Structural variation of arabinoxylans in endosperm and bran of durum wheat (*Triticum turgidum* L.)

Ilaria Marcotuli[1], Yves SY Hsieh[2,3], Jelle Lahnstein[2], Kuok Yap[2], Rachel A. Burton[2], Antonio Blanco[1], Geoffrey B. Fincher[2] , Agata Gadaleta[1*]

[1]Department of Soil, Plant and Food Sciences, Section of Genetics and Plant Breeding, University of Bari 'Aldo Moro', Via G. Amendola 165/A, 70126 Bari, Italy.

[2]Australian Research Council Centre of Excellence in Plant Cell Walls, School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Glen Osmond, SA 5064, Australia.

[3]Division of Glycoscience, School of Biotechnology, Royal Institute of Technology (KTH), AlbaNova University Centre, Stockholm, SE-10691, Sweden

*Corresponding author Agata Gadaleta; University of Bari 'Aldo Moro', Via G. Amendola 165/A, 70126 Bari, Italy

Email: agata.gadaleta@uniba.it

ABSTRACT

Arabinoxylans (AX) are one of dietary fibre components in cereal grains and numerous health benefits have been linked with their molecular fine structures and hence with physicochemical properties such as solubility in aqueous media. In bread wheat, AXs account for the majority of non-cellulosic cell wall polysaccharides but there is little information available on the levels and structures of AXs from durum wheat (*Triticum turgidum ssp.*). In order to identify lines with better fibre quality for functional foods, starchy endosperm and bran fractions from 11 durum wheat lines were analysed for total and water-soluble AX, (1,3;1,4)-β-glucan and bound ferulic acid. The AX contents ranged from 11% to 16.4% (w/w) in bran and from 1.5% to 1.8% in the starchy endosperm. Of the starchy endosperm AX, 37% was soluble in water. No correlation was found between AX content and bound ferulic acid in bran, although a relatively high level of this antioxidant was found in endosperm (38.3 μg/g of endosperm flour). Enzymatic fingerprinting was performed to define the major fine structural features of AXs from both regions of the grain.

Keywords: arabinoxylan structure – cell walls - dietary fibre – oligosaccharides - polysaccharides

INTRODUCTION

Over the past decade it has become clear that complex polysaccharides from cell walls of various parts of the grains of barley (Hordeum vulgare), oats (Avena sativa) and wheat (Triticum aestivum) fall under the definition of dietary fibre and can significantly lower the risk of serious, diet-related chronic diseases (1). A large clinical study during the European Prospective Investigation into Cancer and Nutrition (EPIC) showed that dietary fibre consumption reduces the risk of colon cancer and diverticular diseases (2). A regular fibre diet can also reduce serum cholesterol and postglandial blood glucose levels and thus lower the risk of obesity, type II diabetes, colorectal cancer and cardiovascular disease (3). The NHMRC's Australian Dietary Guidelines recommend an increased intake of high fibre cereal foods to reduce the risk of diet-related chronic diseases, to improve individual and public health, and to curtail escalating healthcare costs.

The most important dietary fibre components in cereal grains are arabinoxylans (AX) and (1,3;1,4)-β-glucans (BG), the structural properties of which are strongly linked to food digestibility, bulking and fermentability.

Arabinoxylans consist of a backbone of β-D-xylopyranosyl units (β-D-Xylp) substituted with single α-L-arabinofuranosyl (α-L-Araf) units, situated at O-3 and O-2 of Xylp residues, giving rise to Xylp residues that may be unsubstituted, monosubstituted or disubstituted.

In wheat and rye arabinoxylans, only small amounts of O-2 substituted Xylp residues are present (4). The molecular structure will therefore adopt an extended chain conformation similar to cellulose, albeit with more flexibility than a cellulosic chain, and chains will have a propensity to aggregate through the formation of extensive interchain hydrogen bonding (5).

Cereal arabinoxylans, also have hydroxycinnamic acid substituents, including ferulic acid and p-coumaric acids, at O-5 of Araf substituents attached to the O-3 atoms of backbone Xylp residues (6). The feruloyl moiety is susceptible to oxidative cross-linking through a free-radical

mechanism catalysed by enzymes and oxidizing agents such as peroxidase/H2O2. This can lead to gel formation through dimerization of neighbouring arabinoxylan chains (7). The presence of AX-bound phenolic acids might influence the solubility of AX through the oxidative cross-linked of AX chains (17) and it has been suggested that the release of FA under acidic conditions or as a result of esterase activity in the human digestive system could contribute to anti-oxidant effects that are believed to lower the risk of colorectal cancer (18). In addition, acetyl ester groups can be present on Xylp or Araf residues but the locations are not well-defined because of the labile nature of the esters (7). Other substituents on Xylp, including glucuronosyl residues, 4-O-methyl-glucuronosyl residues and short oligomeric side chains consisting of two or more arabinosyl residues, or an arabinosyl residue with a terminal xylosyl residue, have been reported at low levels for some cereal arabinoxylan extracts (8, 9). For example, in developing barley coleoptiles, the ratio of substituted to unsubstituted 4-linked xylosyl units changes from about 4:1 to 1:1 over about 3 days (10). This indicates that about 80% of xylopyranosyl backbone is high substituted with arabinosyl residues, which are progressively removed during growth (10), in a process that might be mediated by the action of arabinoxylan arabinofuranohydrolases (11). Similar changes have been reported in the arabinoxylans of developing maize coleoptiles (12).

An important indicator of the physicochemical properties of arabinoxylans is the distribution of arabinosyl substituents along the xylan backbone. In many cereal arabinoxylans substitution patterns appear to be non-random, with different regions showing different substitution patterns (13, 14). In some regions, mono- and di-substituted Xylp residues are clustered together, often separated by 1–2 unsubstituted Xylp residues. Other regions contain relatively few arabinosyl substituents and therefore are susceptible to hydrolysis by xylanases.

The Araf and other substituents sterically inhibit aggregation of the (1,4)-β-D-xylan chains and lead to the formation of an extended, asymmetrical polysaccharide that has physicochemical properties suited to its function as a major matrix phase component of walls in grasses.

The arabinose-to-xylose (Ara/Xyl) ratio influences the ability of chains to aggregate and hence affect solubility. Thus, highly substituted arabinoxylans result being more soluble than those with fewer arabinosyl substituents. For example, when a water-soluble arabinoxylan from wheat flour was treated with α-L-arabinofuranosidase, the resulting products have fewer Araf substituents and more readily aggregate into insoluble complexes (15).

