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Structural characteristics of arabinoxylan in barley, malt, and beer

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Abstract

Non-starch polysaccharides (NSP) content, partially water-soluble and high-molecular-weight polymers, are related to beer viscosity. Thus, arabinoxylan alone, or synergistically with β-glucan, may be involved in some of the undesirable effects in the brewing process, such as influencing the rate of wort separation. Three malting barley samples were studied to determine glycosyl linkage composition of highly substituted arabinoxylans by analysis of partially methylated alditol acetate (PMAA) sugar derivatives using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). Arabinoxylan, total non-starch polysaccharide and β-glucan levels between all samples were not significantly different ($P < 0.05$). The NSP fractions decreased significantly ($P < 0.05$) during malting and brewing. No apparent differences in arabinoxylan structure were found between malt and beer samples. Structurally, four major glycosyl derivatives, *t*-Glu-*p*, 4-Xyl-*p*, 4-Glu-*p*, and 2,3,4-Xyl-*p*, were identified. Arabinoxylans appear to contain randomly branched regions, mostly consisting of an unsubstituted and a double arabinofuranosylated xylose residue. The number of xylose residues *O*-2,3 disubstituted, and *O*-2 and *O*-3 monosubstituted were similar in all varieties.

Keywords: Arabinoxylan; Structure; GC–MS; Barley; Malt; Beer

1. Introduction

In view of the importance of non-starch polysaccharides in the malting of barley and in subsequent steps in the brewing process, including the possible influence of residual cell walls on the rate of wort separation, it is desirable to have a more detailed knowledge of cell walls in barley endosperm. In a previous study, the endosperm arabinoxylans from three barley samples of differing brewing quality were studied and the non-starch polysaccharide composition of the samples determined (Han & Schwarz, 1996). Although the composition and structure of β-glucan have been extensively studied (Bathgate & Dalgliesh, 1975), determination of the structure of arabinoxylans, especially from beer, has been incomplete (Åman & Graham, 1987; Henry, 1988b). It is therefore important to obtain more information about the structure and properties of barley endosperm cell wall arabinoxylans.

Henry (1988a), Luchsinger (1967), and Viëtor, Voragen, Angelino and Pilnik (1991b) reported that arabinoxylan contains a backbone structure of D-xylopyranose residues linked by β-(1→4)-glycosidic bonds with units such as L-arabinofuranose attached as branches by β-(1→2)- or β-(1→3)-linkages. Typically, L-arabinofuranose or other side chains are carried on the main chain as non-reducing end groups.

Forrest and Wainwright (1977) reported that the pentosans isolated from endosperm cell walls are not covalently linked to the β-glucans. They had a higher xylose/arabinose ratio than previously reported for barley pentosans. Pentosan molecules extracted by water are smaller than those extracted by alkali (Fincher, 1975; Forrest & Wainwright, 1977). Little difference was observed in the chemical or physical properties of cell wall materials of barley cultivars of different malting qualities (Henry, 1988a).

Although the major structural differences of β-glucan and arabinoxylan have been reported (Bathgate & Dalgliesh, 1975; Fincher & Stone, 1986; Luchsinger, 1967), the knowledge of barley non-starch carbohydrates still

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remains incomplete in many areas and much of the early quantitative work must be questioned because of the materials and methods used at the time. The complexity of the carbohydrates of barley grains and the inadequacies of available methods have prevented complete analysis of the carbohydrate composition of barley grains.

Diabete, Geyer and Stirm (1984), Edge, Langehove and Reinhold (1986), Fournet, Leroy, Wieruzeski, Montreuil, Poretz and Goldberg (1987), and Geyer, Geyer, Kuhnhardt, Mink and Stirm (1983) reported that methylation analysis, using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS), are suitable tools in determining the positions of the glycosidic linkages between monosaccharide units in polysaccharides, oligosaccharides, glycopeptides, and glycolipids. GC has been used to separate and quantitate the partially methylated alditol acetate (PMAA) derivatives of monosaccharides obtained in the linkage analysis of permethylated oligosaccharides (Carpita & Shea, 1989).

Previously, sugar composition, soluble- and insoluble-NSP and ratio of arabinose to xylose of the total NSP were reported on enzyme-treated flours of selected commercial six-rowed malting barley, malt and pilot-brewed beer (Han & Schwarz, 1996). In the present study, the objectives were to obtain qualitative and quantitative information on total NSP using GC and GC–MS methods. The identity and levels of methylated glycosyl components of total NSP, arabinoxylan and β-glucan, were determined because of their great importance in the malting and brewing of barley.

