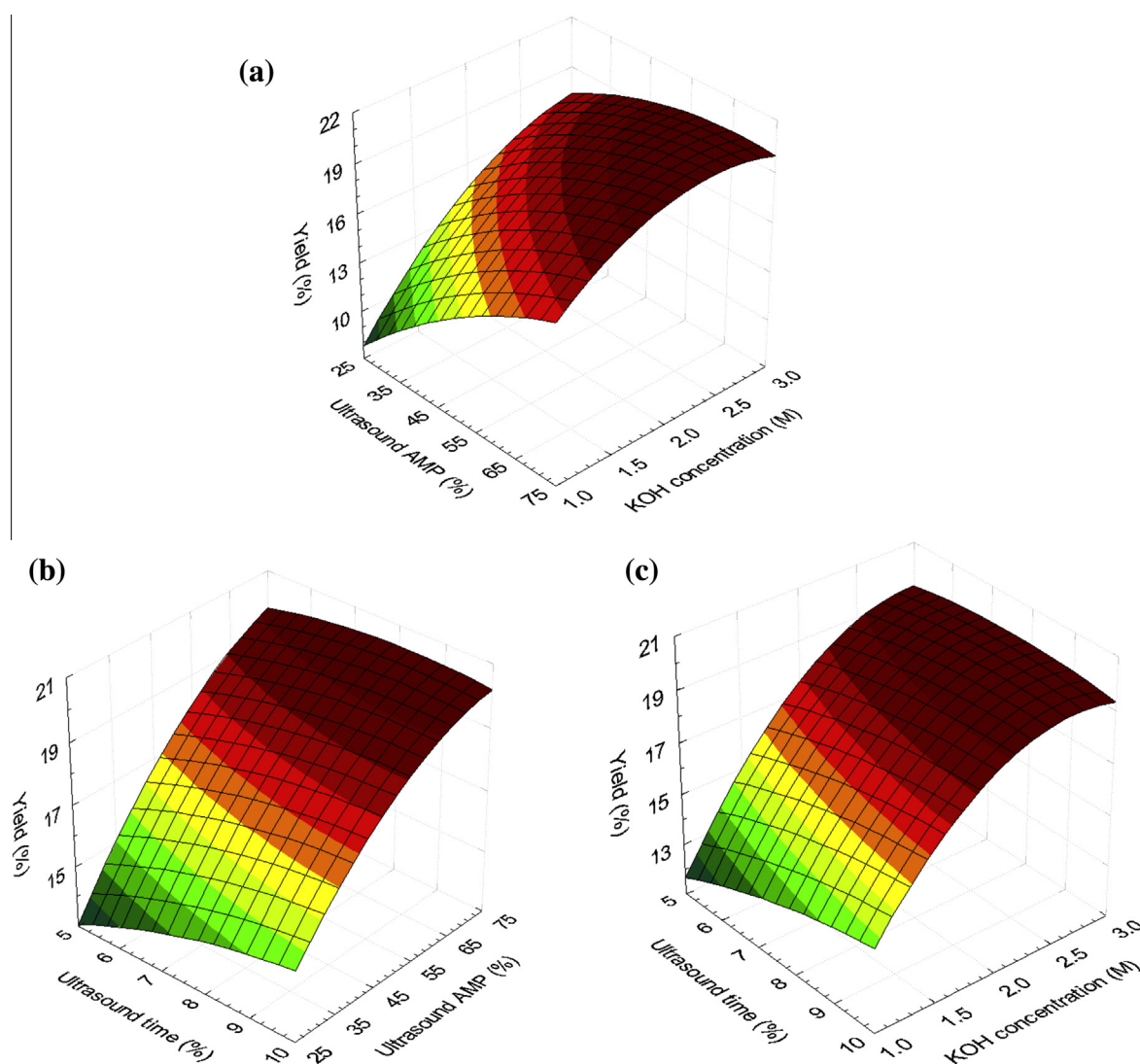

Fig. 4. Estimated response surfaces for the effect of (a) ultrasound amplitude (AMP) and KOH concentration, (b) ultrasound extraction time and ultrasound amplitude and (c) ultrasound extraction time and KOH concentration on the yield of ethanol insoluble material (USAE) obtained.