The solubility of the arabinoxylan in wheat and other cereal is the most important feature of these non-starch polysaccharides. In fact, its structural properties are strongly linked to potent effects on innate and acquired immune response, antitumor activity, increase of faecal bulk, beneficial health effect in patients with impaired glucose tolerance (16).

Moreover, these unique physicochemical properties allow arabinoxylan to have a considerable effect on cereals food industry, including bread making (17), gluten-starch separation (18), refrigerated dough syruping (19, 20), and in animal feeds (21).

Durum wheat is a tetraploid Triticum species that is a dominant crop in many temperate countries, where it is widely used for human food such as pasta and for livestock feed, particularly in Mediterranean regions.

The objective of the present work was primarily to study the variation of AX content in grains from durum wheat breeding lines and assess the arabinoxylan structure. At the same time, the levels of associated ferulic acid residues and the (1,3;1,4)-β-glucans contents were defined. Through this approach, it was expected that varieties with higher concentrations of soluble dietary fibers (AX) and ferulic acid could be identified for the production of pasta and other durum wheat products with enhanced benefits for human health and nutrition.

MATERIALS AND METHODS

Plant material

A total of 108 advanced breeding lines and 12 commercial varieties from the University of Adelaide durum breeding program (dr. Jason Able, personal communication) was evaluated for grain weight, AX content and fine structure, total starch content and (1,3;1,4)-β-glucan content. From 120 initial collections, 11 lines, including two cultivars (Hyperno, Tamaroi) and nine breeding lines (UAD1151046, UAD1151101, UAD1151118, UAD1152076, UAD1153207, UAD1154007, UAD1154021, UAD1154055, and UAD1154229) were selected for detailed analyses of dietary fibre and FA contents in starchy endosperm and bran fractions. Plant material was harvested in 2011 and 2012 in two different regions of South Australia, namely Wandearah (designated Wan2012) and Mallalah (designated Mal2011 and Mal2012). Grains (20g) for each line in three biological replicates were ground using a Quadrumat Junior laboratory mill, which separates endosperm from the bran and seed coat layers. The endosperm fraction, which consists predominantly of starchy endosperm, was passed through a 71 µm sieve to obtain the superfine fraction. All grain analyses were performed on the bran and superfine endosperm fractions.

Measurement of (1,3;1,4)-β-glucan and starch contents

A small-scale version of the Megazyme Total Starch Assay (20 mg of samples), which is based on the amyloglucosidase⁄α-amylase method (McCleary 1994), was developed to estimate starch content in the flour samples. Analyses of (1,3;1,4)-β-glucan in wheat endosperm and bran were performed using a scaled down Mixed-Linkage β-Glucan Assay Kit (Megazyme International Ireland Ltd, Wicklow, Ireland), which is based on the industry standard method for barley (22), with 15 mg of samples. To determine the ratio of the degree of polymerization (DP) of major oligosaccharide components of the (1,3;1,4)-β-glucan, which are expressed as a DP3:DP4 ratio, the oligosaccharides released by lichenase digestion during the Megazyme

assay were subjected to solid phase extraction on Varian Bond Elut Carbon 50 mg/1ml columns, eluted with 55% acetonitrile, before they were analysed with the HPAEC-PAD (Dionex, model ICS-5000 LC).

Monosaccharide analysis

The total AX content for each line was measured on 20 mg samples of the bran and endosperm fractions by reverse phase HPLC (23). Samples were initially hydrolysed in 1 M sulphuric acid at 97°C for 3 hours. The hydrolysate was diluted 1:20 times and released monosaccharides were derivatized with 1-phenyl-3-methyl-5-pyrazolone (PMP). Separation of PMP-monosaccharide derivatives was effected on a Phenomenex Kinetex 2.6 μm C18 100 x 3 mm 100Å column at 30°C and a flow rate of 0.8 mL/min. The eluent contained 40 mM ammonium acetate in 10% acetonitrile (pH 6.8), with a gradient of 8% (v/v) to 100% (v/v) acetonitrile over 18.5 min. The standard curve was generated using a 0.5 M 2-deoxyglucose internal standard. Total AX was calculated on a molar basis from the total amount of L-arabinose and D-xylose.

Extraction of water-soluble polysaccharides

Bran and endosperm flours (20 mg) were incubated with 80% ethanol (1 mL) in a thermomixer at 90°C and 1000 rpm to remove sugars and other low molecular weight material. After 30 min, the suspension was cooled down for 10 minutes at room temperature, centrifuged at 3500 rcf for 10 minutes and the supernatant discarded. The pellet was washed with 100% ethanol (1 mL), centrifuged 3500 rcf for 10 minutes and the supernatant discarded. The pellet was dried in an oven at 38°C for 30 minutes. The water-soluble polysaccharides were extracted in 200 μL water at 40°C for 2 hours at 1000 rpm. Following centrifugation, the supernatant (50 μL) was subjected to monosaccharide analysis as described above.

AX oligosaccharide purification

Bran and endosperm samples (2 g) were pre-treated with ethanol and heated at 100 oC to inactivate endogenous enzymes before hydrolysis with 22.5 U endo-(1,4)-β-xylanase M6

(Megazyme) in 25 mM sodium acetate buffer (pH 6.5). Released oligosaccharides were fractionated by HPLC (Agilent Technologies, 1200LC) using a graphitised carbon column (Hypercarb 5um 100x4.6 mm, Thermo Scientific) and an aqueous normal phase column (Prevail Carbohydrate ES 5um 150x4.6 mm, Alltech), with UV (192nm) and evaporative light scattering detection (ALLTECH 800 ELSD). The eluents for both separations were (A) 1mM ammonium hydroxide and (B) 70% acetonitrile. The graphitized carbon column was eluted at a flow rate of 0.8 mL/min at 25°C, with a gradient of 10% to 40% (B) over 30 minutes. The normal phase column was eluted at 0.6 mL/min at 20°C with a gradient of 80% to 62.5% (B) over 30 minutes. Oligosaccharide fractions were further characterized by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and matrix-assisted laser desorption-ionization time-of-flight mass (MALDI-TOF MS) (24).

