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# Isolation and characterization of glucuronoarabinoxylans from wheat bran obtained by classical and ultrasound-assisted extraction methods

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**Abstract**

*Introduction* Plant biopolymers like arabinoxylans possess valuable potentials as food additives with health-promoting properties or as basic products for the production of chemically functionalized polymers with unexploited technical or physiological properties. Wheat bran as an abundant milling by-product can serve as a cheap renewable source of cereal arabinoxylans. Isolation yields of xylans from plant materials are traditionally only moderate or low. *Objectives* A study was undertaken to investigate the effect of short-ultrasound application on the extractability of arabinoxylans from wheat bran suspended in aqueous alkaline solutions on a laboratory scale. The influence of the ultrasonic treatment on the overall extraction yield, purity and chemical structure of bran arabinoxylans under conditions of different sonication intensities and alkaline media was compared with conventional procedures not applying ultrasound. *Results* Extraction of xylans from bran using alkaline peroxide solutions without sonication for 240 min at 60 °C and subsequent purification provided polymers of 83% purity of a total yield of about 14%. Products of the same purity could be isolated by ultrasonication of bran suspensions in alkaline peroxide solutions for 10 min at 60 °C. Structural details of the recovered xylans were verified by high-pressure liquid chromatography-based monomer analysis, Fourier transform infrared, $^{13}$C-nuclear magnetic resonance spectroscopy and molecular weight distribution analysis by high-performance size exclusion chromatography with triple detection. Ultrasound-assisted extraction lead to a partial depolymerization of the isolated arabinoxylan. *Conclusion* Applying ultrasound for the extraction of xylans from plant cell walls can reduce the extraction time considerably but has no influence on the maximum extraction yield.

## Introduction

Next to cellulose arabinoxylans (AX) are the quantitatively second most abundant biopolymers in plant kingdom. They are found as component of the cell walls not only in wood but also in grasses, cereals and herbs. While cellulose as the main cell wall component is a β-1-4 linked polyglucan differing in the degree of polymerization only, AX represent a large class of branched heteropolysaccharides ('hemicelluloses') containing a xylan backbone highly substituted with sugar side chains and with acetyl feruloyl and cumaroyl and other side chains, depending on the botanical origin (Ebringerova & Heinze, 2000). Xylans found in cereal plants like maize, rice, oats, sunflower, barley and wheat have a 1, 4-β-D-xylopyranosyl main chain predominantly substituted with arabinose. Galactose, 4-O-methyl-glucuronic acid or

galacturonic acid are minor substituents. The functional properties of AX in the field of baking are known for many years while their health-related properties as a dietary fibre has been investigated only recently (Garcia *et al.*, 2007). The technical and bread making properties of glucuronoarabinoxylans (GAX) are related to their physicochemical properties such as water solubility, oxidative cross-linking, gel and viscosity forming, water-binding capacity, emulsion or foam stabilizing effects. Because of their chemical structure and physiological functionality, e.g. as food-added dietary fibre, AX may also have beneficial health effects such as promotion of satiety feeling or as a natural stimulant of digestion. Because of its properties as typical soluble dietary fibre it may also influence positively blood glucose and insulin levels in people suffering from type II diabetes (Lu *et al.*, 2004).The prebiotic potential has already been investigated (Glei *et al.*, 2006) and initiated the manufacture of functional food substituted with AX with suspected colon-health promoting effects. The industrial applications include use as viscosity modifiers and gelling agents. There is also an increasing interest in exploiting the technological potential of chemically modified AX as arabinoxylanesters or ethers (Izydorczyk & Biliaderis, 2006).

AX occur in particular large amounts in wheat and rye bran (up to 35% of dry matter). Wheat and rye bran are abundantly available by-products (14–20% of dry matter) of the wheat and rye milling industry and are utilized mainly as a feedstuff. The isolation of AX from wheat and rye bran is laborious and involves aqueous alkaline media for solubilization (Saulnier *et al.*, 2007). The polysaccharide chains are cross-linked by both esterified and etherified hydroxycinnamates and other phenolic substances. Etherified linkages represent complexes of xylan chains and cellulose and neighbouring feruloylated AX are cross-linked by diferulate bridges. But as non-hydrolysable ether linkages restrict the solubilization of xylans from the lignin matrix, an additional delignification step is necessary to increase the extraction yield of isolated polymers. Delignification of plant material can be achieved by using sodium hypochlorite, chlorine, chlorine dioxide or peroxyacetic acid or alkaline hydrogen peroxide.