2. Materials and methods

2.1. Barley and malt samples

Two barleys and two commercially malted samples of the cultivar Robust and one sample each of the cultivar Excel were used for this study. The samples were obtained from Froedtert Malt Corporation, Milwaukee, WI. Barley was from the 1992 crop year. These cultivars had been part of a blend causing beer filtration problems during commercial brewing. Filtration flow rate was significantly slowed due to the plugging of filter pores.

A modification of high pressure air-abrasion (Harris & Scott, 1947) and solvent washing method (Viëter, et al., 1991) was used to avoid contamination of the sample with husk arabinoxylans, husk and radicles. Dehusked barley was ground to pass through a 0.5 mm mesh screen on UDY cyclone sample mill (UDY Co., Boulder, CO). Ground barley and malt samples were further solvent washed for removal of husk particles, as described in Han and Schwarz (1996) and air-dried.

2.2. Pilot brewing

Three commercially malted samples were ground, mashed, lautered, and boiled in tandem at the North Dakota State University pilot brewery according to a modification of the procedure of Figueroa, Madsen and D'Appolonia (1987). Detailed pilot-brewing methods are described in Han and Schwarz (1996). Each pilot brew used 800 g of malt in 2400 ml of brewing water. No adjunct materials were incorporated. The total yield of wort after mashing and lautering was 4.5 l. Cascade hop pellets (4.0 g) were added during boiling. Cooled wort (12°C) was adjusted to 15°P by the addition of distilled water. The wort was fermented with lager yeast (9.0 g) at 12°C for 7 days.

Fermented beer was filtered through Celite and a Millipore micromembrane filter (Opticap filter with milligard media, 0.5 μm nominal hydrophilic capsule pre-filter, Millipore Co., Bedford, MA). For analysis, the filtered beer was degassed at 20°C. Specific gravity, alcohol content and real extract were measured according to the *Methods of Analysis of the American Society of the Brewing Chemists* (ASBC, 1992). Beer (2 l) was distilled with reduced pressure to remove alcohol, and freeze-dried.

2.3. β-Glucan, glycosyl components, and arabinoxylan determination

The mixed linkage β-glucan content of barley, malt, and freeze-dried beer were determined according to the method of McCleary (1991) using a Megazyme Mixed-linkage β-Glucan Assay kit (Megazyme International, Ireland Limited, County Wicklow, Ireland).

The glycosyl composition and arabinoxylan content of barley, malt, and beer were determined by gas chromatography (GC) using a modification of the method of Carpita and Shea (1989). Solvent-washed barley and malt flours were treated with α-amylase, pullulanase, and protease prior to hydrolysis according to Englyst and Cummings (1998). Freeze-dried beer samples were hydrolyzed for GC analysis without enzyme treatments.

For determination of total non-starch polysaccharides (Total NSP), the finely ground samples (100 mg) were incubated in 2.0 ml of dimethyl sulfoxide (DMSO) at 100°C for 1 h. Sodium acetate buffer (0.1 M, pH 5.0, 2.0 ml) was added, followed by 500 units of α-amylase and 0.5 units of pullulanase. Samples were incubated at 42°C for 16 h, then precipitated for 1 h with 40 ml of ethanol. Precipitated samples were centrifuged at 1500 *g* for 10 min. Ethanol (85% v/v) precipitation was repeated twice more. Samples were washed with acetone for 5 min and air-dried.

The acetylated alditol derivatives of enzyme-treated total NSP samples were prepared along with those from

a standard sugar mixture using the method of Han and Schwarz (1996). Samples were hydrolyzed with 2.0 M trifluoroacetic acid, containing myo-inositol as an internal standard, at 121°C for 1 h. The hydrolysed samples were reduced to the corresponding alditols with sodium borodeuteride ($NaBD_4$) in dimethylsulfoxide (DMSO). The reduced alditols were *O*-acetylated by acetic anhydride in the presence of 1-methylimidazole. After acetylation, the per-*O*-acetylated alditols were partitioned from water into methylene chloride, dried, and dissolved in acetone for injection. The HP-5890 series II gas chromatograph (Hewlett Packard, Inc., Palo Alto, CA) equipped with a SP-2380 type fused silica capillary column (30 m length, 0.25 mm ID, and 0.20 μm film thickness) was used for analysis (Supelco, Inc., Bellefonte, PA). Sugars of interest were expressed as the normalized percent using internal normalization. The arabinoxylan was calculated according to the following formula (Henry, 1986): total arabinoxylan = (% arabinose + %xylose) × 0.88.