HPAEC-PAD

AX-derived oligosaccharides (AXOS) were separated and quantified by HPAEC-PAD (Dionex, model ICS-5000 LC) at 30°C on a CarboPac PA200 analytical anion-exchange column with an electrochemical detector, using 0.1M NaOH (solvent A) and a linear gradient from 1% to 100% and 0.1M NaOH with 1M sodium acetate (solvent B) over 35 min at 0.5 mL/min. The mole percentages of the three most abundant AX oligosaccharides (xylobiose, DP5 and DP6) that are structurally characterized described below, were calculated by based on each peak area of the corresponding oligosaccharide (25).

MALDI-TOF mass spectrometry

Molecular weights of the sodium adducts ions [M + Na]+ of the purified xylan digestion-derived oligosaccharides were determined using a BioTOF Ultraflex II (Bruker Daltonics). The oligosaccharides were dissolved in 10 mM 2,5-dihydroxybenzoic acid and 10 mM NaCl in the ratio of 5:5:3. The mixture was dried on sample plates and the MS operated in the reflectron

mode at an acceleration voltage of 20 kV. Each spectrum consisted of data from an average of 2000 laser shuts (26).

NMR spectral analysis

1H NMR spectra of purified oligosaccharides were recorded using an Agilent 600 MHz NMR spectrometer equipped with a cryoprobe. Samples were dissolved in 1 mL of D2O for analysis. Chemical shifts are given in δ values relative to the HOD signal (δH 4.8 at 25℃) (26).

Analysis of phenolic compounds

Bound ferulic acid extraction was performed following the method described by (27). Bran and endosperm (20 mg) were pre-treated with 80% ethanol (500µL) for 10 min at room temperature. After centrifugation the supernatant, which would contain any free ferulic acid, was discarded. This pre-treatment step was repeated twice. For the release of bound ferulic acid, saponification was performed by mixing residual pellets with 2M NaOH (600 µL) under a N2 atmosphere for 16 hours in the dark, followed by acidification with 36% HCl to pH 2.0. Released ferulic acid was extracted three times with ethyl acetate; extracts were pooled and dried in a vacuum centrifuge. Before characterization, the extract was resuspended in 50% methanol (100µL), and 10µL was loaded onto a HPLC. For separation of phenolic acids, a Poroshell 120SB-C18 3x100 mm 2.7 µm column was used at 30°C and a flow rate of 0.7 mL/min. The separation gradient was performed as following: 5% (v/v) to 55% (v/v) of 1 mM TFA in 40% acetonitrile and 40% methanol over 10 minutes.

Statistical analysis

Data were reported as means ± standard errors (SE) for three independent environments for each assay. Results were processed using ANOVA.

RESULTS AND DISCUSSION

Arabibnoxylans and (1,3;1,4)-β-glucans are key components of the cereal cell walls. Their structural features are closely linked to pasta and bread dough quality (37, 38). Herein, we report the variation in AXs in the two milling fractions, endosperm and bran, from 11 selected durum wheat lines chosen from a set of 120 genotypes on the basis of high AX and (1,3;1,4)-β-glucan content. These durum wheat lines were bred in South Australia as potential cultivars for the production of spaghetti and bread with higher soluble dietary fibre contents and therefore with a range of potential health benefits for the consumer, such as a lower the risk of heart disease or colorectal cancer (39).

Selection of durum varieties for high arabinoxylan and (1,3;1,4)-β-glucan contents

Total starch, (1,3;1,4)-β-glucan and AX contents, together with grain weights, of 120 durum wheat lines from the University of Adelaide breeding program were evaluated and they are shown in Table S1. Eleven of the lines with the highest AX and (1,3;1,4)-β-glucan contents were selected for further analysis. These included two cultivars (Hyperno and Tamaroi), and nine breeding lines (UAD1151046, UAD1151101, UAD1151118, UAD1152076, UAD1153207, UAD1154007, UAD1154021, UAD1154055, and UAD1154229). To address potential environmental effects on levels of AX and (1,3;1,4)-β-glucans, the selected durum lines were grown in and harvested from two different sites and harvests, namely Mallalah 2011 (Mal2011), Mallalah 2012 (Mal2012) and Wandearah (Wan2012). Thus, values reported here represent the means of values from three batches of grain grown under the three different conditions. It is worth noting that in previous large scale field trails of durum wheats across four seasons and six different sites in Australia, only small effects were reported on dough and other pasta technological characteristics (28).

(1,3;1,4)-β-glucan content and DP3:DP4 ratios determination

The (1,3;1,4)-β-glucans in bread wheat, T. aestivum, are primarily located in the aleurone layer, which is recovered in the bran fraction, with relatively small amounts in the starchy endosperm walls (29). This is consistent with our data, where bran from durum wheat also has the highest abundance of (1,3;1,4)-β-glucans (0.71-1.08%, w/w), compared with the starchy endosperm (0.12-0.16%, w/w) (Table 1). In addition, we treated the endosperm and bran flour with lichenase to determine DP3:DP4 ratios through the molar ratios of the released trisaccharide (G4G3G; DP3) and tetrasaccharide (G4G4G3G; DP4). Bran flour (1,3;1,4)-β-glucans had DP3:DP4 ratios of 3.2:1 to 3.4:1, whereas (1,3;1,4)-β-glucans from endosperm flour had ratios between 2.5:1 and 3.0:1 (Table 1), which are similar to barley and oat (1,3;1,4)-β-glucans, but lower than the DP3:DP4 ratios of (1,3;1,4)-β-glucans from the grain of bread wheat Triticum aestivum, which range from 3.0:1 to 4.5:1 (42, 43). The ratios were also lower in durum wheat bran (3.2:1 to 3.4:1), compared with those from the (1,3;1,4)-β-glucans of T. aestivum bran (4.5). The DP3:DP4 ratio of (1,3;1,4)-β-glucans in grasses and cereals strongly are correlated with the solubility of this polysaccharide, because the random insertion of (1,3)-β-glucopyranosyl residues along (1,3;1,4)-β-glucans is believed to create irregularly space kinks in the (1,3;1,4)-β-glucan chain and therefore to prevent aggregates. Thus, DP3:DP4 ratios close to 1:1 are indicative of increased (1,3;1,4)-β-glucan solubility, compared with (1,3;1,4)-β-glucans with very high or very low DP3:DP4 ratios (30). For example, the (1,3;1,4)-β-glucans, lichenin, from Icelandic moss has a DP3:DP4 ratio of 24 and these are relatively insoluble (31), while the (1,3;1,4)-β-glucan in horsetail ferns has a DP3:DP4 ratio of 0.1:1 and these are also insoluble (32).