Several extraction procedures for isolation of AX from cereal bran (Bergmans *et al.*, 1996; Roubroeks *et al.*, 2000; Karppinen *et al.*, 2001; Maes & Delcour, 2001; Hollmann & Lindhauer, 2005), wheat straw (Sun & Tomkinson, 2002) have been described. Today, some of the proposed extraction solvents are no longer acceptable with respect to environmental protection issues and occupational safety and thus not suited for isolation of these plant biopolymers in an industrial scale. Beyond that, the extraction yields and the purity of the products were only moderate to low.

Ultrasonication has been applied in the course of procedures for extracting hemicelluloses from plant cell walls by several authors, e.g. by isolation of hemicelluloses from maize bran (Ebringerova & Hromadkova, 2002), from buck wheat (Hromadkova & Ebringerova, 2003), wheat straw (Sun & Tomkinson, 2002) and wheat bran (Hromadkova *et al.*, 2008). It was shown that ultrasonication can help separating coextracted starch and protein from the isolated hemicelluloses and by splitting of α-ether linkages between lignin and hemicellulose chains thus improving the extraction with respect to yield and purity.

The present work describes the influence of ultrasonication on the chemical structure of GAX isolated from wheat bran in alkaline suspensions assisted by ultrasonic irradiation in comparison to xylans obtained by extraction procedures using only alkaline hydrogen peroxide, only.

## Material and methods

Wheat grain (cv. Bussard, harvested in 2001) was milled and the coarse bran fraction separated (mean particle size diameter 0.5 mm) on a pilot plant mill (MIAG/Buehler AG, Switzerland). Hydrogen peroxide, hydrochloric acid and sulphuric acid were obtained from Merck (Darmstadt, Germany), sodium hydroxide from Carl Roth GmbH & Co. (Karlsruhe, Germany), industrial 96% ethanol from Alcohol Handelskontor (Lippstadt, Germany), the antifoaming agent Witafrol 7456 C from Sasol GmbH (Witten, Germany). Lichenase [endo 1,3-(1,4)-β-D-glucanase, *Bacillus* sp., E.C. 3.2.1.73] and β-glucosidase (*Aspergillus niger*, E.C. 3.2.1.21) were obtained from Megazyme (Bray, Ireland). Heat-stable α-amylase (Termamyl 120L) and protease (Alcalase 2,4L) were kindly provided by Novozymes A/S (Bagsvaerd, Denmark).

Moisture content was determined by International Association for Cereal Science and Technology (ICC) standard method no. 110/1 (ICC, 1991). The content of uronic acids was assayed colorimetrically using the 3-phenylphenol reagent according to the method of Blumenkrantz & Asboe-Hansen (1973). Protein ($N \times 5.7$) and nitrogen content were determined using the Dumas combustion method according to ICC standard method no. 167 (ICC, 1991). Total starch content was measured according to Association of Official Analytical Chemists (AOAC) standard method no. 996.11 (AOAC, 2003) using Megazyme assay kit K-TSTA (Megazyme, Ireland). Total fat content was determined in a Soxhlet apparatus by extraction with petroleum ether (Association of Cereal Research, 1994). Mixed-linkage β-glucan

content was quantified by ICC standard method no. 166 (ICC, 1991) with Megazyme assay kit K-BGLU. For determination of total dietary fibre (TDF) according to AOAC standard method no. 991.43 (AOAC, 2003) Megazyme assay kit K-TDFR was used. All experiments were repeated triplicate under the same conditions. All yields and composition analyses were calculated on moisture-free basis and the results given as mean values. The neutral sugar composition of wheat bran and isolated AX, respectively, were quantified by high-performance anion-exchange chromatography with pulsed amperometric detection on a Dionex Biolc system (Dionex Corporation, Sunnyvale, CA, USA) as described by Hollmann & Lindhauer (2005). Monosaccharides were separated on a CarboPac PA1$^{®}$ column (10 μm, $4 \times 250$ mm) with guard column CarboPac PA1$^{®}$ (10 μm, $4 \times 50$ mm) isocratically at 20 mM NaOH at 20 °C at a flow rate of 0.8 mL min$^{-1}$. Injected sample volume was 25 μL. Analytes were detected by an electrochemical detector ED50 with gold electrode as working electrode and an Ag/AgCl reference electrode. Chromatography management and data analysis was accomplished by Dionex Chromeleon Software 6.5. Before the analysis samples were hydrolysed with 1 M sulphuric acid at 105 °C for 2 h, then neutralized by adding 0.25 M aqueous barium hydroxide and analysed as described. Phenolic acid analysis of wheat bran and isolated AX fractions was performed according to a published procedure after saponification of samples with 2 M NaOH for 18 h at 20 °C under a N$_2$ atmosphere in the dark (Hollmann & Lindhauer, 2005).

