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Extractability and chemical and enzymic degradation of psyllium (*Plantago ovata* Forsk) seed husk arabinoxylans

Valerie Van Craeyveld, Jan A. Delcour, Christophe M. Courtin *

Laboratory of Food Chemistry and Biochemistry, Leuven Food Science and Nutrition Research Centre (LFoRCe), Katholieke Universiteit Leuven, Kasteelpark Arenberg 20 bus 2463, B-3001 Leuven, Belgium

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ABSTRACT

To assess the potential of arabinoxylan (AX)-rich psyllium (*Plantago ovata* Forsk) seed husk (PSH) as a source for production of arabinoxylo-oligosaccharides (AXOS), the parameters determining PSH AX extraction yield and the chemical and enzymic hydrolysis of PSH AX were investigated. The seed husk material had a high content of AX (62.5%) with an arabinose to xylose ratio of 0.41. The water extractability of PSH AX was affected by suspension concentration rather than by temperature. Maximally, 27% of all AX could be extracted, even when using very dilute suspensions (0.1% w/v). When subjected to alkaline conditions, a pH of at least 12 was needed to significantly increase extractability (up to 77% of all AX). The extractability-increasing effect of alkali was, however, reversible, as extractability decreased again when lowering the pH. Acid hydrolysis at high temperature drastically increased AX extraction yields (up to 97% of all AX), released monomeric arabinose (up to 38%), and lowered the average degree of polymerisation (DP_{avg}) of the AX (down to 31). The presence of substituents (arabinose, xylose, rhamnose and galacturonic acid) on the xylan backbone was an important factor limiting degradation by xylanases. Enzyme preparations containing substituent-removing enzymes were far better for enhancing the extractability of a large portion of the AX population (up to 82%) and for degrading the extracted fragments (down to DP_{avg} 14) than preparations lacking such activities. The above results show that PSH is a good source for the production of AXOS, since both chemical and enzymic treatments significantly increase the extractability of PSH AX and convert PSH AX molecules into small fragments.

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1. Introduction

Psyllium (*Plantago ovata* Forsk) is an annual plant, grown primarily in India (Dhar, Kaul, Sareen, & Koul, 2005; Iqbal, 1993). It has a long history of use in both conventional and traditional systems of medicine. Psyllium seed husk (PSH) is mechanically removed from the seed (Anderson, Allgood, Turner, Oeltgen, & Daggy, 1999). It is a well-known laxative (Marlett & Fischer, 2002; Marlett, Kajs, & Fischer, 2000) and lowers serum total and LDL cholesterol concentrations in hypercholesterolemic adults (Anderson et al., 1999; Arjmandi, Sohn, Juma, Murthy, & Daggy, 1997; Olson et al., 1997), as well as all-day and post-lunch postprandial glucose concentrations in subjects with type 2 diabetes (Anderson et al., 1999). It is not clear to what extent these properties can be ascribed to the most abundant polysaccharide in PSH, i.e. a complex heteroxylan with, as main monosaccharides, arabinose and xylose, and which is further referred to as arabinoxylan (AX) (Fischer et al., 2004; Kennedy, Sandhu, & Southgate, 1979; Marlett & Fischer, 2002, 2003), or possible degradation products.

PSH AX are highly branched non-starch polysaccharides with a main chain of densely substituted β-(1,4)-linked xylopyranose residues. Single arabinofuranose and xylopyranose residues, or short side chains consisting of these monosaccharides, are attached at positions 2 and/or 3 of the main chain xylopyranose residues (Edwards, Chaplin, Blackwood, & Dettmar, 2003; Fischer et al., 2004). In addition, PSH AX contain additional residues such as rhamnose (3–5%) and galacturonic acid (5–8%) (Edwards et al., 2003; Kennedy et al., 1979; Marlett & Fischer, 2002). A schematic representation of the PSH AX structure is given in Fig. 1. Due to their complex structure, PSH AX resist enzymic degradation (Edwards et al., 2003; Fischer et al., 2004). Fischer et al. (2004) suggested that the resistance of the polysaccharide to the action of glycoside hydrolases is due to an unusual linkage pattern in the molecule and/or the high density of its branching. Only 9% of all PSH AX was found to be water-extractable (Marlett & Fischer, 2002). Unlike wheat bran AX, PSH AX form strong gels in water (Gelissen, Brodie, & Eastwood, 1994; Sandhu, Hudson, & Kennedy, 1981). The gel dissociates almost completely in the presence of sodium

* Corresponding author. Tel.: +32 16 321917; fax: +32 16 321997.
E-mail address: christophe.courtin@biw.kuleuven.be (C.M. Courtin).

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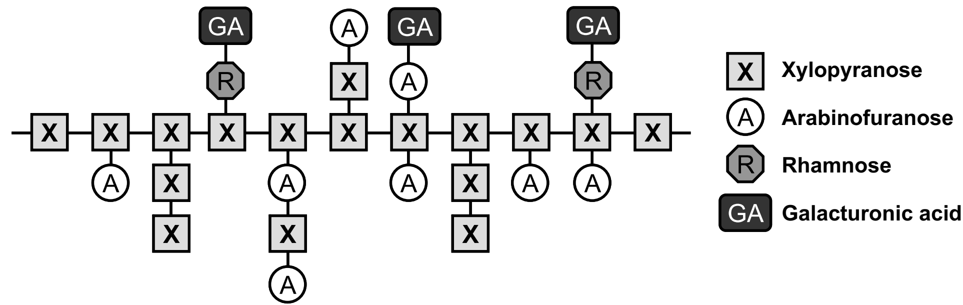

Fig. 1. Schematic representation of part of the PSH AX structure (based on results from Edwards et al. (2003) and Fischer et al. (2004)).

hydroxide (Sandhu et al., 1981). PSH is used as a dietary fiber-rich ingredient (Edwards et al., 2003). One of its applications is in treatment of constipation (Edwards et al., 2003; Gelissen et al., 1994; Sandhu et al., 1981).