As noted above, differences in DP3:DP4 ratios are correlated with wide variations in the solubility of the (1,3;1,4)-β-glucans. For example, the (1,3;1,4)-β-glucans of barley and oat have DP3:DP4 ratios of 1:1 to 2:1, which are lower than those of the (1,3;1,4)-β-glucans of T. aestivum. Thus, 20% of the (1,3;1,4)-β-glucans from barley and oat grain are extractable with

water at 40°C and about 50-70% of (1,3;1,4)-β-glucans are soluble in water 65°C (33, 34). In contrast, it is difficult to extract (1,3;1,4)-β-glucans from T. aestivum with water, even at 65°C (35). The data we present here indicate that the (1,3;1,4)-β-glucans in durum wheat have similar solubilities to those of barley and oat (1,3;1,4)-β-glucans, based on their similar DP3:DP4 ratios. This unique property for durum wheat (1,3;1,4)-β-glucans has been used to increase the content of soluble (1,3;1,4)-β-glucans in products such as bread and pasta, and have been shown to provide health benefits such as reduced glycemic response, obesity and metabolic syndrome (36).

Arabinoxylan content and degree of substitution in durum wheat

The arabinoxylans of cereal grains are key dietary fibre components of grain that influence diverse physicochemical properties, including water solubility, viscosity, gelation and hydration properties. All these affect end-use properties, nutritional quality and health benefits of the fibre within human foods (37). This study reported the amount of total AX content in the starchy endosperm and bran of durum wheat lines. No significant variations were detected in endosperm between the 11 lines. However, for the bran fractions, significant differences were revealed with values ranged from 11.0% to 16.4% (w/w) (Figure 1A). It has been reported previously that durum wheats have average AX contents of between 4-6% (w/w) (38), but all of the selected high AX lines in the present study had values >10% (w/w).

The degree of substitution of AXs was defined as the ratio of arabinofuranosyl (L-Araf) substituents to xylopyranosyl (D-Xylp) residues (A:X ratio). The A:X ratio measured for starchy endosperm from the 11 durum wheat lines was between 0.5:1 and 0.6:1, while for bran the ratios were in the range 0.6:1 to 0.7:1 (Table 1). There were no significant differences in A/X ratios between the two tissue types consistent with literatures previously reported (39, 40).

Solubility of arabinoxylans from bran and endosperm

Based on commercial processes rather than biological functions, cereal AXs have been broadly classified into two groups, namely the water extractable (WE-AX) and water unextractable (WU-AX) arabinoxylans. We reported the WE-AXs amount extracted from bran and endosperm of the 11 durum lines and quantified by HPLC. Total WE-AXs in the starchy endosperm ranged between 0.43% and 0.68% (w/w) (Table 1), whereas in bran the values ranged from 0.58% to 0.93% (w/w) (Table 1) (Figure S1-B). Our results suggested that the WE-AXs were more abundant in the bran, compared with the starchy endosperm, although the differences were relatively small. However, the WE-AXs accounted for more than 30% of total endosperm AXs, whereas only 5.4% of the bran AXs were water-extractable.

This extractability and solubility of the WE-AX can be correlated with the content and distribution of L-Araf substituents on the AX chains. For example, it has been reported that AXs with a higher proportion of L-Araf are more soluble in water and hence more 'water-extractable' than those with fewer L-Araf (41).

Oligosaccharides released from AX by endo-(1,4)-β-xylanase

In the endosperm flour preparations from the 11 durum lines, the total amount of AX released by xylanase treatment was 0.9-1% (w/w), whereas the remaining residual pellets accounted for 0.2-0.3% (w/w) of the total AX in grain (Figure 2A). Thus, the majority of AXs in endosperm flour were degraded by the xylanase, with only 15-23% of the AX remaining in the insoluble pellets (Table 2).

In contrast, the total weights of bran flour AXs hydrolysed by the xylanase were 3-4 fold higher (3.1-4.4% w/w), compared with endosperm flour (0.9-1% w/w) (Table 2). Overall weights for non-hydrolysable AXs were much higher in residual pellets from xylanase-treated bran fractions (6.1-12%, w/w) compared with residual pellets from starchy endosperm flour (0.2-0.3% w/w) (Figure 2B). Thus, the majority of AX in the bran flour was resistant to xylanase

digestion. More specifically, only 20-35% of bran AXs were digested by the xylanase treatment, whereas 74-85% of endosperm AXs were digested (Table 2).

Variations were observed in the xylanase-hydrolysable AXs in the bran, with UAD1153207 having the highest value among the 11 lines (4.4% w/w), which is 40% greater in abundance when compared with UAD1151118 and UAD1154229. Proportions by weight of non-hydrolysable AX in the brans from 11 durum lines were also analysed. The UAD1151046 and UAD1154229 lines had the lowest proportions of non-hydrolysable AX, measured at 6.6 % (w/w) and 6.1% (w/w), respectively. These were about half the values observed for UAD1151118 and UAD1151101, which had the highest non-hydrolysable AX content (12% w/w) (Table 2).

No significant differences were found for the total AX contents in either the enzyme extracts or the residual pellets among endosperm of the 11 lines. There was some variation in bran preparations, where the durum lines UAD11510436 and UAD1154229 had much lower overall enzyme hydrolysable AX levels, compared with the other nine lines (Table 2).

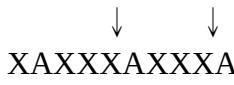
Analysis of oligosaccharides released by xylanase treatment

Xylanase extracted fractions from endosperms had A:X ratios ranging from 0.63:1 to 0.7:1, whereas in bran the A:X ratios were 0.33:1 to 0.38:1 (Table 2). For the xylanase-indigestible residual pellets, high levels of Ara were found. These could arise from the inability of the enzyme to hydrolyse highly substituted AXs, or possibly to the presence of water-soluble arabinogalactan-proteins (AGPs), which would not be hydrolysed by the xylanase (42).