Table 5
Experimental design and corresponding response values for AKE of BSG AX.

	X_1		X_2		X_3		X_4		Y
1	0.12	(0)	3	(0)	65	(2)	7	(0)	4.1
2	0.16	(1)	2	(−1)	55	(1)	10	(1)	4.6
3	0.16	(1)	2	(−1)	55	(1)	4	(−1)	5.8
4	0.12	(0)	3	(0)	45	(0)	7	(0)	18.3
5	0.16	(1)	4	(1)	35	(−1)	10	(1)	5.8
6	0.16	(1)	4	(1)	35	(−1)	4	(−1)	7.3
7	0.08	(−1)	4	(1)	35	(−1)	10	(1)	15.5
8	0.04	(−2)	3	(0)	45	(0)	7	(0)	20.8
9	0.12	(0)	3	(0)	45	(0)	7	(0)	21.5
10	0.16	(1)	2	(−1)	35	(−1)	10	(1)	9.2
11	0.08	(−1)	2	(−1)	35	(−1)	10	(1)	11.0
12	0.12	(0)	3	(0)	45	(0)	7	(0)	21.5
13	0.12	(0)	3	(0)	45	(0)	7	(0)	21.6
14	0.08	(−1)	4	(1)	55	(1)	10	(1)	17.3
15	0.08	(−1)	2	(−1)	55	(1)	4	(−1)	14.3
16	0.08	(−1)	4	(1)	55	(1)	4	(−1)	19.2
17	0.12	(0)	3	(0)	25	(−2)	7	(0)	10.3
18	0.16	(1)	4	(1)	55	(1)	10	(1)	12.9
19	0.12	(0)	5	(2)	45	(0)	7	(0)	25.0
20	0.12	(0)	3	(0)	45	(0)	7	(0)	22.1
21	0.08	(−1)	2	(−1)	35	(−1)	4	(−1)	15.8
22	0.16	(1)	4	(1)	55	(1)	4	(−1)	11.9
23	0.12	(0)	3	(0)	45	(0)	1	(−2)	20.7
24	0.20	(2)	3	(0)	45	(0)	7	(0)	15.0
25	0.16	(1)	2	(−1)	35	(−1)	4	(−1)	7.0
26	0.08	(−1)	4	(1)	35	(−1)	4	(−1)	9.9
27	0.08	(−1)	2	(−1)	55	(1)	10	(1)	12.9
28	0.12	(0)	3	(0)	45	(0)	13	(2)	21.6
29	0.12	(0)	1	(−2)	45	(0)	7	(0)	9.5

X_1 = sample/solvent ratio (w/v); X_2 = KOH concentration (M); X_3 = temperature (°C); X_4 = time (h); Y = yield (%).

Table 6
Regression coefficients and analysis of variance of uncoded units for AKE yield.

Coefficient	Estimate	*p*-Value
$\beta0$	−79.90	
$\beta1$	200.00	0.0181
$\beta2$	3.37	0.0485
$\beta3$	3.59	0.8264
$\beta4$	1.13	0.9939
$\beta11$	−1054.00	0.1566
$\beta12$	5.54	0.8519
$\beta13$	−0.88	0.7674
$\beta14$	1.46	0.8823
$\beta22$	−1.85	0.1237
$\beta23$	0.18	0.1559
$\beta24$	0.17	0.6607
$\beta33$	−0.04	0.0024
$\beta34$	−0.01	0.7884
$\beta44$	−0.10	0.4491
R^2	74.5	

matrix by a proteinaceous barrier [29]. The ultrasound treatment seems to break the proteinaceous barrier in only 5 min at room temperature in contrast to a 24 h treatment using proteases at 40 °C to recover these water soluble AX [29].