Production of AXOS from PSH AX requires knowledge on the optimal AX extraction conditions and the degradation of PSH AX into smaller fragments. However, very little is known of the parameters determining the extractability of PSH AX, or the chemical and enzymic degradation of these polysaccharides. The aim of this study was therefore to investigate the extractability of PSH AX as a function of temperature, pH and suspension concentration. Furthermore, chemical and enzymic treatments for degradation of the PSH AX into arabinoxylo-oligosaccharides (AXOS) were studied.

2. Materials and methods

2.1. Materials

PSH (85% husk material) was obtained from Biofiber–Damino (Gesten, Denmark). Chemicals and reagents were purchased from Sigma–Aldrich (Bornem, Belgium) and were of at least analytical grade. Azurine-cross-linked arabinoxylan (AZCL-AX) tablets were purchased from Megazyme (Bray, Ireland).

All crude enzyme preparations used were commercially available. They all contained xylanase as main or side activity. Shearzyme 500L, containing only an *Aspergillus aculeatus* glycoside hydrolase family (GH) 10 xylanase (further referred to as Shearzyme), Pectinex 3XL, a pectolytic *Aspergillus niger* enzyme preparation containing mainly pectin transeliminase, polygalacturonase and pectin esterase, and small levels of hemicellulase and cellulase activities (further referred to as Pectinex) and Pulpzyme HC, a *Bacillus* sp. xylanase preparation (further referred to as Pulpzyme), were from Novozymes (Bagsvaerd, Denmark). Grindamyl H640, containing mainly the *Bacillus subtilis* GH 11 xylanase, but also showing β-glucanase activity (further referred to as Grindamyl), was from Danisco (Brabrand, Denmark). Ecopulp TX-200A, a fungal, thermostable xylanase preparation (further referred to as Ecopulp) was obtained from AB Enzymes (Darmstadt, Germany). Crystalzyme APXL Concentrate, an *A. niger* enzyme preparation, including pectin esterase, pectin depolymerase, cellulase, hemicellulase and arabinase (further referred to as Crystalzyme), was from Valley Research (Angers, France).

2.2. Standard analyses

Moisture and ash contents of PSH were analysed in triplicate according to AACC methods 44–19 and 08–01, respectively (Anonymous, 2000). Protein content was determined in duplicate, using the Dumas combustion method, an adaptation of the AOAC official method (AOAC, 1995) to an automated Dumas protein analysis system (EAS VarioMax N/CN, Elt, Gouda, The Netherlands), using 6.25 as a factor for conversion of nitrogen to protein contents.

2.3. Analysis of total non-cellulosic, reducing end and monomeric monosaccharide contents

Total non-cellulosic monosaccharide content was determined, in triplicate, by gas chromatography of alditol acetates obtained after acid hydrolysis and reduction and acetylation of the resulting monosaccharides. PSH (approximately 15 mg accurately weighed) was hydrolysed with 2.0 M trifluoroacetic acid (TFA, 5.0 ml) at 110 °C for 60 min. Hydrolysis of AX-containing supernatants (2.5 ml) was with 4.0 M TFA (2.5 ml) at 110 °C for 60 min. The resulting monosaccharides were reduced with sodium borohydride and converted to alditol acetates (Delcour, Van Win, & Grobet, 1999), of which 1.0 µl was injected (split ratio 1:20) and separated on a Supelco SP-2380 column (30 m × 0.32 mm ID, 0.2 mm film thickness) (Supelco, Bellefonte, PA, USA) with helium as carrier gas in an Agilent 6890 series chromatograph (Agilent, Wilmington, DE, USA) equipped with an autosampler and flame-ionisation detector. Separation was at 225 °C with injection and detection temperatures at 270 °C. β-D-Allose was used as internal standard and calibration samples, containing the expected monosaccharides, were included with each set of samples. The AX content was defined as the sum of the contents of the monosaccharides arabinose and xylose times 0.88 to correct for hydration water (Swennen, Courtin, Lindemans, & Delcour, 2006). The content of complex PSH AX, taking into account also rhamnose residues and uronic acids, was calculated using the following formula, with the factors 0.88, 0.89 and 0.92 to correct for hydration water

$$\text{Complex AX level} = 0.88 \times (\%\ \text{arabinose} + \%\ \text{xylose}) + 0.89 \times (\%\ \text{rhamnose}) + 0.92 \times (\%\ \text{uronic acids})$$

Analysis of the reducing end monosaccharide contents was very similar to that of total monosaccharides. However, samples (2.5 ml) were reduced prior to hydrolysis and acetylation to alditol acetates (Courtin, Van den Broeck, & Delcour, 2000). Separation was at 210 °C. Analysis of monomeric monosaccharide contents was similar to that of reducing end monosaccharides, except that, after reduction, samples were immediately acetylated without hydrolysing them first. Combination of data for total, reducing end and monomeric monosaccharides allowed calculation of the arabinose to xylose ratio (A/X ratio) (Swennen et al., 2006), which is an indication of the degree of substitution, and the average degree of polymerisation (DP_{avg}) of the AX fragments (Swennen et al., 2006)

$$\text{A/X ratio} = \frac{\text{total arabinose level} - \text{monomeric arabinose level}}{\text{total xylose level} - \text{monomeric xylose level}}$$

$$DP_{avg} = \frac{\text{total xylose level} - \text{monomeric xylose level}}{\text{reducing end xylose level} - \text{monomeric xylose level}}$$

The coefficient of variation for the determination of total non-cellulosic, reducing end and monomeric monosaccharide contents was less than 5%.