The Ara:Xyl ratios calculated for the durum AXs do not provide detailed structural information on the substitution patterns of AXs with L-Araf residues. For example, the L-Araf residues could be mono-substituted through (1,2)- or (1,3)-linkages to the xylan backbone or the D-Xylp residues could be doubly substituted with L-Araf residues at both the C2- and C3-positions of D-Xylp residues (Fincher and Stone, 2004). To obtain more detailed structural

information, highly abundant oligosaccharides released from the AXs by xylanase treatment were profiled by HAPEC-PAD, in which the column was calibrated with linear xylo-oligosaccharide standards (DP 2-6).

The endo-(1,4)-β-xylanase M6 hydrolyses glycosidic linkages within AXs at positions where at least two adjacent non-substituted D-Xylp residues are present (40, 43-45), as indicated by arrows in the following:



The enzyme digests of the bran and endosperm samples from the 11 durum lines gave rise to xylose, xylobiose and 19 other putative arabinoxylo-oligosaccharides (AXOs) in these analyses. For example, Figure 3 shows the oligosaccharide profiles of the xylanase hydrolysate of UAD1151101 endosperm and bran preparations, indicating that the xylanase extract from endosperm possesses a higher proportion of L-Araf substituted oligosaccharides (DP5 and DP6) than the bran, with endosperm A:X ratios of 0.63:1 to 0.7:1, compared with bran, where the A:X ratios were 0.33:1 to 0.38:1. The manipulation of α-(1,2)- and/or α-(1,3)-arabinosyltransferase genes and their encoded enzymes might allow the manipulation of A:X ratios and hence lead to increased or decreased AX solubility in these durum wheat lines.

In addition, the variation in substitution patterns of AXs might have significant implications in human health, via the effects of AXs on small intestine viscosity (46). Traditionally, viscosity has been reported to be dependent upon molecular weight (MW) and concentration of AXs (37), insofar as higher MWs are generally associated with higher viscosities. However, the report by Shelat et al. (2012) (46) demonstrated that the AX substitution pattern with L-Araf residues also affects AX conformation in solution through the aggregative/entanglement behaviour of AXs, which in turn influences micro-viscosity. In our study, oligosaccharide profiling showed the larger AXOs were present in relatively higher abundance than xylose and xylobiose in the bran hydrolysates, while the endosperm hydrolysates had much higher

amounts of xylobiose. In addition, lines UAD1152076 and Tamaroi endosperm had a greater abundance of mono-substituted arabinoxylosides of DP5, compared with all other lines (Table S3) and this was also observed in the bran AXs of the durum lines. These AXs are likely to possess lower proportions of di-substituted Xylp residues, which tend to adopt a random coil conformation, and hence have a tendency to aggregate (lower micro-viscosity) (46). Therefore, these data could be useful in the selection of durum wheat lines for the capacity of their AXs to aggregate and thus influence the viscosity in the human gut and could have implication in human health.

Two of most abundant AXOs, which had estimated sizes of DP5 and DP6, were successfully purified to homogeneity (Figure S1), together with an AXO of DP7. These were subjected to detailed structural characterization.

Oligosaccharides structures

MALDI analysis of purified oligosaccharides 1 (DP5), 2 (DP6) and 3 (DP7) gave molecular ions with m/z values of 701, 833, and 965, and these values corresponded to the [M + Na]+ adduct ions of oligosaccharides containing 5, 6 and 7 pentosyl residues, respectively (Figure S2-A). Structures of these purified oligosaccharides were assigned by 1H NMR and are summarized in Table S2, based on signature anomeric proton signals (47).

The H-1 signals of unsubstituted β-Xyl residues can be readily distinguished within chemical shift ranges between δ 4.4 - δ 4.6 (48-50). These characteristic signals are derived from the H-1 of the reducing end β-Xylp, the non-reducing end β-Xylp residues and signals from internal unsubstituted β-Xylp, and showed little differences between the three samples examined here (Table S2). The addition of α-Araf to an internal β-Xylp has a significant effect on the chemical shift. Using compound 1 as an example (1), the H-1 signal of substituted β-Xylp-3II (δ 4.56) has shifted to a higher field compared with the unsubstituted β-Xylp-2 (δ 4.52) residues. An even more dramatic effect was observed in compound 2, where the H-1 of β-Xylp-3III (δ 4.68)

had an even higher field shift when two α-Araf residues were attached (Table S2). In the case of α-Araf H-1 signals, the chemical shift has an upper field shift above δ 5.2, as is evident by H-1 of at δ 5.43, δ 5.31 and δ 5.43 for compound 1, 2 and 3, respectively. It is clear that the addition of α-Araf-A2x3 to compound 2 had a dramatic downfield shift effect on the H-1 of α-Araf-A3x3 (Figure S2-B), as reported elsewhere (47) .

Having structurally characterized three AXOs, together with the commercially available standard xylo-oligosaccharides of DP 2-6, we have identified the five major oligosaccharides released by xylanase hydrolysis of endosperm and bran AXs from the 11 durum lines. Structural heterogeneity of AXs was detected between endosperm and bran tissues, on the basis of the relative proportions of the three most abundant oligosaccharides from xylanase digests, namely mono-subsituted (DP5), disubstituted (DP6) and unsubstituted oligosaccharides (xylobiose). The proportions of DP5 and DP6 were significantly higher in the endosperm than in the bran (Figure 3). The fine structural variation among the durum wheat lines could be demonstrated by comparison of the amounts and structures of the five assigned structural units (xylose, xylobiose, DP5, DP6 and DP7), where the endosperm reference was Hyperno and bran was UAD1151101 (Table S3).

Phenolic compound analysis

Cereal grain AXs can also be covalently cross-linked through ferulic acid dehydrodimers and trimers, suggesting a role for feruloyl residues in wall assembly, tissue cohesion and cell expansion ((37, 51, 52). We have detected a positive correlation (0.89***) between the content of bound ferulic acid and arabinoxylan in durum wheat bran.