3.1.2. Ultrasound alkaline extraction

The ultrasound alkaline extraction experimental design (USAE) started with the use of the optimum conditions achieved for the previous experimental design; 100% ultrasound amplitude for 5 min which was followed by 15 min autoclave treatment. The experimental design optimised the ultrasound extraction time (X_1), ultrasound amplitude (X_2) and alkali solution concentration (X_3) in the ranges of 5–10 min, 25–75% and 1–3 M KOH, respectively (Table 1).

The model fitted to the USAE experimental design explains 95.4% of the variability in the yield and the *p*-values of regression and ANOVA analysis showed that four effects have significant influence on the yield (Table 2), at 95% confidence level. The main effect is the alkali solution concentration ($\beta3$), followed by ultrasound amplitude ($\beta2$), the quadratic factor of alkali solution concentration ($\beta33$) and the interaction of the ultrasound amplitude and alkali solution concentration ($\beta23$). Estimate response surfaces based on the experimental data (Fig. 4) shows that the yield increases with the increase of ultrasound amplitude, alkali solution concentration and ultrasound extraction time. Alkali solution concentration and extraction time have been reported as important parameters that strongly affect the UAE yield of xylans [14,16–18]. However, while at low ultrasound amplitudes the increase in alkali concentration increases strongly the yield, at high ultrasound amplitudes the increase in alkali concentration does not affect significantly the yield obtained (Fig. 4a). Although at a low level of significance, it was observed that prolonged ultrasound extraction time increases the yield with the increase of ultrasound amplitude (Fig. 4b). Also the increase in ultrasound extraction time at low alkali solution concentration increases significantly the yield, yet at high alkali solution concentration it showed no influence on the yield (Fig. 4c). These results suggest that higher ultrasound amplitudes promote the degradation of polymeric material, which is probably soluble in ethanol solutions or could be lost during the dialysis step and consequently leads to a decrease in the yield obtained. The results also suggest that the extent of degradation is a compromise between the alkali solution concentration and the ultrasound amplitude applied.

The optimum conditions chosen, using the regression equation fitted to the data, for maximising the yield of ethanol insoluble material were: ultrasound extraction time of 5 min at 100% ultrasound amplitude and 2 M KOH. The predicted yield by the regression Eq. (3) was 20.2%, and the experimental value observed was 20.3 ± 0.4% (*n* = 5). The ultrasound intensity average calculated by Eqs. (1) and (2) at this optimum point was 3 W cm^{-2}.

The sugar analysis of the optimised extracts (Table 3) shows that they were composed of 47% polysaccharides rich in xylose and arabinose residues. These extracts also contained residual amounts of uronic acids and galactose. In contrast to the previous extracts no glucose was detected. Methylation analysis (Table 4) confirmed the presence of AX due to the highest molar percentage of 4-Xyl*p* and the t-Ara*f*, together with all other sugar residues characteristic of AX, namely 2,4-Xyl*p*, 3,4-Xyl*p* and 2,3,4-Xyl*p* considered as the common substitution linkages to the arabinofuranose residues. The presence of 5-Ara*f* indicates that the extracted AX were feruloylated [1]. The presence of 4-Glc*p* could be considered as a residual when compared to the previous US and AC extracts. The presence of 3-Gal*p*, 6-Gal*p* and 3,6-Gal*p* simultaneously with 2-Ara*f* and 3,5-Ara*f* suggests the presence of a residual type II arabinogalactans (AG). Residual AG have also been reported in literature in barley and barley malt [28].

3.2. Optimisation of AKE of arabinoxylan-rich extracts from BSG by RSM

The conventional alkaline extraction (AKE) was also optimised by RSM for the optimum yield of polymeric material, obtained by ethanol precipitation followed by dialysis. The experimental design and corresponding response values are presented in Table 5. The independent variables optimised were (i) weight of sample to the volume of solvent (w/v ratio, in the range of 0.08–0.16), (ii) alkali solution concentration (2–4 M), (iii) temperature (35–55 °C) and (iv) extraction time (4–10 h).