2.4. Determination of uronic acid contents

Uronic acid content in PSH was determined, in triplicate, using the colorimetric method of Blumenkrantz and Asboe-Hansen (1973). Samples (100 mg) were suspended in 6.0 ml of 13.0 M sulphuric acid and placed in an ice bath for 180 min to allow hydrolysis (Ahmed & Labavitch, 1977). After careful addition of deionised water (20.0 ml), samples were filtered and diluted to 100 ml. Sodium tetraborate (12.5 mM in concentrated sulphuric acid; 3.6 ml) was slowly added to 600 µl of hydrolysate. Samples were mixed and placed in a water bath at 100 °C for 5 min. After cooling and filtration, 60 µl of meta-hydroxydiphenyl reagent, prepared as outlined by Blumenkrantz and Asboe-Hansen (1973), were added. The samples were mixed exactly for 1 min and, after exactly 1 min, extinction measurements were made at 520 nm with an Ultraspec III UV/Visible spectrophotometer (Pharmacia Biotech, Uppsala, Sweden). Glucuronic acid solutions (0–100 µg/ml) were used as a standard.

2.5. Extractability of PSH AX under different suspension concentration, temperature and pH conditions

PSH was suspended in water at different concentrations (0.1%, 0.2%, 0.4%, 1.0% and 2.0% w/v), mixed and shaken for 60 min at room temperature (RT). After centrifugation (24,000*g*; 10 min; RT), supernatants were analysed for total monosaccharide contents. Since this experiment showed that suspension concentration was an important factor for determining the yields of extracted PSH AX, for each following experiment, the most suitable concentration was chosen.

PSH suspensions in water (1.0% w/v) were mixed well and left at the right temperature (6 °C, 22 °C, 30 °C, 40 °C, 50 °C, 60 °C and 70 °C) for 60 min, with intermediate shaking. After cooling and centrifugation (24,000*g*; 10 min; RT), supernatants were filtered and analysed for total, reducing end and monomeric monosaccharide contents.

PSH was suspended (1.0% w/v) in sodium hydroxide solutions of concentrations ranging from 0 to 0.2 M. After shaking (60 min; RT), the pH of the suspensions was determined and, after subsequent centrifugation (24,000*g*; 10 min; RT), supernatants were analysed for total, reducing end and monomeric monosaccharide contents.

All measurements were done at least in duplicate.

2.6. Chemical degradation of PSH AX: Mild acid hydrolysis

PSH was suspended in deionised water (0.2% w/v). The pH of the suspensions (typically 6.7) was adjusted with HCl (0.2 M) to 2.8, 3.0, 3.3, 3.6, 4.0 and 6.0. Samples were incubated for 24 h at different temperatures (60 °C, 70 °C, 80 °C and 90 °C). After neutralisation (0.1 M NaOH) and centrifugation (24,000*g*; 10 min; RT), supernatants were analysed for total, reducing end and monomeric monosaccharide contents. Measurements were performed in duplicate.

2.7. Enzymic degradation of PSH AX

PSH was incubated with different hydrolytic enzyme preparations. To allow all enzymes to work under optimal temperature and pH conditions, different buffers were used. A control sample was analysed for each temperature-pH condition. Incubation with Shearzyme and Grindamyl was in 25 mM NaOAc (pH 5.0) at 40 °C. Incubation with Ecopulp and Pulpzyme was in 25 mM Tris–HCl (pH 7.0) at 70 °C. PSH was incubated with Pectinex in 25 mM NaOAc (pH 4.7) at 55 °C and with Crystalzyme in 25 mM NaOAc (pH 5.0) at 55 °C.

Under these conditions, xylanase activity was determined with insoluble AZCL-AX, as described in Megazyme Data Sheet 9/95. All enzyme preparations were diluted in the appropriate buffer containing bovine serum albumin (0.5 mg/ml) and then equilibrated for 10 min before addition of an AZCL-AX tablet. After 10 min of incubation, the reaction was stopped by adding 10.0 ml of 1.0% (w/v) Tris solution, followed by vigorous vortex stirring. After 10 min at RT, the solutions were filtered, and the extinction values at 590 nm (E_{590}) [Ultraspec III UV/vis spectrophotometer (Pharmacia Biotech)] were measured against a control, prepared by incubating the diluted enzyme preparations without the AZCL-AX tablet. Activities were expressed in xylanase units (U_x). One U_x is the amount of enzyme required to yield an E_{590} of 1.0 per 10 min under the conditions of the assay (above-mentioned temperature and buffer).

To investigate the potential of xylanases to hydrolyse PSH AX, enzyme dosages were standardised for xylanase activity using the above assay. For most of the enzyme preparations used, and particularly for Pectinex and Crystalzyme, which have small levels of xylanases, this implies that enzymes other than xylanases were also present in the incubation experiment. PSH was suspended in the indicated buffers (0.2% w/v) and enzyme preparations (3 U_x/mg PSH) were added after pre-incubation at the appropriate (*cfr. supra*) incubation temperature (10 min). The suspensions were left at these temperatures for 16 h and then cooled and centrifuged (24,000*g*; 10 min; RT). After filtration and inactivation (100 °C; 30 min) of the supernatants, analysis of saccharides was done as outlined above. Measurements were performed in duplicate.