A wide range of bound FA contents was detected for both bran (1095-2192 µg/g) and endosperm (30.6-44.9 µg/g) (Table 1), which suggested a very high genetic variability for the trait. In comparisons of bound FA and AX contents within the bran flour, a correlation of 0.89 ($P \leq 0.001$) were detected, while no apparent correlation was found between bound FA and WS-

410 AX. No relationship was revealed among the three traits in the endosperm (Table 3). Our
411 observation is consistent with previous studies on milling fractions enriched in bran or starchy
412 endosperm from durum wheat (38, 53). The lower proportions of extractable WE-AXs in bran
413 described previously are consistent with efficient phenolic cross linkages (38, 53).
414 When feruloylated AXs were consumed as components of dietary fibre, esterases from the
415 human intestine are able hydrolyse the ester-linkage between the feruloyl residues and the Ara
416 residues of the AX. The released FA is known to possess antioxidant properties that might
417 provide some protection against colorectal cancer (54, 55). Having selected durum wheat lines
418 with highly abundant bound FA and similarly high levels of AXs could provide better dietary
419 fibre of higher abundance and improved quality quantity in durum wheat related products, such
420 as “super spaghetti” that decreases the risk of diet related diseases and potentially decreases
421 risk of colorectal cancer (55).

Conclusions

In conclusion, the results of this work suggest that there are differences in the molecular fine structures for AXs and in two durum wheat milling fractions: starchy endosperm and bran. The extractability in water of the major polysaccharides from endosperm of durum wheat grain are likely to be controlled by their physical and/or fine chemical structures (56). Specifically, high contents of soluble AX and (1,3;1,4)-β-glucans in endosperm durum wheat lines (UAD1151046, UAD1153207 and UAD1154021), when used in pasta for daily consumption, are likely to increase the bulk viscosity of the contents of the small intestine and might thereby impede the actions of α-amylase and protease-mediated starch and protein breakdown. Those results provide an alternative avenue for slowing glucose absorption and reducing glycaemic index and give us additional information with which to assess durum varieties that could be useful for the production of functional end-use products associated with human health benefits.

Abbreviations Used

AX	Arabinoxylan
BG	β-glucan
β-D-Xylp	β-D-xylopyranosyl
α-L-Araf	α-L-arabinofuranosyl
Ara/Xyl	Arabinose-to-Xylose ratio
WE-AX	Water extractable arabinoxylan
WU-AX	Water unextractable arabinoxylan
AX-OS	Arabinoxylan-oligosaccharides
bFA	Bound ferulic acid
Mal11	Mallala 2011
Mal12	Mallala 2012
Wan12	Wandearah 2012
X	Xilose
XX	Xylobiose
A	Arabinose
DP	Degree of polymerization

Acknowledgment

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Figure captions

Figure 1 Total amount of AX (A) and WE-AX (B) in endosperm and bran of 11 Australian durum wheat lines. No variability was found for AX content in endosperm tissue.
Primary y-axis values (left side) are reported for bran, while secondary y-axis values (on the right side) are referred to endosperm. *, **, ***: significant differences at 0.05P, 0.01P and 0.001P.

Figure 2 Total AXs in xylanase extract and residual pellet of endosperm (A) and bran (B). Eleven different durum wheat lines are marked in x-axis, and measured amounts of AXs (% w/w) are showed on the y-axes.

Figure 3 AXOs fingerprinting in durum wheat endosperm and bran tissues. A) Oligosaccharides profile obtained from hyperno endosperm preparation treated with endo 1,4 xylanase. B) AXOs derived by UAD1151101 bran preparation with treatment of endo 1,4 xyalanase.

608 # Tables

609 **Table 1** Composition of carbohydrate moieties and ferulic acid in endosperm and bran of 11
610 selected durum wheat lines. AX, A/X, WE-AX, BG and DP3/DP4 are expressed as % w/w;
611 bFA is expressed as µg/g of flour.

Compound	Kernel compartment	Mean	SD	Min-Max
Arabinoxylan (% w/w)	Endosperm	1.63	0.08	1.52-1.75
	Bran	13.7	1.76	11.0-16.4
A/X	Endosperm	0.60:1	0.01	0.50-0.60:1
	Bran	0.69:1	0.16	0.60-0.74:1
Water soluble AX (% w/w)	Endosperm	0.55	0.08	0.42-0.68
	Bran	0.74	0.14	0.54-0.95
A/X from WSAX	Endosperm	0.60:1	0.10	0.50-0.70:1
	Bran	0.60:1	0.06	0.50-0.90:1
(1,3;1,4)-β-glucan (% w/w)	Endosperm	0.15	0.01	0.12-0.16
	Bran	0.85	0.10	0.71-1.08
DP3/DP4	Endosperm	2.55	0.11	2.37-2.72
	Bran	3.28	0.06	3.17-3.43
Ferulic acid (µg/g)	Endosperm	38.3	4.32	30.6-44.9
	Bran	1654	137.6	1095-2192

612

Table 2 Monosaccharide analysis of enzyme digestion products (xylanase extract and residual pellet) in endosperm and bran tissues of 11 durum wheat lines.

	Endosperm						Bran					
Line	Enzyme[a] extract	Ratio[b]	Residual[a] pellet	Ratio[b]	Enzyme extract fraction	Pellet fraction	Enzyme[a] extract	Ratio[b]	Residual[a] pellet	Ratio[b]	Enzyme extract fraction	Pellet fraction
HYPERNO	0.96	0.67	0.28	0.91	77%	23%	3.30	0.35	11.39	1.12	22%	78%
TAMAROI	0.98	0.70	0.23	0.94	81%	19%	3.68	0.38	10.69	1.10	26%	74%
UAD1151046	1.07	0.64	0.19	0.93	85%	15%	3.52	0.35	6.59	1.12	35%	65%
UAD1151101	0.93	0.68	0.33	0.81	74%	26%	3.66	0.33	11.91	1.03	24%	76%
UAD1151118	0.91	0.69	0.26	0.91	78%	22%	3.09	0.37	11.97	1.00	20%	80%
UAD1152076	0.96	0.70	0.28	0.98	77%	23%	3.30	0.37	11.39	1.01	22%	78%
UAD1153207	1.03	0.63	0.18	0.91	85%	15%	4.42	0.35	9.55	1.03	32%	68%
UAD1154007	1.05	0.70	0.28	0.87	79%	21%	4.23	0.36	11.03	1.00	28%	72%
UAD1154021	0.96	0.65	0.28	0.96	77%	23%	3.30	0.37	11.39	1.10	22%	78%
UAD1154055	1.09	0.67	0.20	0.97	85%	15%	4.25	0.37	9.35	1.14	31%	69%
UAD1154229	1.04	0.66	0.32	0.93	77%	23%	3.08	0.37	6.11	1.08	34%	66%

[a] Quantities of AXs were expressed as % w/w; [b] A/X ratio

618 **Table 3** Correlation analysis among arabinoxylans (AXs), water soluble arabinoxylans (WS-
619 AXs) and bound ferulic acids (bFAs) in bran and endosperm of 11 durum wheat lines.