2.4. Glycosyl-linkage analysis for arabinoxylans

Glycosyl-linkages were determined using the per-*O*-methylated method of Carpita and Shea (1989), a modification of the Hakomori (1964) procedure as shown in Fig. 1. Samples were per-*O*-methylated in the presence of *n*-butyllithium with methyl iodide (CH_3I), and the per-*O*-methylated sugars partitioned into chloroform and dried. Then the partially-*O*-methylated, partially-*O*-acetylated alditols (PMAA) were prepared by hydrolysis, reduction, and acetylation as described above (Han & Schwarz, 1996). Methylated samples were hydrolyzed with trifluoroacetic acid, reduced with sodium borodeuteride and *O*-acetylated with acetic anhydride. GC–MS analysis of the methylated derivatives was on a SP-2330 fused silica capillary column (30 m length, 0.25 mm ID, 0.20 μm film thickness), in the splitless mode (Supelco, Bellefonte, PA). The GC–MS system consisted of a HP-5890A Gas Chromatograph and HP-5970B Series Mass Selective Detector (MSD) (Hewlett-Packard Instrument Inc., Palo Alto, CA). The GC temperature program was 3 min at an initial temperature of 80°C, increasing temperature to 170°C at 30°C min^{-1}, immediately increasing to 240°C at a rate of 4°C min^{-1}, and holding at 240°C for 8.4 min. The temperature of both injection port and detector was 270°C. The flow rate and pressure of helium carrier gas were at 118.0 ml min^{-1}, and 878.8 g cm^{-2}, respectively.

A retention time map of PMAAs of each neutral sugar standard was constructed. Configuration of the sugar composition of each sample was identified by comparing retention time to the retention time map and by mass spectra of each fragment of the PMAA of sugar standards.

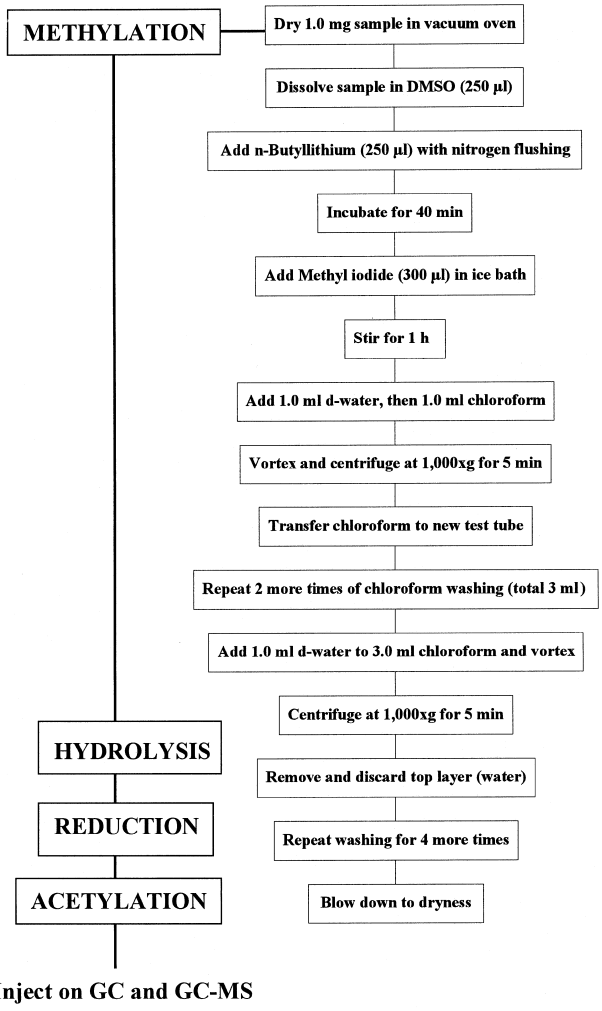

Fig. 1. Flow chart of methylation procedure for partially-*O*-methylated, partially-*O*-acetylated alditols (PMAA) derivatives of sugar.

2.5. Quantification of partially-O-methylated and partially-O-acetylated alditol (PMAA) derivatives of sugars

PMAA derivatives of the glycosyl residues of samples, prepared by the method of Carpita and Shea (1989), were analyzed on the GC. The acetylated PMAA of neutral sugar derivatives were used as a standard.