3. Results

3.1. Composition of PSH

The largest part of PSH consisted of carbohydrates (70.7%), while ash and protein contents accounted for only 3.4% and 7.1%, respectively (Table 1). The most abundant monosaccharide moieties were arabinose (20.7%) and xylose (50.3%). Minor monosaccharides were galactose (4.8%), glucose (2.0%), mannose (1.1%) and rhamnose (1.1%). The uronic acid content was 5.0%. These results agree well with the findings of other authors, who reported PSH to contain 17–20% arabinose, 50–54% xylose and 5–8% galacturonic acid (Kennedy et al., 1979; Marlett & Fischer, 2002). The

Table 1
Chemical composition of psyllium seed husk

	Psyllium seed husk
Ash (% dm)	3.4 ± 0.1
Protein (% dm)	7.1 ± 0.1
Total non-cellulosic carbohydrate (% dm)[a]	**70.7 ± 0.5**
Arabinose (% dm)	20.7 ± 0.1
Xylose (% dm)	50.3 ± 0.4
AX (% dm)[b]	**62.5 ± 0.4**
A/X ratio[c]	0.41 ± 0.01
Mannose (% dm)	1.1 ± 0.1
Galactose (% dm)	4.8 ± 0.1
Glucose (% dm)	2.0 ± 0.1
Rhamnose (% dm)	1.1 ± 0.1
Uronic acid (% dm)	5.0 ± 0.5

Values are expressed as means ± standard deviations.
[a] Total non-cellulosic carbohydrate = 0.88 × (% arabinose + % xylose) + 0.89 × (% rhamnose) + 0.9 × (% mannose + % galactose + % glucose).
[b] AX = 0.88 × (% arabinose + % xylose).
[c] Arabinose to xylose ratio.

sum of non-cellulosic carbohydrate, galacturonic acid, protein and ash accounted for over 85% of the total dry matter.

Although the precise structure of PSH AX is still not fully understood, most authors acknowledge the complex character of these molecules, which consist of xylose (in the main chain, as well as in side chains) and arabinose residues, and, in addition, rhamnose and galacturonic acid residues (Edwards et al., 2003; Fischer et al., 2004; Kennedy et al., 1979; Marlett & Fischer, 2002). Taking into account all of these substituents, the level of complex AX was calculated as 68.0%. However, since arabinose and xylose are the most abundant components of AX, and uronic acid measurement was not carried out for all samples, from here on, AX content is calculated as 0.88 times the sum of only arabinose and xylose. In that way, the AX level was calculated as 62.5%. The A/X ratio was 0.41.

The presence of uronic acids implies the presence of potential charges in the AX structure, meaning that the polysaccharide is a polyelectrolyte.

3.2. Influence of suspension concentration on extractability of PSH AX

Even low PSH concentrations resulted in suspensions that were difficult to handle and process, due to the gel-forming capacity of PSH. Hence, strong dilution was necessary to make processing feasible. We therefore further investigated the effect of suspension concentration on the extractability of PSH AX (Fig. 2).

In control experiments, the highest suspension concentration used (2.0%) extracted only 3% of all PSH AX. An estimated 75% of the suspension water was retained in the gel network. Under the experimental conditions, only a small portion of supernatant could be separated from the gel, and hence, only a small part of the AX population could be extracted. The use of more diluted suspensions increased the level of extractable AX. The lowest suspension concentration (0.1%) resulted in the extraction of 27% of all PSH AX.

3.3. Influence of temperature on extractability of PSH AX

To investigate the effect of temperature on the extractability of PSH AX, aqueous extracts of 1.0% (w/v) suspensions were prepared at different temperatures. At 6 °C, only 8% of the AX was extractable. Their A/X ratio and DP_{avg} were 0.19 and 340, respectively (Table 2). These data correspond well with the findings of Marlett and Fischer (2002), who isolated (0.5% w/v, RT) a viscous, water-soluble PSH fraction containing 9% of all PSH AX and having an A/X ratio of 0.20 (Marlett & Fischer, 2002).

With temperatures increasing up to 50 °C, both the levels of extracted AX and the DP_{avg} of the AX fragments increased only to a limited extent. Maximal values were obtained at 50 °C, at which 16% of the AX was extracted and the DP_{avg} was 400. Irrespective of the temperature applied, the A/X ratio of the extracted fragments remained constant (0.19). The higher extraction yields of AX with increasing temperature were consistent with earlier results obtained for wheat bran AX (Bataillon, Mathaly, Cardinali, & Duchiron, 1998), rye flour AX (Cyran, Courtin, & Delcour, 2003) and corncob xylan (Wang & Zhang, 2006).

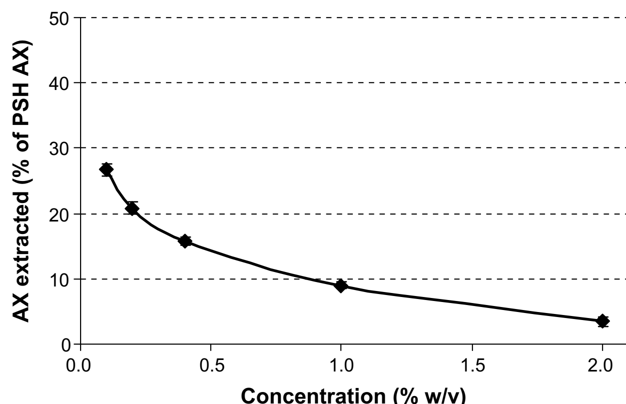

Fig. 2. Effect of suspension concentration on the extractability of PSH AX at room temperature (extraction time = 60 min). ♦ AX extracted in supernatant, expressed as % of total PSH AX. Error bars indicate standard deviations.