## Gel permeation chromatography (GPC)/size exclusion chromatography

Molecular weight distribution of the isolated AX was determined by HPSEC-RI-LALS-Visc using two ViscoGel GMPWXL mixed bed columns in series (Viscotek GmbH, Waghausel, Germany). Fifty microlitre samples (2 mg mL$^{-1}$) were injected and eluted at 35 °C with an aqueous solution (0.1 M NaNO$_3$, NaH$_2$PO$_4$), pH 7, at a flow rate of 0.7 mL min$^{-1}$. The elution profiles were determined using a Triple Detector Array TDA 302 (refractive index/viscometer/low-angle light scattering). Weight-average molar mass ($M_w$) and number-average molar mass ($M_n$) were calculated based on the viscosimetry and light scattering measurements using d$n$/d$c = 0.147$ mL g$^{-1}$ (Dervilly *et al.*, 2002). The detectors were calibrated with a narrow molecular weight range pullulan standard of $M_w = 1\,12\,000$ g mol$^{-1}$ (Showa Denko Europe, Munich, Germany) due to commercial unavailability of xylan standards. Intrinsic viscosities IV$_w$ were calculated based on the viscosity signals of the viscosity detector. All calculations including hydrodynamic diameter Rh$_w$ and the *a*-value from the Mark–Houwink plot were made using the TriSEC GPC software (Viscotek GmbH).

## Spectroscopic methods

Fourier transform infrared (FT-IR) spectra (in KBr pellets, 1.5–2.5 mg sample/260–270 mg KBr, $d = 13$ mm) were measured using the FT-IR spectrophotometer IFS 28, (Bruker-Franzen Analytik GmbH, Bremen, Germany) operating at $1$ cm$^{-1}$ resolution. The spectral range was 4000–400 cm$^{-1}$ and the scan rate was 30 scans sec$^{-1}$. Proton-decoupled $^{13}$C-nuclear magnetic resonance (NMR) spectra of AX were recorded with a Varian Inova 500 spectrometer at 60 °C at a frequency of 125 MHz. Samples were measured in DMSO-d$_6$ at a concentration of 2% (w/v). The solvent served as an internal reference at 39.5 p.p.m. Spectra were recorded by inverse-gated-decoupling technique, spectral width was set to 25 kHz and the pulse delay was 3 sec and the data acquisition time 1.3 sec. 25 000 scans were accumulated within 24 h.

## Purification of wheat bran

The coarse wheat bran fraction (mean particle size diameter 0.5 mm) was collected, purified with ethanol for removal of coloured contaminations and for inactivating AX degrading enzymes and dried for 24 h at 40 °C as described before (Hollmann & Lindhauer, 2005). The purified bran was analysed according to ICC standard methods (ICC, 1991) and applied for isolation of GAX.

## Extraction of GAX from wheat bran without ultrasonication ('classical' extraction procedure)

The classical procedure is based mainly on the procedural instructions published by Maes & Delcour (2001) and Hollmann & Lindhauer (2005) for isolation of these biopolymers from cereal bran. In a typical experiment, 20 g of purified wheat bran were suspended in 1 L of 2% aqueous hydrogen peroxide, adjusted to pH 11.5 with 25% aqueous sodium hydroxide, warmed up to 60 °C with continuous stirring. Strong foaming was avoided by adding 1 mL of Witafrol 7456 C. The suspension was kept for 4 h at 60 °C under continuous stirring and then cooled to ambient temperature under tap water. Isolation of xylans was performed as described below.

## Extraction of GAX from wheat bran with ultrasonication

In a typical experiment for ultrasound-assisted extraction of AX from purified wheat bran, 20 g of purified bran were suspended in 400 mL of aqueous alkaline solvent. The ultrasound generating system consisted of an Ultrasound homogenizer Sonopuls HD 200 at 20 kHz ultrasonic frequency (Bandelin Elektronik, Berlin, Germany) in combination with an ultrasonic converter UW 200, with a probe Boosterhorn SH 225 titanium flat tip TT25 at 120 W HF output and maximum ultrasonic intensity of 24 W cm$^{-2}$. The influence of different alkaline media, concentration of hydroxide and ultrasound intensity was investigated with respect to maximum extraction yield and product purity. The suspensions were ultrasonicated in a water bath at temperatures between 45 and 50 °C under continuous stirring for 10 min and then cooled to ambient temperature followed by isolation of xylans as described below.