A regression analysis was carried out to fit the mathematical models to the experimental data and the regression coefficients
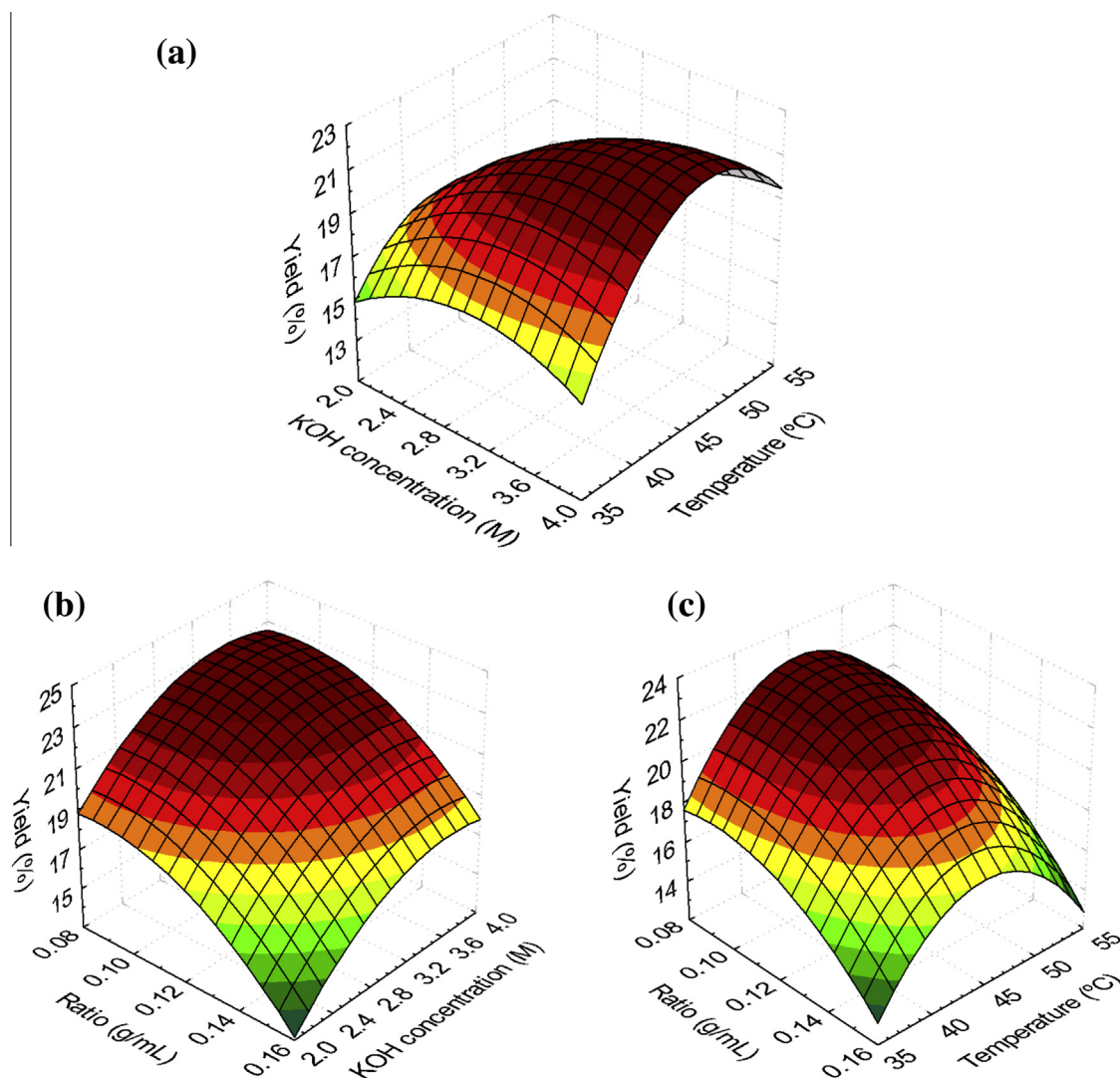
Fig. 5. Estimated response surfaces for the effect of (a) KOH concentration and temperature, (b) weight sample to volume solvent ratio (w/v) and KOH concentration and (c) w/v ratio and temperature on the yield of ethanol insoluble material (AE) obtained.