Each methylated sample was dissolved in acetone and analyzed on a HP-5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and Integrator (Hewlett-Packard Instrument Inc., Palo Alto, CA). The column was a Supelco SP-2380 fused silica capillary column of 30 m length, 0.25 mm ID, and 0.20 μm film thickness (Supelco Inc., Bellefonte, PA). Injections of the PMAA derivatives (1.0 μg) were made, using the splitless mode with 949.2 g cm^{-2} column head pressure. The initial oven temperature was 80°C with 3 min holding time, and the temperature was increased to 170°C at a rate of 30°C min^{-1}. The temperature was

then increased immediately to 240°C at a rate of 4°C min^{-1}, and the temperature was held at 240°C for 20 min. The temperature of both injection port and detector was 275°C.

Each PMAA GC sugar peak of the chromatogram was identified by comparing of mass spectra from GC-MS. The MRF was calculated with the "effective carbon response (ecr)" theory, originally based on the predicted ionization potential of organic constituents in a flame ionization detector (FID) (Sweet, Shapio & Albersheim, 1975), and the peak areas of identified fractions were converted into the mole percentage. The quantity of methylated sugar derivatives was expressed as the mole percentage (mol%).

2.6. Statistical analysis

The levels of each glycosyl-linkage derivative present in barley, malt, and beer were evaluated by the Analysis of Variance and Least Significant Difference (PROC ANOVA/LSD) procedure of the Statistical Analysis System (SAS, 1985). All analyses were carried out on duplicate samples, and were reported on a dry weight basis.

3. Results and discussion

Three commercial malting barley samples, Robust A, Robust AB, and Excel, were used in this study. Table 1 shows the total NSP (non-starch polysaccharide), arabinoxylan and β-glucan contents in barley, malt, and beer samples. The composition of total NSP in barley, malt and beer, including the ratio of arabinose to xylose, was reported in an previous study by Han and Schwarz (1996). No significant differences ($P < 0.05$) in levels of total NSP, arabinoxylan, and β-glucan were observed between varieties except for the β-glucan level in malt flour and beer. The total NSP was reduced to an average 34% of barley flour after malting. Robust AB barley flour contained the highest amount (18.2%) of total NSP; it decreased significantly ($P < 0.05$) by 71% after malting, compared to 63 and 64% in Robust A and Excel samples, respectively. The arabinoxylan contents in barley and malt flour were 7.1–8.0 and 3.1–4.0%, respectively. There were no statistically significant differences in arabinoxylan levels between the barley and malt flour and those present in beer. The average β-glucan levels in all samples were in agreement with the range reported by Henry (1986, 1988a).

Barley, malt, and pilot brewed beer from three selected six-row malting barleys were methylated (Fig. 1) to determine the glycosyl linkage composition of their non-starch carbohydrates. The complete chemical names and their abbreviated names of deduced linkage of each sugar derivative, are found in Table 2, followed by the quantitative results in Tables 3 through 5. The gas chromatogram of partially-*O*-methylated, partially-*O*-acetylated alditols (PMAA) of Robust AB beer are shown, as an example, in Fig. 2 A and B. After methylation of the enzyme treated samples (Han & Schwarz, 1996) and preparation of the acetylated alditol derivatives of sugars released by hydrolysis, the sugar linkages were determined by identifying the resulting partially-*O*-methylated, partially-*O*-acetylated alditol acetates (PMAA) by GC–MS using retention time and mass spectra recorded in the literature (Bjorndal, Lindberg & Svensson, 1967; Carpita & Shea, 1989; Jansson, Kenne, Liedgren, Lindberg & Lonngren, 1976; Sweet, Shapiro & Abersheim, 1975).

In chromatogram (Fig. 2A), terminal galactopyranose (*t*-Gal-*p*), identified through mass spectra identification,

Table 1
Total non-starch polysaccharides, arabinoxylan and β-glucan in barley, malt and beer

	Robust A	Robust AB	Excel	LSD[a]
Barley (%, w/w)				
Total NSP[b]	17.8	18.2	17.3	2.2
Arabinoxylan	8.0	7.5	7.1	2.1
β-Glucan	3.7	4.0	3.9	1.1
Malt (%, w/w)				
Total NSP	6.7	5.4	6.2	3.9
Araninoxylan	4.0	3.1	3.7	1.7
β-Glucna	0.6	0.5	0.5	0.0
Beer (%, w/v)				
Total NSP	0.5	0.5	0.3	0.3
Arabinoxylan	0.3	0.3	0.2	0.2
β-Glucan	0.1	0.1	0.0	0.0

[a] Least significant difference.
[b] Non-starch polysaccharide.