## Isolation of GAX

After cooling to ambient temperature the alkaline bran extracts were sieved with a sieve with 0.4 mm mesh width and washed thoroughly with distilled water. The aqueous extract was then neutralized with 32.5% hydrochloric acid, heated to 85 °C, 4.5 mL of amylase Termamyl 120 L was added and heated for 2 h at 85 °C under continuous stirring. After cooling to ambient temperature the pH of the extract was raised to pH 8.5 by adding 2 M NaOH and 5 mL of protease Alcalase 2.4 L added. The extract was stirred at ambient temperature for 16 h, heated to 95 °C for 15 min to precipitate proteins and recooled to ambient temperature. Precipitated proteins were removed by centrifugation for 10 min at $4500 \times g$ (Centrifuge Varifuge F, Heraeus Holding GmbH, Hanau, Germany). The supernatant was recovered and AX were precipitated with ethanol at a final concentration of 80% (v/v), washed with 80% ethanol, redissolved in distilled water, precipitated again with ethanol at 80% final concentration, dried for 12 h at 40 °C in a circulating air dryer (Tuttlingen, Binder GmbH, Germany) and analysed for neutral sugar composition and other analytes. All values given are means of duplicate experiments. For GPC, FT-IR and $^{13}$C-NMR analysis isolated AX were further purified to remove residual β-glucans with a combination of lichenase and β-glucosidase as described (Hollmann & Lindhauer, 2005).

## Results

Chemicals used for developing a procedure either on a laboratory or a technical scale, for isolating natural products from any raw material should be chosen under the aspects of occupational health and environmental protection. Thus chlorine-containing organic chemicals used in previous decades for disintegrating the lignin matrix of the cell walls of plant material were not used in this study. Neither were alkaline media like aqueous ammonia or barium hydroxide used due to their inherent toxicity. Previous work had shown that wheat bran washed with hot aqueous ethanol for removal of coloured constituents and low molecular weight carbohydrates provided AX as white or slightly yellow precipitates (Hollmann & Lindhauer, 2005). Using untreated bran only yellow-brown or grey products resulted. The chemical composition of the purified bran used for extraction is shown in Table 1.

Analysis shows that the fraction of insoluble dietary fibre of the total fibre content is very high and a substantial portion consists of GAX. Therefore, wheat bran is an interesting raw material for isolation of these polysaccharides. AX are usually isolated from plant raw material using alkaline solvents (Darvill *et al.*, 1980). In order to find a suitable alkaline solvent for dissolving AX from bran assisted by ultrasonication three different alkaline media were tested, the extraction yield determined and xylose and arabinose composition identified (Table 2).

The results show that only sodium hydroxide is able to extract up to 20% of the total AX content of the bran and that the sodium ions play an effect on the extractability of

**Table 1** Chemical composition of purified wheat bran

| Component (% d.m.) | |
|---|---|
| Protein | 17.4 |
| Ash | 6.4 |
| Fat | 2.8 |
| Starch | 5.8 |
| Total dietary fibre | 70.4 |
|    Insoluble fibre | 66.0 |
|    Soluble fibre | 4.4 |
| Glucose | 6.5 |
| Galactose | 0.7 |
| Xyl/Ara | 1.4 |
| AX | 39.4 |
| Ferulic acid (mg g$^{-1}$) | 0.9 |
| Uronic acid | 2.7 |
| GAX | 42.1 |
| β-glucan | 2.3 |
| Cellulose + lignin | 26.0 |

(cellulose + lignin) = total dietary fibre − (GAX + β-glucan).
AX = 0.88 × (% Ara + % Xyl); GAX = 0.88 × (%Ara + % Xyl + % uronic acid).
AX, arabinoxylans; d.m., dry matter; GAX, glucuronoarabinoxylans; Xyl/Ara, xylose to arabinose ratio.

the biopolymers from the cell wall lignin matrix. Therefore, based on the highest extraction yield of AX with sodium hydroxide solution, in subsequent experiments the influence of the concentration of sodium hydroxide on the recovery yield and purity and neutral sugar composition was investigated. The isolation and chemical characterization was performed as described (Table 3).

These results show that increasing alkali concentration influences the amount of released xylans from the cell walls. Extracting AX with 3% or 5% NaOH gave nearly similar high yields. Thus the following extractions were conducted in 3% NaOH. Extraction trials with hydroxide concentrations of 5% and above led to dark brown suspensions from which the isolation of xylans was laborious (data not shown). The yield of the yellow-brown xylan isolates was drastically diminished indicating decomposition of polymers under these conditions. In order to test which impact ultrasonication intensity has on the extraction potential of sodium hydroxide, suspensions of wheat bran in 3% NaOH

were sonicated at different intensities and the amount of extracted AX was determined (Table 4).