Table 2
Effect of temperature and pH on the extractability of PSH AX (extraction time = 60 min)

	AX extracted[a] (% of PSH AX)	DP_{avg}[b]	A/X ratio[c]
Temperature (°C)			
6	7.9 ± 0.4	344 ± 7	0.19 ± 0.01
22	10.1 ± 0.1	349 ± 23	0.19 ± 0.01
30	14.2 ± 0.1	386 ± 6	0.19 ± 0.01
40	15.1 ± 0.1	380 ± 39	0.19 ± 0.01
50	15.6 ± 0.1	402 ± 33	0.19 ± 0.01
60	12.7 ± 0.1	391 ± 18	0.18 ± 0.01
70	10.2 ± 0.1	319 ± 7	0.19 ± 0.01
pH			
7.1	9.4 ± 0.3	430 ± 19	0.19 ± 0.01
7.6	9.5 ± 0.1	415 ± 16	0.19 ± 0.01
9.5	9.3 ± 0.1	405 ± 17	0.19 ± 0.01
10.4	7.8 ± 0.1	330 ± 11	0.19 ± 0.01
11.4	7.5 ± 0.1	324 ± 6	0.20 ± 0.01
12.0	7.8 ± 0.5	417 ± 26	0.20 ± 0.01
12.3	18.6 ± 0.5	725 ± 36	0.21 ± 0.01
12.7	61.1 ± 3.1	996 ± 2	0.26 ± 0.01
13.0	77.3 ± 7.1	1038 ± 27	0.26 ± 0.01

Values are expressed as means ± standard deviations.
[a] Expressed as % of total PSH AX.
[b] Average degree of polymerisation of the extracted fragments, corrected for the presence of monomeric monosaccharides.
[c] Arabinose to xylose ratio of the extracted fragments, corrected for the presence of monomeric monosaccharides.

Further increase of the temperature (to above 50 °C) resulted in decreasing levels of extractable AX and decreasing DP_{avg} values. At 70 °C, only 10% of the AX was extracted and the DP_{avg} was 320. At this temperature, only a small portion of supernatant could be separated from the gel. After shaking the PSH suspensions at 80 °C or 90 °C, no supernatant could be separated from the gel anymore. These findings may indicate an enhanced water-holding capacity at higher temperatures.

With increasing temperature, the levels of extractable galactose changed in the same way as the AX levels, while the levels of extractable glucose and mannose continuously decreased with increasing temperature (results not shown). Whether this indicates that galactose units are associated with the AX molecules, whereas glucose and mannose are not, is unclear at present.

3.4. Influence of pH on extractability of PSH AX

PSH was incubated in sodium hydroxide solutions of different concentrations to investigate the impact of alkaline pH on extractability of PSH AX. Table 2 shows that, at pH 6.7 (deionised water), only 9% of all PSH AX was extracted (1.0% w/v), while the A/X ratio and the DP_{avg} were 0.19 and 430, respectively. Below pH 12.0, the levels of extracted AX and both the A/X ratio and DP_{avg} of the extracted fragments remained more or less constant. At pH values exceeding 12, the levels of extracted AX increased with pH. At the same time, both the A/X ratios and DP_{avg} of the extracted fragments increased, indicating extraction of material of different structures. The largest level of extracted AX (77% of PSH AX) was obtained at pH 13.0. The extracted fragments were clearly more substituted (A/X ratio = 0.26) and they were larger (DP_{avg} > 1000) than the fragments in solution at pH 7.0. The profiles of extractable

galactose, glucose and mannose levels, as a function of pH, were similar to those of the extractable AX levels. The highest levels of extracted galactose, glucose and mannose (69%, 31% and 22% of their total levels, respectively) were obtained at pH 13.0 (results not shown). The extraction of PSH galactose and AX in similar high amounts (69% and 77%, respectively) and of PSH glucose and mannose in similar lower amounts (31% and 22%, respectively) by the addition of alkali may again indicate that galactose units are associated with the AX molecules, whereas glucose and mannose are not.

Alkaline media impact PSH AX in a reversible way. PSH AX gel when the alkaline solution is neutralised. This behaviour is somewhat comparable to that of hemicelluloses of type A, usually consisting of xylans with some uronic acid residues, although such hemicelluloses do not gel after neutralisation but precipitate instead (Southgate, 1991). In contrast to this, solubilisation of cereal AX in alkaline media is irreversible. Most of the solubilised AX molecules remain in solution when the pH is lowered (Cyran, Courtin, & Delcour, 2004; Trogh, Croes, Courtin, & Delcour, 2005; Viëtor, Angelino, & Voragen, 1992), which is typical for hemicelluloses of type B (Southgate, 1991).

3.5. Chemical degradation of PSH AX: Mild acid hydrolysis

3.5.1. Mild acid hydrolysis at 60 °C

PSH was subjected to mild acid hydrolysis (0.2% w/v). The data obtained following acid hydrolysis at 60 °C (Fig. 3) demonstrate that lowering the pH increased extraction yields of both xylose and arabinose. When the pH was decreased from 6.0 to 2.8, the total levels of xylose and arabinose in the supernatant (expressed as % of the total levels of xylose or arabinose, respectively, in PSH) increased from 34% to 93% (xylose) (Fig. 3a) and from 17% to 64% (arabinose) (Fig. 3b). The difference between maximally extractable xylose and arabinose levels (93% and 64%, respectively) did suggest that PSH contains a fraction, consisting mainly of arabinose residues, which resists acid hydrolysis. This acid resistant PSH fraction may be the PSH fraction isolated by Marlett & Fischer, 2002 as the residue after alkaline extraction (0.5% w/v in 0.2 M KOH, RT) and described as consisting primarily of arabinose residues (Marlett & Fischer, 2002).