Table 2
Chemical names and their abbreviations of PMAA sugar derivatives

Chemical names	Deduced linkage end
1,4-di-*O*-acetyl-(1-deuterio)-2,3,5-tri-*O*-methyl arabinitol	*t*-Ara-f
1,3,4-di-*O*-acetyl-(1-deuterio)-2,5-tri-*O*-methyl arabinitol	3-Ara-f
1,4,5-di-*O*-acetyl-(1-deuterio)-2,3-tri-*O*-methyl arabinitol	4-Ara-*p*
1,4-di-*O*-acetyl-(1-deuterio)-2,3,5-tri-*O*-methyl xylitol	*t*-Xyl-*p*
1,2,5-tri-*O*-acetyl-(1-deuterio)-3,4-di-*O*-methyl xylitol	2-Xyl-*p*
1,4,5-tri-*O*-acetyl-(1-deuterio)-2,3-di-*O*-methyl xylitol	4-Xyl-*p*
1,2,4,5-tetra-*O*-acetyl-(1-deuterio)-3-*O*-mehtyl xylitol	2,4-Xyl-*p*
1,3,4,5-tetra-*O*-acetyl-(1-deuterio)-2-*O*-mehtyl xylitol	3,4-Xyl-*p*
1,2,3,4,5-penta-*O*-acetyl-(1-deuterio)-methyl xylitol	2,3,4-Xyl-*p*
1,5-di-*O*-acetyl-(1-deuterio)-2,3,4,6-tetra-*O*-methyl galactitol	*t*-Gal-*p*
1,2,5-tri-*O*-acetyl-(1-deuterio)-3,4,6-tri-*O*-methyl galactitol	2-Gal-*p*
1,4,5-tri-*O*-acetyl-(1-deuterio)-2,3,6-tri-*O*-methyl galactitol	4-Gal-*p*
1,5-di-*O*-acetyl-(1-deuterio)-2,3,4,6-tetra-*O*-mehtyl glucitol	*t*-Glu-*p*
1,4,5-tri-*O*-acetyl-(1-deuterio)-2,3,6-tri-*O*-methyl glucitol	4-Glu-*p*
1,5,6-tri-*O*-acetyl-(1-deuterio)-2,3,4-tri-*O*-mehtyl glucitol	6-Glu-*p*

Table 3
Mol% of partially-*O*-methylated, partially-*O*-acetylated alditols (PMAA) of barley flour

Linkage	Robust A	Robust AB	Excel	LSD[a]
t-Ara-f	1.4	1.5	1.2	1.5
3-Ara-f	2.3	tr[b]	5.8	0.2
4-Ara-f	1.9	2.1	2.0	2.1
t-Xyl-*p*	2.4	1.6	1.7	2.2
2-Xyl-*p*	6.7	7.3	5.4	1.1
4-Xyl-*p*	20.1	21.9	16.2	3.3
2,4-Xyl-*p*	4.3	4.9	4.7	1.3
3,4-Xyl-*p*	4.3	4.9	4.7	1.3
2,3,4-Xyl-*p*	13.5	19.0	11.7	4.8
t-Gal-*p*	1.5	1.7	3.0	2.8
2-Gal-*p*	2.6	2.6	3.4	4.6
4-Gal-*p*	3.8	4.1	3.1	0.4
t-Glu-*p*	17.7	12.9	22.6	6.2
4-Glu-*p*	15.5	12.3	17.8	0.8
6-Glu-*p*	2.0	3.2	1.0	2.6

[a] Least significant difference.
[b] Trace.

is shown as peak e. However, the *t*-Gal-*p* peak in Fig. 2B was only recognized as a trace amount (Tables 4 and 5). The molar response factor (MRF) of partially methylated sugars was used to quantify sugar derivatives of peaks from the flame ionization detector (FID) of the GC. The results are shown in Tables 3–5 and reported as mole percent (mol%)