The results show that extracting xylans from bran without preceding ultrasonication in a suspension of wheat bran in 3% NaOH and with an intensity of 24 W cm$^{-2}$ (the maximum intensity the equipment was able to generate) for 10 min at a temperature of 60 °C can provide nearly 1/3 of the total AX from bran. In a final experiment the extraction of AX from purified wheat bran under classical conditions with alkalized hydrogen peroxide was compared with an ultrasound-assisted extraction in the same solvent. As the isolated xylans had to be characterized with respect to possible ultrasonication-induced structural changes by FT-IR, GPC and $^{13}$C-NMR residual β-glucans were enzymatically removed as described (Hollmann & Lindhauer, 2005). The data in Table 5 show that although the presumed extraction yield-enhancing ultrasonication was applied yield and purity of the isolated AX were not significantly different in comparison with the classical extraction procedure. Thus a maximum of 1/3 of the total arabinoxylan pool of wheat bran can be extracted under alkaline conditions at elevated temperatures and ultrasound assistance at a considerably reduced extraction time of 10 versus 240 min under classical conditions.

## FT-IR spectra

FT-IR spectroscopy can be used for the approximate identification of the structure of the isolated hemicelluloses.

**Table 2** Influence of type of alkaline medium on the ultrasound-assisted extraction of AX from wheat bran

| Solvent | Yield (% of bran) | AX (% of d.m.) | AX (% of bran AX) | Xyl/Ara |
|---|---|---|---|---|
| 1% NaHCO$_3$ | 2.9 | 51.1 | 4 | 2.2 |
| 1% KOH | 12.7 | 40.1 | 13 | 1.5 |
| 1% NaOH | 17.9 | 39.7 | 18 | 2.0 |

See Table 1 for abbreviations.

**Table 3** Influence of sodium hydroxide concentration on the ultrasound-assisted extraction of AX from wheat bran

| Solvent | Yield (% of bran) | AX (% of d.m.) | AX (% of bran AX) | Xylose (% of d.m.) | Arabinose (% of d.m.) | Xyl/Ara |
|---|---|---|---|---|---|---|
| 1% NaOH | 17.9 | 39.7 | 18 | 30.5 | 14.78 | 2.1 |
| 2% NaOH | 14.1 | 58.8 | 21 | 45.2 | 21.6 | 2.1 |
| 3% NaOH | 17.8 | 62.9 | 28 | 46.2 | 25.2 | 1.8 |
| 5% NaOH | 17.4 | 66.4 | 29 | 48.8 | 26.6 | 1.8 |

See Table 1 for abbreviations.

**Table 4** Influence of ultrasound intensity on the extraction of AX from wheat bran with 3% aqueous sodium hydroxide

| Intensity (W cm$^{-2}$) | Yield (% of bran) | AX (% of d.m.) | AX (% of bran AX) | Xylose (% of d.m.) | Arabinose (% of d.m.) | Xyl/Ara |
|---|---|---|---|---|---|---|
| 0 | 12.6 | 60.6 | 19 | 54.5 | 14.4 | 3.8 |
| 4.8 | 12.4 | 72.2 | 23 | 62.0 | 20.1 | 3.1 |
| 9.6 | 13.9 | 65.6 | 23 | 53.9 | 20.6 | 2.6 |
| 14.4 | 11.0 | 73.2 | 20 | 59.2 | 24.0 | 2.5 |
| 19.2 | 13.8 | 70.8 | 23 | 62.4 | 18.1 | 3.5 |
| 24.0 | 17.7 | 62.9 | 28 | 52.7 | 15.6 | 3.4 |

See Table 1 for abbreviations.

Assignment of absorption bands to structural features was based on the work of several authors (Kacurakova *et al.*, 1998; Hromadkova *et al.*, 2002; Hromadkova & Ebringerova, 2003; Sun *et al.*, 2004a, b). Figure 1 illustrates the FT-IR spectra of AX isolated from wheat bran either without

**Table 5** Comparison of conventional and ultrasound-assisted extraction of arabinoxylans from wheat bran with 2% alkaline hydrogen peroxide

| | Extraction procedure | |
| --- | --- | --- |
| Component (% d.m.) | Without ultrasound | With ultrasound |
| Yield (before purification) | 18.0 | 18.4 |
| Yield (after purification) | 14.4 | 14.7 |
| AX (before purification) | 61.8 | 65.6 |
| AX (after purification) | 80.2 | 84.6 |
| GAX | 83.2 | 87.5 |
| GAX (% of total bran AX) | 29 | 32 |
| Xylose | 51.3 | 55.6 |
| Arabinose | 39.9 | 40.5 |
| Glucose | tr. | tr. |
| Galactose | tr. | 0.6 |
| TDF | 84.0 | 87.6 |
| Insoluble DF | 79.6 | 85.8 |
| Soluble DF | 4.4 | 1.8 |
| Uronic acids | 3.0 | 2.9 |
| Nitrogen (dumas) | 0.89 | 0.93 |
| β-glucan | 0.36 | 0.72 |
| Ferulic acid (mg g$^{-1}$) | tr. | tr. |
| Xyl/Ara | 1.3 | 1.4 |

See Table 1 for abbreviations. tr., traces

ultrasonic irradiation (spectrum 1) or with ultrasound assistance (spectrum 2).