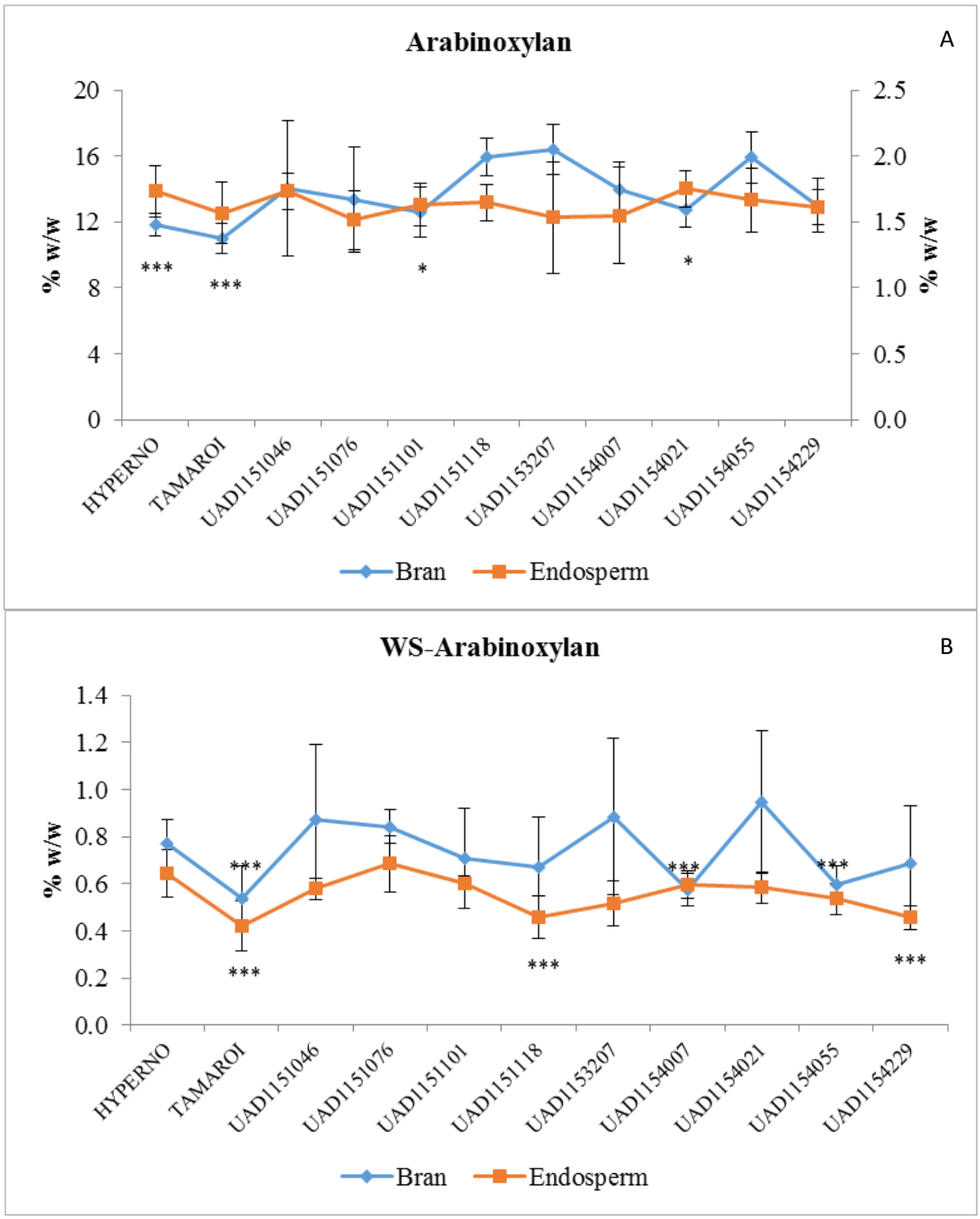
620

	Bran		Endosperm	
	WS-AX	bFA	WS-AX	bFA
AX	0.56	0.89***	0.15	0.56
WA-AX	-	0.35	-	0.32
bFA		-		-