As shown in Fig. 2, four major peaks (c, g, k, and m) in all barley, malt and beer samples were identified by methylation analysis of the neutral sugars. These major peaks were the methylated glycitol peracetates of *t*-Glu-*p*, 4-Xyl-*p*, 4-Glu-*p*, and 2,3,4-Xyl-*p*, respectively. Several sugar derivatives were also present as minor components (less than 5 mol%). From the construction of a retention time map of PMAA of neutral sugar standards, a 1:4 ratio of 2-Xyl-*p* and 4-Xyl-*p* were found in peak g. Although 2-Xyl-*p* residues were not expected in our samples, according to the mass spectra of our standard, 2-Xyl-*p* was present in barley, malt and beer. However, the apparent level may be more than the actual level due to difficulty in our GC–MS procedure. Equal parts (1:1 ratio) of 2,4-Xyl-*p* and 3,4-Xyl-*p* were found in peak l. A possible linkage composition is predicted, based on the results obtained, in which arabinoxylans are envisaged as containing randomly branched regions, mostly consisting of an unsubstituted and a double arabinofuranosylated xylose residue. The branched regions of arabinoxylans are enriched in both *O*-2,3 disubstituted (2,3,4-Xyl-*p*), and *O*-2 (2,4-Xyl-*p*) and *O*-3 (3,4-Xyl-*p*) monosubstituted, xylose residues.

The galactose derivatives found in these samples were believed to be methylated fractions from arabinogalactan. The small amounts of galactose found in isolated barley arabinoxylan factions have been reported to be fractions from arabinogalactan in both barley and malt (Fincher, 1975; Henry, 1988a). Although the starch was hydrolyzed from the samples by enzyme treatment during sample preparation (Han & Schwarz, 1996), it is possible that the starch was not completely removed by subsequent washing steps, shown as a small amount of 6-Glu-*p* derivatives. The considerable amount of *t*-Glu-*p* in pilot-brewed beer could be from dextrins, possibly maltose and isomaltose. Both methylated 3-Glu-*p* and 4-Glu-*p* derivatives from the β-glucan fractions in barley and malt were expected to be present; however 3-Glu-*p* residues were not detected in our sample. The ratio of 3-Glu-*p* and 4-Glu-*p* in barley and malt was estimated at 3:7, but only the 4-Glu-*p* was detected, possibly because of its higher pronounced presence in β-glucan (Bathgate & Dalgliesh, 1975). Since the focus of study was on the arabinoxylan structure in barley, malt and beer, the inconsistency relating the β-glucan was not pursued. Further, research on the subject, using nuclear magnetic resonance, is recommended.

The mol% of PMAA from dehusked and solvent-washed barley flour is shown in Table 3. Comparing the ratio between PMAA derivatives, the (1→4)-β-xylan chain in Robust AB was the longest, followed by Robust A and Excel barley sample. The lengths of (1→4)-β-xylan chains in Robust A and AB varieties were significantly different ($P < 0.05$) from the Excel variety. In all three barley samples, however, approximately the same number of xylose units were substituted and twice as many of xylose were disubstituted (C-2 and C-3 position) as were single-substituted, (C-2 or C-3 position). The Robust AB contains significantly more ($P < 0.05$) disubstituted xylose than the other samples. On the other hand, the terminal arabinose (*t*-Ara-f) was not identified clearly enough to account for substitution through GC–MS and GC analyses. A lower occurrence of non-reducing terminal Xyl-*p* units is reported in endospermic arabinoxylan (Bengtsson & Åman, 1990). We also found that at least one branch arabinose was ester-linked with another compound, possibly 4-hydroxy-3-methoxy-cinnamic acid (ferulic acid), through the C-3 position of arabinose, previously suggested by Nishitani and Nevins (1988).

In Table 4, the mol% of PMAA from solvent-washed malt flour is shown. Although similar patterns of arabinoxylan structure in the barley flours were anticipated, longer (1→4)-β-xylan chain length was predicted from the ratio of their mol%. The configurational patterns of each malt sample show a pattern similar to corresponding barley samples, except that higher disubstitution was found in Robust A and AB. Robust A and AB varieties contained longer and more dibranched configurations of 4-Xyl-*p* and 2,3,4-Xyl-*p* residues compared to Excel. Table 5 shows the mol% of PMAA from pilot-brewed beer. Robust AB, again, contained the most