All spectra show typical absorption patterns of hemicelluloses. The broad absorption band at 3404 cm$^{-1}$ in both spectra belong to the stretching of polymeric hydrogen-bonded OH-groups, the bands at 2930 cm$^{-1}$ arise from C–H stretching. The intensive and sharp absorptions at 1635 cm$^{-1}$ are assigned to OH-groups of adsorbed water and sharp bands at 1043 cm$^{-1}$ originates from glycosidic C–O–C-stretching vibrations typical for xylan chains. The peaks at 1458 and 1400–1424 cm$^{-1}$ are associated with C–H and O–H bending vibrations. The low intensity absorption at 1322 cm$^{-1}$ can be attributed to C–C– and C–O– skeletal vibrations. The presence of xylan chains may also be indicated by absorptions of low intensity at 1066–1077 cm$^{-1}$ and caused by C–O–C vibrations, as already shown for xylo-oligosaccharides (Kacurakova *et al.*, 1998). The small sharp peak at 900 cm$^{-1}$ is characteristic for the presence of β-glycosidic bonding between different sugar moieties in all AX. No absorptions typical for carboxylic esters in the range of 1700–1800 cm$^{-1}$ could be detected. This implies that at least based on FT-IR analysis, the AX contain no acetyl or uronic ester or ferulic ester groups, which have been completely saponified under the alkaline extraction conditions.
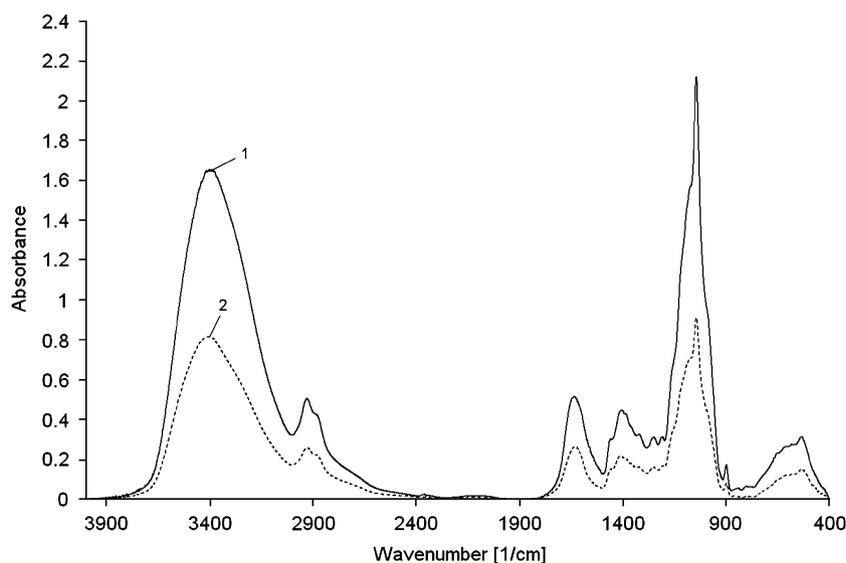
## GPC analysis

Molecular weights and polydispersity of AX extracted from wheat with and without ultrasonic irradiation were



**Figure 1** Fourier transform infrared (FT-IR) spectra of arabinoxylans isolated from alkaline peroxide suspensions of wheat bran without ultrasound assistance (spectrum 1) or by ultrasonic irradiation (spectrum 2) for 10 min.

**Table 6** Molecular characteristics of arabinoxylans from wheat bran extracted conventionally or with ultrasound-assistance with 2% alkaline hydrogen peroxide

| Extraction procedure | Polymer content (%) | $M_W$ (g mol$^{-1}$) | $M_n$ (g mol$^{-1}$) | PD | IV$_w$ (dL g$^{-1}$) | Rh$_w$ (nm) |
|---|---|---|---|---|---|---|
| Classical | 98.4 | 491747 | 177811 | 2.77 | 0.94 | 15.76 |
| With ultrasound | 95.4 | 325809 | 85868 | 3.80 | 1.03 | 13.57 |

$M_w$, weight-average molecular weight; $M_n$, number-average molecular weight; PD, polydispersity ($M_w/M_n$); Rh$_w$, radius of gyration, IV$_w$, intrinsic viscosity.

determined using GPC/size exclusion chromatography with a Viscotek$^{TM}$ triple detector array consisting of a differential refractive index detector, a four-capillary differential viscometer detector and a low-angle light scattering detector.