Only negligible levels of the xylose and arabinose in the supernatants were found as monomeric residues. After incubation at pH 2.8, the levels of monomeric xylose (Fig. 3c) and arabinose (Fig. 3d) (expressed as % on the total level of xylose or arabinose, respectively, in PSH) were only 0.1% and 2.2%, respectively. Increasing release of monomeric arabinose with decreasing pH was already reported for corn hull AX (Zhang, Zhang, & Whistler, 2003). Since arabinofuranosyl groups are particularly sensitive to hydrolysis under acidic conditions (Whistler & Corbett, 1955; Zhang et al., 2003), they were preferentially released.

The total level of galactose in the supernatant (expressed as % of the total galactose level in PSH) also increased with decreasing pH. At pH 2.8, twice as much galactose was extracted (44%) than at pH 6.0 (22%). In contrast, the influence of acid on the extractabilities of glucose and mannose was less pronounced. Decreasing the pH from 6.0 to 2.8 increased the extractability of glucose from 66% to 75% and that of mannose from 25% to 36% (expressed as % of the total levels of glucose or mannose, respectively, in PSH) (results not shown).

The difference between the total and the monomeric monosaccharide levels provided information of the structure of the AX fragments in the supernatants. With decreasing pH, the A/X ratio of these AX fragments (Fig. 3e) increased from 0.20 (pH 6.0) to 0.27 (pH 2.8). This suggested that more highly substituted fragments were solubilised when the hydrolysis conditions became more drastic (i.e. at lower pH). In contrast to the A/X ratio, the DP_{avg} of the solubilised fragments remained more or less constant with decreasing pH (Fig. 3f). Thus, no significant hydrolysis of the xylan backbone occurred at 60 °C. Zhang et al. (2003), in a similar way, observed that AX molecular weight did not change at either pH 2.0 or 3.0 when incubating corn hull AX for 180 min at 37 °C.

3.5.2. Mild acid hydrolysis at 70 °C and higher

Acid hydrolysis at 70, 80 or 90 °C (0.2% w/v) yielded trends comparable to those observed upon acid hydrolysis at 60 °C (Fig. 3). With decreasing pH, yields of both soluble xylose and arabinose increased, as did the levels of monomeric xylose and arabinose residues in the supernatant (Fig. 3a–d). At each pH value, the levels of both total and monomeric soluble saccharides increased with temperature. Thus, higher incubation temperatures mainly strengthened the effect of the acidic conditions. The most drastic condition (pH 2.8; 90 °C; 24 h) released almost all PSH xylose (97%) and arabinose (96%) in the supernatant. Only part of it was released as monomeric monosaccharides, namely 8% of all PSH xylose and 38% of all PSH arabinose.

While the total extraction yield of galactose clearly increased with increasing temperature (up to 90% at 90 °C and pH 2.8), the extractabilities of glucose and mannose were less dependent on temperature.

The changes in the A/X ratio of the AX fragments, as a function of pH, were not similar for all temperatures. The A/X ratio of AX fragments solubilised at 60 °C and 70 °C continuously increased with decreasing pH (Fig. 3e). However, for incubations at 80 °C and 90 °C, the A/X ratio of the fragments first increased with decreasing pH, and then remained constant or even decreased again. This phenomenon may be the result of two activities. The solubilisation of more and/or more substituted fragments may have resulted in increasing A/X ratio values, while the release of arabinose substituents from the xylan backbone (mainly at the higher temperatures) may have decreased A/X ratio values.

The DP_{avg} values of the AX fragments in the supernatant (Fig. 3f) also showed different trends for different incubation temperatures. At 60 °C, the DP_{avg} remained more or less constant, irrespective of the pH at which the hydrolysis was executed. However, incubation at 70 °C, 80 °C and 90 °C resulted in DP_{avg} values which decreased with decreasing pH. Higher temperatures gave rise to an increased degradation of the xylan backbone. Thus, bonds between xylose units can be hydrolysed in sufficiently acidic circumstances, although they are less sensitive to acid than are those involving arabinofuranosyl residues. The lowest DP_{avg} values (31) were obtained at the lowest pH (2.8) and the highest temperature (90 °C). In summary, both low pH values and high temperatures were needed to depolymerise or degrade the xylose backbone into smaller fragments with smaller DP_{avg} values.

3.6. Enzymic degradation of PSH AX

PSH was incubated with a set of commercially available enzyme preparations. Different incubation conditions allowed the different preparations to be active at their optimal temperature and pH. The effect of the enzymes on the PSH AX structure was investigated by monitoring the levels of AX in the supernatant after incubation (expressed as % of total PSH AX). This provided information on the solubilising capacity of the enzyme. The DP_{avg} of the solubilised AX fragments, which represents the capacity of the enzyme to depolymerise the xylose backbone of PSH AX, was evaluated as well. As mentioned above, temperature has a strong influence on the extent of solubilisation and depolymerisation when working with strongly diluted suspensions (0.2% w/v). Therefore, results will be discussed separately for each incubation temperature.

PSH was incubated with Shearzyme and Grindamyl at 40 °C (Table 3). Shearzyme had only limited solubilising capacity, as the