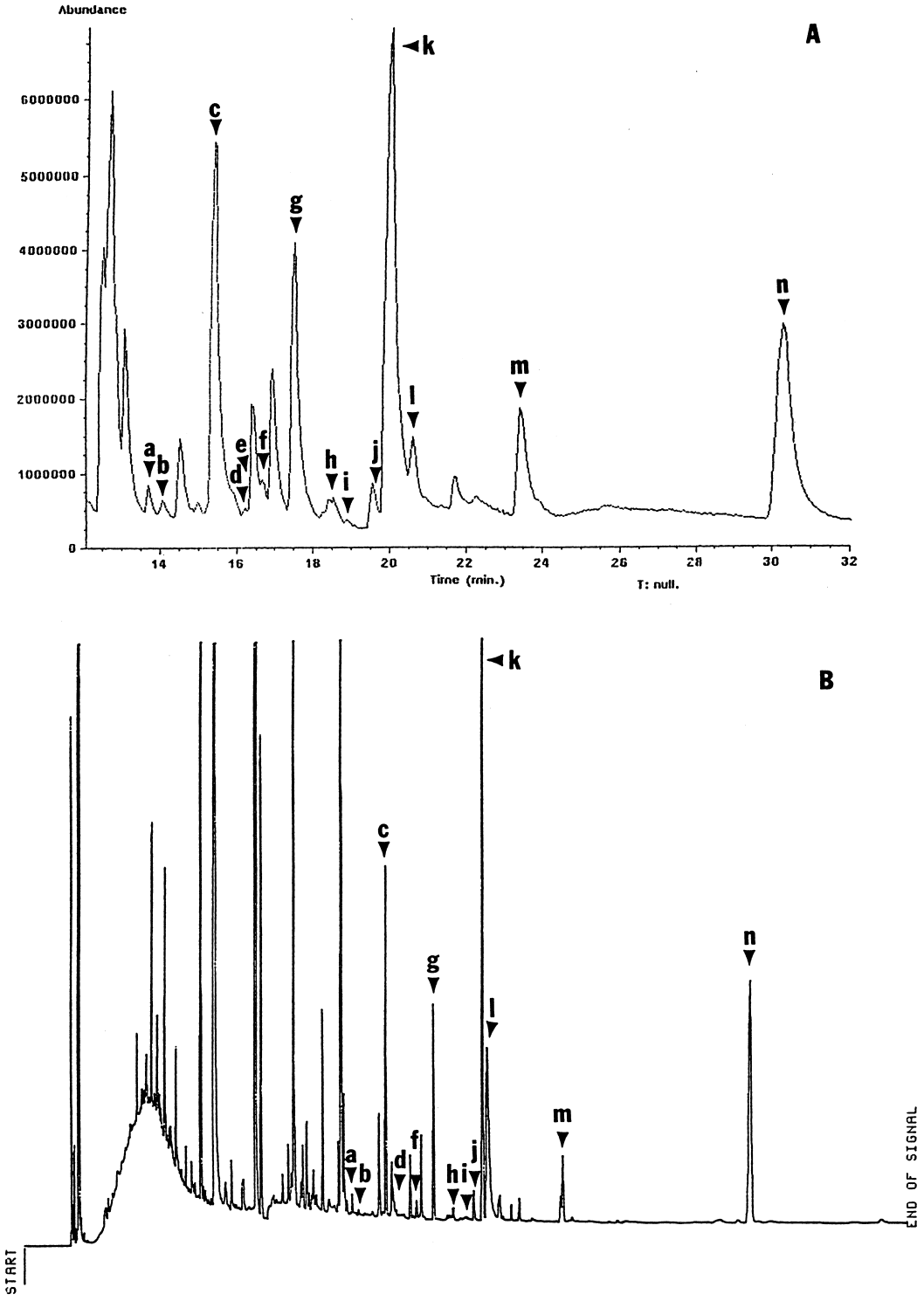

Fig. 2. Gas chromatogram from GC and GC–MS of partially-*O*-methylated, partially-*O*-acetylated alditol (PMAA) derivatives of Robust AB beer. A: GC–MS chromatogram of PMAA of Robust AB beer. B: GC chromatogram of PMAA of Robust AB beer. a: *t*-Ara-f, b: *t*-Xyl-*p*, c: *t*-Glu-*p*, d: 3-Ara-f, e: *t*-Gal-*p*, f: 4-Ara-*p*, g: 2-Xyl-*p* and 4-Xyl-*p* (1:4), h: 2-Gal-*p*, i: 4-Gal-*p*, j: 6-Glu-*p*, k: 4-Glu-*p*, l: 2,4-Xyl-*p* and 3,4-Xyl-*p* (1:1), m: 2,3,4-Xyl-*p*, and n: Inositol (internal standard).

branched configuration. No apparent differences were shown among the three beer samples.