for the uncoded variables are shown in Table 6. The model fitted explain 74.5% of the yield variability and the *p*-values of regression and ANOVA analysis showed three effects significantly influencing the yield, at 95% confidence level. The quadratic factor of extraction temperature ($\beta 33$) is the main influence on yield ($p \leqslant 0.05$), followed by the w/v ratio ($\beta 1$) and concentration of the alkali solution ($\beta 2$).

The estimate response surfaces based on the experimental data (Fig. 5) shows that increasing temperature and alkali solution concentration promoted a yield increase. However, at higher temperatures there was a significant decrease in the yield (Fig. 5a and c). The influence of temperature on the xylans extraction was also reported by Hromádkova et al. [14], who suggested that the increase in the temperature and alkali concentration increased the degradation of xylans, and subsequently promoted the depolymerisation into shorter xylans that were soluble in ethanol solution. The increase in the w/v ratio shows a decrease in the yield (Fig. 5b and c). Increasing the concentration of the alkali solution at the highest ratio did not show an influence on the final yield obtained (Fig. 5b), since increasing the sample weight for the same solvent volume seems to decrease the hydration and swelling processes.

The optimum yield, predicted by the regression Eq. (3) fitted to the data, was 23.2% by the combination of the factors, which maximised the yield over the studied region. This combination was 0.09 (w/v), 3.7 M KOH, 47 °C and 7.2 h.

Since in UAE the ratio was limited to 0.04, the alkaline extraction was performed using this ratio for the purposes of comparing the two methods. To maximise the yield using this ratio, the combination of the other factors were 3 M KOH, 45 °C and 7 h and the predicted yield by the regression equation was 20.1% while the experimental value obtained was 20.3 ± 1.7% (*n* = 3).

The extracts obtained under the optimised conditions were composed of 76% polysaccharides and the major sugar residues identified were xylose followed by arabinose and residual amounts of uronic acids, glucose and galactose (Table 3). These extracts were richer in sugars than the USAE (47.4%), although the major sugars and their glycosidic-linkage composition were similar. The highest molar percentages of 4-Xyl*p* and t-Ara*f* were indicating the major composition of AX (Table 4). The lower relative percentages of 2,4-Xyl*p*, 3,4-Xyl*p* and 2,3,4-Xyl*p* in AE as compared to USAE showed the presence of less branched structures. The 4-Glc*p* linkage, indicative of the presence of residual starch, accounted for 8.5% in AE whereas it was almost absent in USAE (0.8%), indicating

Table 7

Content of polysaccharides (AX, AG, β-glucans and starch) and protein of AX-rich extracts obtained from BSG.

	Yield (%)	Polysaccharides (%)				AX		Protein (%)
		AX	AG	β-glucans	Starch	$\frac{Xyl_{total}}{t\text{-}Xyl}$	$\frac{Xyl_{ram}}{Xyl_{total}}$	
US	2	31	2	0.6	8	24	0.4	4
AC	1	21	1	0.1	16	22	0.4	8
USAE	20	45	2	–	0.4	12	0.5	25
AE	20	66	2	0.5	7	14	0.3	17

US – ultrasound extract; AC – autoclave extract; USAE – ultrasound alkaline extract; AE – alkaline extract; AX – Arabinoxylans ($Xyl_{total} + Ara_{total} - (Gal_{total} \times 0.7 \times 180/150)$); AG – Arabinogalactans ($Gal_{total} + (Gal_{total} \times 0.7 \times 180/150)$); Xyl_{ram} – (2,4-Xyl + 3,4-Xyl + 2 × 2,3,4-Xyl).

that the USAE is able to recover an extract almost devoid of starch. This could be attributed by the US and AC pre-treatments. In addition, a 3-Glc*p* linkage was also identified, also present in both US and AC extracts, suggesting the presence of residual β-glucans. The residual galactose linkages were also identified in these extracts but in low amounts, reflecting the presence of residual AG. The 2-Man*p*, 6-Man*p* and 2,6-Man*p*, which are typical linkages of mannoproteins found in the yeast used for production of the beer [30,31], were also present in residual amounts in the extract obtained under the optimised AKE conditions. The presence of mannoproteins may reflect some contamination of *Saccharomyces* in the BSG analysed.