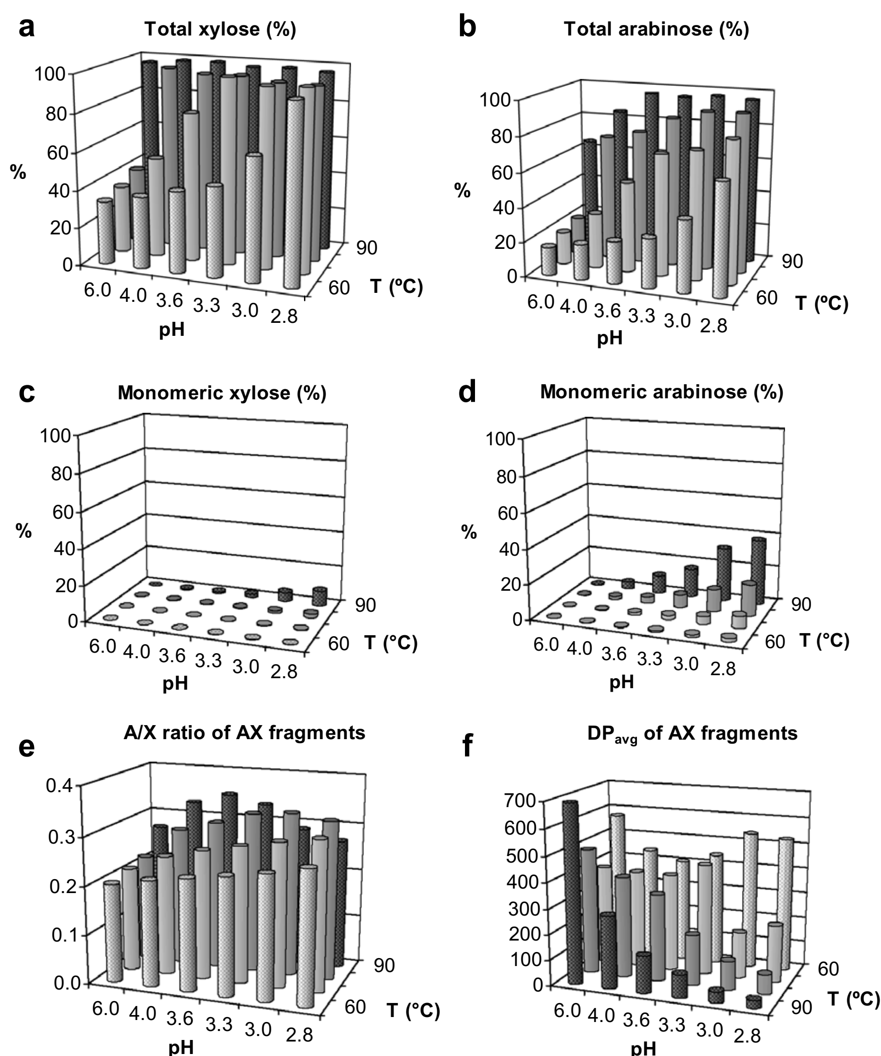

Fig. 3. Effect of acid hydrolysis (during 24 h) on PSH AX. Incubation was at different pH values (6.0, 4.0, 3.6, 3.3, 3.0 and 2.8) and different temperatures (60 °C, 70 °C, 80 °C and 90 °C). Total and monomeric xylose and arabinose contents in supernatants were expressed as % of total xylose or arabinose levels in PSH. Both the A/X ratio and DP_{avg} of the AX fragments were corrected for the presence of monomeric monosaccharides. (a) Total extracted xylose, (b) total extracted arabinose, (c) monomeric xylose in supernatant, (d) monomeric arabinose in supernatant, (e) A/X ratio of extracted AX fragments and (f) DP_{avg} of extracted AX fragments.

Table 3
AX extracted as a result of incubation of PSH suspensions (0.2% w/v) with different enzyme preparations (3 U_x/mg PSH), carried out at optimal temperature and pH conditions for 16 h

		AX extracted[a] (% of PSH AX)	DP_{avg}[b]	A/X[c]
40 °C	Control	30.0 ± 0.2	320 ± 3	0.21 ± 0.01
	Shearzyme	40.8 ± 0.2	120 ± 7	0.21 ± 0.01
	Grindamyl	30.6 ± 0.5	290 ± 51	0.21 ± 0.01
55 °C	Control	33.7 ± 1.5	450 ± 8	0.21 ± 0.01
	Pectinex	74.8 ± 0.0	21 ± 6	0.08 ± 0.01
	Crystalzyme	81.9 ± 9.3	14 ± 1	0.08 ± 0.01
70 °C	Control	35.9 ± 4.2	580 ± 1	0.22 ± 0.01
	Ecopulp	38.1 ± 0.1	590 ± 25	0.22 ± 0.01
	Pulpzyme	37.3 ± 1.6	520 ± 22	0.22 ± 0.01

Values are expressed as means ± standard deviations.
[a] Expressed as % of total PSH AX.
[b] Average degree of polymerisation of the extracted fragments, corrected for the presence of monomeric monosaccharides.
[c] Arabinose to xylose ratio of the extracted fragments, corrected for the presence of monomeric monosaccharides.

treatment added only 11% AX in solution to the level (30%) already solubilised in the control sample. Grindamyl brought hardly more AX into solution. Both enzyme preparations were only able to depolymerise the xylan backbone to a limited extent. After incubation with Shearzyme and Grindamyl, AX fragments showed DP_{avg} values of 120 and 290, respectively. These values were lower than the DP_{avg} of the fragments in the control sample (320). Treatment with both enzyme preparations yielded AX fragments with A/X ratios comparable to those of the control sample (0.21).

Treatment of PSH with Ecopulp and Pulpzyme at 70 °C resulted in hardly more AX (38% and 37% for Ecopulp and Pulpzyme, respectively) in the supernatant than for the control sample (36%). The DP_{avg} values after incubation were similar (Ecopulp) to those of the control (580) or just slightly lower (Pulpzyme, 520) (Table 3). The A/X ratio of the AX fragments extracted after incubation with both enzyme preparations was similar to that of the control (0.22).