In all cases, not enough terminal α-L-arabinofuranosyl (*t*-Ara-f) residues were identified to account for all branches of β-D-xylopyranosyl residues. Methylated/acetylated derivatives of terminal arabinofuranosyl residues are volatile and easily lost during analysis, which may explain the low molecular percent of this

Table 4
Mol% of partially-*O*-methylated, partially-*O*-acetylated alditols (PMAA) of malt flour

Linkage	Robust A	Robust AB	Excel	LSD[a]
t-Ara-f	3.4	1.5	1.4	1.9
3-Ara-f	3.3	1.5	2.6	2.6
4-Ara-f	2.0	1.6	1.5	1.5
t-Xyl-*p*	2.1	2.1	0.8	2.4
2-Xyl-*p*	9.4	7.2	6.5	0.8
4-Xyl-*p*	28.3	21.6	19.5	2.5
2,4-Xyl-*p*	3.8	5.1	5.1	1.4
3,4-Xyl-*p*	3.8	5.1	5.1	1.4
2,3,4-Xyl-*p*	17.3	17.0	16.0	5.1
t-Gal-*p*	1.8	tr[b]	1.6	0.0
2-Gal-*p*	1.2	2.1	2.1	2.4
4-Gal-*p*	0.9	1.9	1.5	1.5
t-Glu-*p*	11.1	13.7	19.1	2.9
4-Glu-*p*	8.9	17.7	16.2	1.4
6-Glu-*p*	1.0	1.7	1.9	0.8

[a] Least significant difference.
[b] Trace.

Table 5
Mol% of partially-*O*-methylated, partially-*O*-acetylated alditols (PMAA) of pilot-brewed beer

Linkage	Robust A	Robust AB	Excel	LSD[a]
t-Ara-f	1.0	1.1	1.8	0.8
3-Ara-f	0.6	0.5	4.0	3.2
4-Ara-f	1.5	1.5	3.6	2.3
t-Xyl-*p*	0.9	0.5	1.3	1.1
2-Xyl-*p*	3.3	4.0	3.9	0.4
4-Xyl-*p*	10.0	12.0	11.8	1.0
2,4-Xyl-*p*	3.0	2.9	2.8	2.1
3,4-Xyl-*p*	3.0	2.9	2.8	2.1
2,3,4-Xyl-*p*	7.7	9.0	8.3	3.7
t-Gal-*p*	tr[b]	tr	1.7	0.6
2-Gal-*p*	1.7	1.5	2.8	1.2
4-Gal-*p*	0.5	0.8	0.5	1.4
t-Glu-*p*	25.6	23.0	20.4	6.6
4-Glu-*p*	39.1	37.8	32.9	5.2
6-Glu-*p*	2.1	2.4	2.3	1.1

[a] Least significant difference.
[b] Trace.

component compared to the branched xylose residues (Bengtsson & Åman, 1990). However, the substitution patterns are in agreement with previously published results by Viëtor, Angelino and Voragen (1990a). Although the amount of substituted xylosyl residues was comparable, the linkage composition in wort arabinoxylan was very different from other arabinoxylans. Viëtor, Angelino and Voragen (1990a) reported that the wort arabinoxylan contains more double substituted xylose residues than single-substituted xylose residues. Viëtor and Voragen (1993) also speculated that these differences in xylose substitution may be related to the differences in water-extractability, although other factors, such as covalent interactions and physical entanglement, may have been involved.

4. Conclusions

The possible structure of arabinoxylans in selected commercial six-rowed barley and malt flour, and pilot-brewed beers were studied. The levels of total NSP, arabinoxylan and β-glucan were not significantly ($P < 0.05$) different between varieties and were found to be similar to previously published data (Henry, 1986; Henry, 1988a; Viëtor, Angelino & Voragen, 1991a). Arabinose and xylose accounted for 70% of the composite sugars of total NSP in pilot-brewed beer.

Four major glycosyl derivatives, *t*-Glu-*p*, 4-Xyl-*p*, 4-Glu-*p*, and 2,3,4-Xyl-*p*, were identified along with several minor glycosyl components in all barley, malt, and beer samples. Arabinoxylans are envisaged as containing randomly branched regions, mostly consisted of an unsubstituted and a double arabinofuranosylated xylose residue. The apparent length of (1→4)-β-xylan chains in Robust A and AB were significantly longer ($P < 0.05$) than Excel. Approximately the same numbers of xylosyl residues were *O*-2,3 disubstituted as were *O*-2 and *O*-3 monosubstituted in all varieties. Because of the high degree of substitution and linkage with other compounds, arabinoxylans alone, or synergistically with β-glucan, could result in lautering and/or filtering problems during brewing.

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