3.3. Ultrasound assisted extraction (UAE) vs alkaline extraction (AKE)

A similar yield of polymeric material (20%) was obtained in UAE when compared to the AKE reference method used. The final residue (FR) after the application of UAE was composed of fewer sugars than the FR after AKE but the quantity of AX remaining (30%) was similar (Table 3). This means that the UAE was more efficient in extracting polysaccharides from the BSG than the AKE, and as efficient as AKE in extracting AX. However, the final extract USAE was composed of less sugars than the AE and the composition in AX was 45%, while AE was composed of 66% AX (Table 7). The lower amount of AX and the results obtained in Section 3.1.2 suggests that UAE, although more efficient in the extraction of the polysaccharides from the cell walls of BSG, promotes their degradation and generates shorter polymers which are probably soluble in ethanol and/or lost in the dialysis step used in this work. The ratios of Xyl_{total}/t-Xyl and Xyl_{ram}/Xyl_{total}, determined for the USAE indicates that the AX in this extract were slightly smaller and more branched than those recovered in the AE (Table 7). Optimisation reflecting AX yields using UAE should be done in order to improve the amount of AX in the extracts obtained.

The composition of USAE and AE also differs with respect to the quantity of protein present and in the type and quantity of other polysaccharides (Table 7). In addition to the AX, USAE was also composed of 2% AG and residual starch, while AE in addition to AX also contained 2% AG, 7% starch and residual amounts of β-glucans. The difference in the quantity of starch in the USAE, values these extracts when compared to the AE.

The extracts obtained with the pre-washing treatment revealed a considerable amount of AX, 31% in US extracts and 21% in AC extracts (Table 7). AX extracted with water showed a higher Xyl_{total}/t-Xyl, which is in accordance with Dervilly et al. [28] for barley and Mandalari et al. [6] for BSG. This type of AX has been correlated with higher prebiotic activity, however the high quantity of starch in these extracts and the high costs associated with its removal could hinder the application of these extracts as prebiotics.

As an example, using 1 tonne of BSG would result in 90 kg of AX being obtained using UAE, compared to 132 kg of AX using conventional alkaline method. However, the extracts obtained by UAE were almost absent of starch, which is a considerable economic advantage. The extract obtained with the conventional alkaline extraction from 1 tonne of BSG will have 14 kg of associated starch while the extract obtained with UAE will have only 0.8 kg of starch. The costs associated with starch removal will be much higher in the conventional method. These costs will also be compounded by energy costs of 7 h extraction at 45 °C, and the amount of alkaline reagent required in contrast with the 25 min UAE at room temperature and with less alkaline reagent used. Moreover, the amount of AX in the extracts produced by UAE can be improved if the optimisation reflecting AX yields will be considered.

The recovery of AX using the extracts obtained with the pre-washing treatment seems to be much too expensive due to the presence of starch. However, the pre-washing treatment should not be skipped because it helps washing the residue for the following ultrasound alkaline treatment, leading to the production of extracts depleted of starch, which could enhance their potential prebiotic applications.

4. Conclusion

The efficiency of UAE in the production of AX-rich extracts from BSG was shown by the significant reduction in the extraction time and energy consumption, for possible industrial applications. Another advantage of the application of UAE is the removal of starch from the final AX-rich extract. The AX recovered by UAE were more branched than those recovered by AKE, which allows to set other extraction procedures in order to obtain AX structures for different possible applications.

Acknowledgements

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