PSH was finally incubated with Crystalzyme and Pectinex (both at 55 °C). These preparations contained different types of enzyme activities (Table 3). Treatment with Crystalzyme, containing pectinase, arabinase, xylanase, xyloglucanase and rhamnogalacturonase activities, yielded 82% AX in solution compared to 34% for the control. This preparation depolymerised the fragments to a DP_{avg} of 14

Table 4
Release of monomeric monosaccharides upon incubation of PSH (0.2% w/v) with Crystalzyme and Pectinex (3 U_x/mg PSH), carried out at optimal temperature (55 °C) and pH (5.0 and 4.7 for Crystalzyme and Pectinex, respectively) for 16 h

	Crystalzyme	Pectinex
Arabinose (%)[a]	43.3 ± 4.3	40.7 ± 0.5
Xylose (%)	26.0 ± 2.7	21.3 ± 0.1
Galactose (%)	17.5 ± 0.7	13.6 ± 0.2
Rhamnose (%)	10.1 ± 0.7	35.9 ± 2.2

Values are expressed as means ± standard deviations.
[a] Monomeric monosaccharide levels are expressed as % of the total level of the particular monosaccharide in PSH.

(control: 450). Pectinex, comprised of pectinase, arabinofuranosidase and xylanase activities, brought 75% of the AX into solution (control: 34%) and degraded the fragments to a DP_{avg} of 21 (control: 450). In contrast to the earlier-mentioned enzyme preparations containing mainly xylanases, Crystalzyme and Pectinex produced AX fragments with a much lower A/X ratio (0.08) than the control (0.21). This decrease in average A/X ratio could be explained by the release of large levels of monomeric monosaccharides, especially arabinose residues, by these enzyme preparations (Table 4). Crystalzyme released 43% of all PSH arabinose and 26% of all PSH xylose as monomeric residues. For Pectinex, these levels were 41% and 21% for arabinose and xylose, respectively. Besides arabinose and xylose, other monosaccharides, such as galactose and rhamnose, were also detected as monomeric residues in the extracts. While Crystalzyme released some more monomeric galactose (18% of all PSH galactose) than did Pectinex (14%), monomeric rhamnose was released much more by Pectinex (36% of all PSH rhamnose) than by Crystalzyme (10%). Release of monomeric monosaccharides by the other enzyme preparations was negligible.

4. Discussion

PSH was found to consist mainly of carbohydrates, with xylose and arabinose being the most abundant building blocks. The presence of rhamnose and uronic acids was also detected. These findings correspond well with literature data, as PSH is reported to contain a high AX level (Kennedy et al., 1979; Marlett and Fischer, 2002), and structural features of PSH AX are a xylose backbone and side chains containing arabinose and xylose (Edwards et al., 2003; Fischer et al., 2004; Kennedy et al., 1979; Marlett and Fischer, 2002, 2003), and, to a more limited extent, rhamnose and galacturonic acid (Edwards et al., 2003; Kennedy et al., 1979; Marlett and Fischer, 2002).

Under the standard experimental conditions used (1.0% w/v, RT), PSH AX extractability was observed to be less than 10%. This low water-extractability was not unexpected, as the complex PSH AX molecules are known to have strong gel-forming capacity (Fischer et al., 2004; Marlett & Fischer, 2002, 2003; Sandhu et al., 1981), resulting from the specific structural characteristics of the molecules. Electron microscopy showed that the gel network results from parallel alignment of the xylan polymers (Sandhu et al., 1981).

The extractability of PSH AX was slightly increased by increasing the extraction temperature (up to 50 °C) and strongly increased by decreasing the suspension concentration. As lowering the suspension concentration and increasing the temperature are known to weaken network structures, observed higher AX extraction yields under these conditions (especially in a 6–50 °C interval) are not surprising. It is further plausible that galacturonate residues in PSH play a role in the strongly enhanced extraction of PSH AX, which was observed when using alkaline media with a pH of 12 or above. Alkaline conditions render the uronic acid residues in their negatively charged form, which induces repulsion between the different molecules, and hence, the AX molecules become extractable. Acidification brings the uronic acids back to their neutral form, resulting in the reassociation of xylan polymers and the formation of a gel. This reversible extractability/gelation resembles the behaviour of hemicellulose A molecules, which precipitate rather than gel following acidification (Southgate, 1991).

With decreasing pH and increasing temperature, more arabinose residues were released as monomeric residues, leaving less substituted polysaccharides behind. Beside this, incubation at sufficiently high temperatures and decreasing pH continuously decreased DP_{avg} values. Both actions together resulted in the formation of smaller and less substituted AX fragments, which were more readily extractable. The latter was shown by the higher extraction yields of AX under acidic conditions.

Among those tested, only two enzyme preparations, Crystalzyme and Pectinex, solubilised and depolymerised PSH AX to a large extent, while Shearzyme, Grindamyl, Ecopulp and Pulpzyme hydrolysed the PSH AX only to a limited degree. Enzyme mixtures containing different kinds of enzyme activities can solubilise and/or depolymerise the PSH AX. Incubation of PSH AX with Crystalzyme and Pectinex clearly showed that, next to xylanases, other enzymes are also necessary for hydrolysis of the polysaccharide. Enzymes releasing arabinoses or rhamnogalacturonyl side chains can really assist xylanases to hydrolyse the polysaccharide. By hydrolysing the mentioned substituents, these enzymes leave behind a polysaccharide with longer stretches of unsubstituted xylose residues, which can, in turn, be degraded by xylanases, resulting in small AXOS fragments.

In conclusion, PSH is a good source for the production of AXOS, owing to its high AX level. Both chemical and enzymic treatments are effective in the production of AXOS, as they increase the extractability of PSH AX and convert PSH AX molecules into small fragments.

5. Abbreviations used

AX, arabinoxylan; AXOS, arabinoxylo-oligosaccharides; A/X ratio, arabinose to xylose ratio; DP_{avg}, average degree of polymerisation; GH, glycoside hydrolase family; PSH, psyllium seed husk; RT, room temperature.

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