Table 6 shows weight-average ($M_w$) and number-average ($M_n$) molecular weights of both xylans. Polydispersity PD was calculated from $M_w/M_n$. The intrinsic viscosity (IV) is inversely proportional to density of the polymer coil. The extension of the polymer coil is expressed as its radius of gyration (Rh$_w$). Both products show high molecular weights and a high degree of polydispersity. Classically extracted AX have a broad molecular weight dispersion with $M_w$ of 491 000 g mol$^{-1}$ and $M_n$ of 177 000 g mol$^{-1}$. Also, ultrasonicated xylans show a broad molecular weight dispersion but a lower $M_w$ of 325 000 g mol$^{-1}$ and lower $M_n$ of 85 000 g mol$^{-1}$. The lower molecular weights of the ultrasound-irradiated products indicate a sonication induced partial decomposition of long polymer chains producing fractions of shorter xylan chains. This is also supported by the higher polydispersity of this type of product. The hydrodynamic diameters of both products differ only slightly. This ultrasound-induced fragmentation of longer xylan chains with a concomitant higher polydispersity of the molecular weight distribution was also presented by Ebringerova & Hromadkova (2002).

## Structural characterization

$^{13}$C-NMR spectroscopy of AX either obtained with or without ultrasonication allowed a more detailed structural elucidation. Most of the major resonances were assigned according to data from the literature (Hromadkova & Ebringerova, 2003; Sun *et al.*, 2004a; Hollmann & Lindhauer, 2005). The typical structure-forming xylan backbone of β-1-4-linked xylopyranose monomers is represented by the signals at 101.3, 69.3 and 65.4 p.p.m. for the carbon atoms C1, C4 and C5 of the reducing terminal D-xylopyranoses. Three intensive signals at 75.4, 73.8 and 63.0 p.p.m. are assigned to carbon atoms C4, C3 and C5 of β-D-xylopyranoses within the xylan chain. Weak resonances at 77.3, 75.1 and 72.8 p.p.m. are due to C3, C4 and C2 of

xylopyranoses. Branching of linear xylose chains by α-L-arabinofuranose is detected by resonances at 107.1 p.p.m. (C1 of α-L-arabinofuranose linked to *O*-3 of β-D-xylopyranose monomers) and at 101.5 p.p.m. (C1-xylopyranose, disubstituted with arabinose at O–2 and O–3). The signals at 85.8, 83.0, 77.7 und 61.7 p.p.m. are due to carbon atoms C4, C2, C3 und C5 of arabinofuranose. $^{13}$C resonances at 81.3 p.p.m. and at 72.4 p.p.m. arise from C3-xylose and C2-xylose monosubstituted at O3 with arabinose, at 80.3 p.p.m. from xylose monosubstituted at O2 with arabinose. β-D-glucopyranosyl uronic acid residues were only identified by the chemical analysis and by the signals of C1 and C3 at 99.3 and 73.3 p.p.m. The spectra of both ultrasound mediated as well as classically extracted AX from wheat bran show intensive sharp signals at 101.6, 75.4, 73.8, 72.5 and 63.0 p.p.m. of the β-D-xylopyranoses (Table 7).

The signals at 108.7, 107.6 and 100.1 p.p.m. in both products were assigned to C1 of α-L-arabinofuranose, bonded at O2 or O3 to xylopyranose residues. Comparing NMR data from the literature (Sun & Tomkinson, 2002; Sun *et al.*, 2004a) with the resonances of the isolated xylans at 60.3, 97.4, 71.8, 82.1 and 71.6 p.p.m. revealed the presence of 4-*O*-methyl groups and atoms C1, C2, C4, C5 of 4-*O*-methyl-β-D-glucopyranosyl uronic acids (4-*O*-Me-β-D-Glc*p*A), not detected by FT-IR. C4, C2, C3 and C5 of terminal arabinofuranose are seen at 85.8, 83.0, 77.7 and 61.7 p.p.m. $^{13}$C-signals at 81.3, 80.3, 77.3, 76.0 and 72.8 p.p.m. are characteristic for C-atoms C3, C4 and C2 of xylopyranoses monosubstituted at either O2 or O3.

Concluding, a comparative analysis of $^{13}$C-NMR spectra, FT-IR spectra and neutral sugar analysis of GAX extracted with or without ultrasound detected no significant differences between these types of xylans and the main structural features were not affected by the sonication under alkaline conditions.