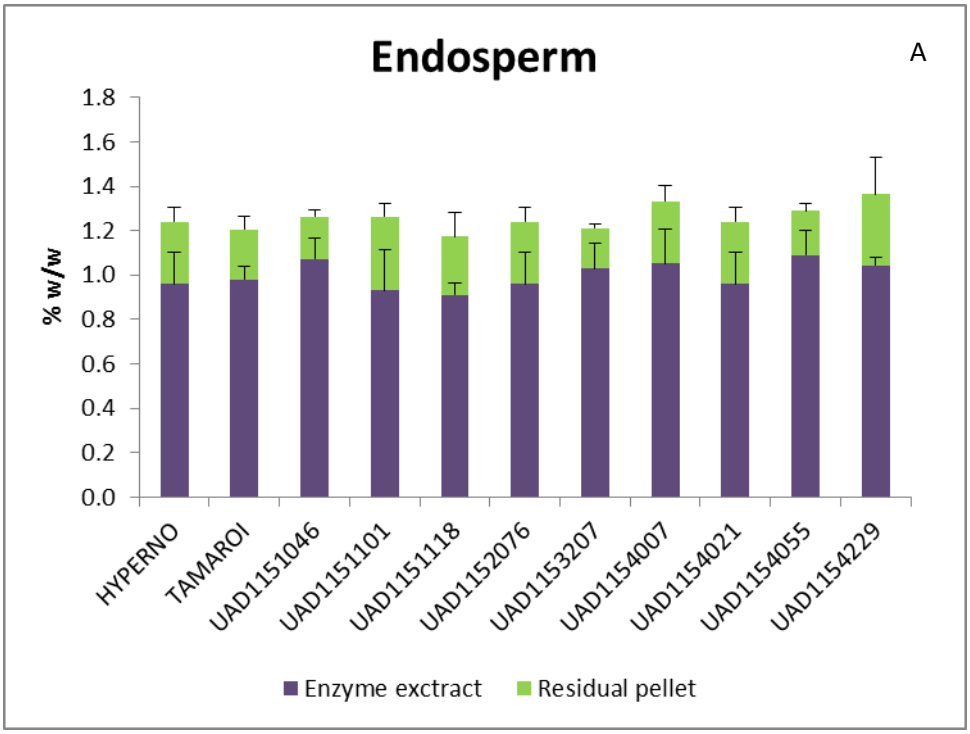
*** significant differences P≤0.001

Figures

Figure 1



626 Figure 2



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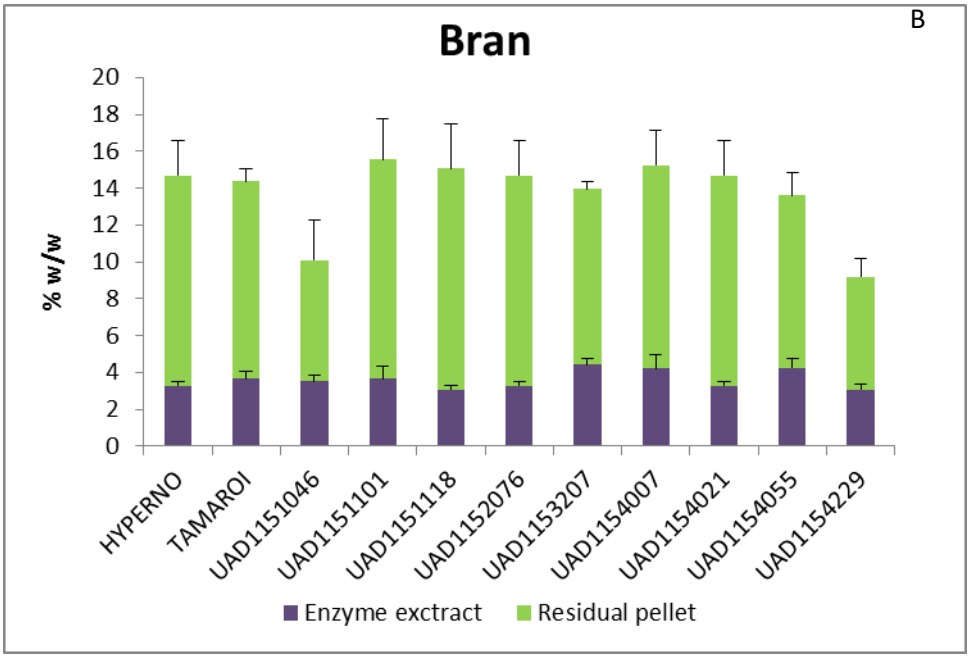

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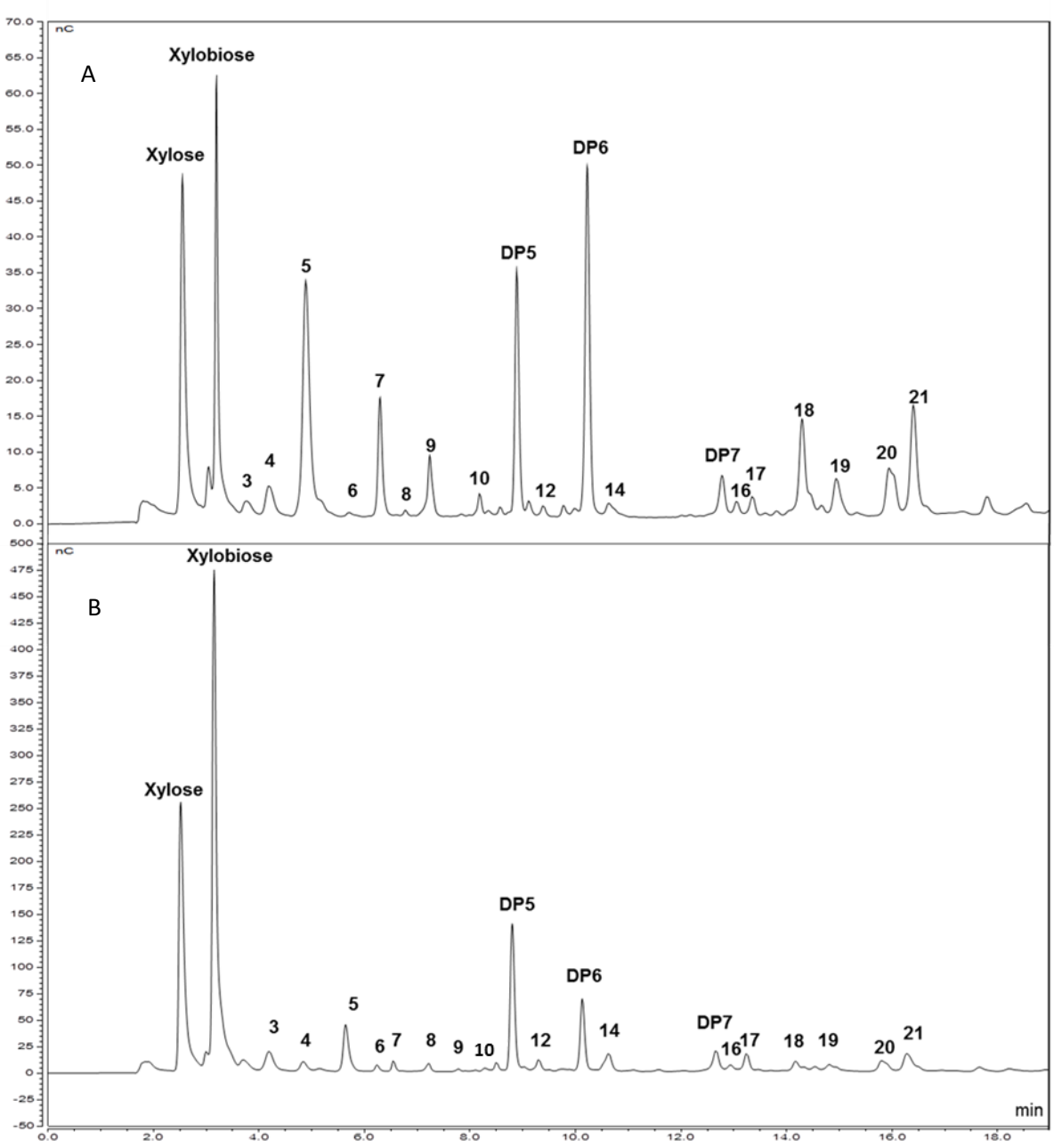
629 Figure 3



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