## Conclusion

Under optimized extraction conditions AX of maximum yield and purity could be extracted by sonication of alkaline bran suspensions for 10 min at 50–60 °C. The maximum

**Table 7** Chemical shifts and their assignments to C-atoms in purified arabinoxylans from wheat bran

| Assigned C-atom | Conventional extraction δ (p.p.m.) | Ultrasound-extraction δ (p.p.m.) |
|---|---|---|
| C1-Ara-O2-Xyl (O3-Ara) | 108.7 | 108.7 |
| C1-Ara-O3-Xyl (O2-Ara) | 107.6 | 107.6 |
| C1-Ara-O3-Xyl | 107.1 | 107.1 |
| C1-Xyl (O2 or O3-Ara) | 101.5 | 101.6 |
| C1-Xyl (terminal) | 101.3 | 101.3 |
| C1-α-D-GlcpA | 99.3 | 99.3 |
| C4-Ara | 85.8 | 85.8 |
| C2-Ara | 83.0 | 83.0 |
| C4-4-OMe-α-D-GlcpA | 82.1 | 82.1 |
| C3-Xyl (O3-Ara) | 81.3 | 81.3 |
| C3-Xyl (O2-Ara) | 80.3 | 80.3 |
| C3-Ara | 77.7 | 77.7 |
| C4-Xyl (O3-Ara) | 77.3 | 77.3 |
| C4-Xyl (O2-Ara) | 76.1 | 76.0 |
| C4-Xyl | 75.4 | 75.4 |
| C3-Xyl (O2- and O3-Ara) | 75.1 | 75.2 |
| C3-Xyl | 73.8 | 73.8 |
| C3-α-D-GlcpA | 73.3 | 73.3 |
| C2-Xyl (O2-Ara) | 72.8 | 72.8 |
| C2-Xyl (O3-Ara) | 72.4 | 72.6 |
| C2-4-OMe-α-D-GlcpA | 71.8 | 71.9 |
| C4-Xyl (terminal) | 69.3 | 69.3 |
| C5-Xyl (terminal) | 65.4 | 65.4 |
| C5-Xyl | 63.0 | 63.0 |
| C5-Ara | 61.7 | 61.7 |

yield was around 30% of the total AX content of the wheat bran. Thus, ultrasonication reduced the time needed for extraction of maximum yield from 240 min under conventional conditions to 10 min but the yield could not be significantly increased. Besides the alkali concentration, neither the extraction temperature nor the sonication time had any marked effect on the extraction efficiency.

Several authors have investigated the influence of ultrasonication on the hemicellulose extraction process from different plant materials. The effect of ultrasonication on the extractability of polysaccharides seems to be strongly dependant on the botanical origin of the extracted plant residues. Thus, ultrasound-mediated extraction of hemicelluloses with alkaline peroxide released 92% of the original hemicelluloses from sugarcane bagasse (absolute yield increase mediated by ultrasound 1.6%) (Sun *et al.*, 2004b), 51% of the original hemicelluloses from corn bran (absolute yield increase mediated by ultrasound 12%) (Ebringerova & Hromadkova, 2002) and 40% of the original hemicelluloses from corn cobs (absolute yield increase mediated by ultrasound 14.1%) (Ebringerova *et al.*, 1998). 41.4% of the

hemicelluloses were extracted from wheat straw (absolute yield increase mediated by ultrasound 1.8%) (Sun & Tomkinson, 2002; Sun *et al.*, 2004a) and 16.5% of the total hemicelluloses from buckwheat hulls could be isolated (absolute yield increase mediated by ultrasound 5.3%; Hromadkova & Ebringerova, 2003). A negative effect of ultrasonication was seen on the efficiency of polysaccharide extraction from valerian roots resulting in a lower yield in the ultrasonication experiments (Hromadkova *et al.*, 2002).

The positive effect of ultrasonication on the extraction of biomolecules from plant raw material is explained by the mechanical disruption of the cell wall–lignin–cellulose–hemicellulose matrix and breakage of some benzyl-ether bonds between lignin and polysaccharides, purported to be triggered by radicals generated from water (Doner & Hicks, 1997). Thus, the dissolution into the alkaline extraction solvent should be facilitated. Consequently, short-time application of ultrasound on alkaline suspensions of wheat bran can reduce the time needed for dissolution of a fraction of AX considerably, a minimum of environmentally friendly chemicals are used and highly purified products can be obtained in a single-step isolation procedure. The chemical compositions of either AX are practically the same but ultrasonication leads to some depolymerization increasing the polydispersity of the isolated products. The yields can only be increased marginally as is in accordance with several other reports trying to enhance extractability of hemicelluloses from plant material, but the extraction efficiency was improved.

Finally, considering on the one hand the low increase in total yield ultrasonication provides and on the other hand the necessity to invest in technical equipment, the ultrasound-assisted extraction of hemicelluloses seems not to be profitable. To the best of the authors knowledge there are still no reports published on a technical-scale extraction procedure for these biopolymers.

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