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Isolation and characterization of high molar mass water-soluble arabinoxylans from barley and barley malt

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Abstract

Water-soluble arabinoxylans have been fractionated from barley and barley malt by size exclusion chromatography to obtain high molar mass arabinoxylans involved in the viscosity of wort and beer. In both cases, five fractions were obtained: AXb_1, AXb_2, AXb_3, AXb_4 and AXb_5 for barley and AXm_1, AXm_2, AXm_3, AXm_4 and AXm_5 for barley malt. For barley and malt fractions, Ara/Xyl ratio, molar mass and intrinsic viscosity decreased as their elution volume increased. $\bar{M}_w$ of barley fractions were higher than that of malt fractions, attesting arabinoxylans degradation during malting. NMR and methylation studies revealed that whatever the fractions, approximately 50% of the xylose residues were un-substituted. 2,3-Xyl level decreased from AXb_1 to AXb_5 and from AXm_1 to AXm_5, whereas 3-Xyl level increased at the same time. A non negligible amount of 2-Xyl was found, this level being almost the same (6–8%) for all the fractions. AXm_1, which exhibited the highest intrinsic viscosity, (240 ml/g) was considered as an interesting fraction with regard to brewing problems. In spite of its highly branched structure, AXm_1 was easily degraded by xylanases. Evidence for the presence of ferulic acid dimers in the water-soluble arabinoxylans from barley and barley malt is provided.

Keywords: Arabinoxylans; Barley; Brewing; Malting; Viscosity

1. Introduction

Non-starch polysaccharides from barley grain are responsible for problems in the brewing industry such as low extract yields, high wort viscosity, decreased rate of filtration or haze formation in beer (Schwarz & Han, 1995; Viëtor, Voragen, Angelino & Pilnik, 1991). Most of these problems have been attributed to β-glucans, but arabinoxylans, which are present in barley, malt and beer also play an important role in these phenomena (Viëtor & Voragen, 1993). Indeed, β-glucans are almost completely degraded by endogenous β-glucanases during malting (Viëtor et al., 1991), and their content in beer is very low (Schwarz & Han, 1995). Some arabinoxylans are solubilized from the cell walls but are not extensively degraded by endogenous enzymes during malting. Solubilized arabinoxylans are, therefore, responsible for high viscosity of malt water-extract, which possibly leads to problems such as a diminished rate of wort or beer filtration (Fincher & Stone, 1986).

Arabinoxylans constitute 4–8% of the barley kernel (Lethonen & Aikasalo, 1987). They represent ≈ 25 and 70% of the cell wall polysaccharides of endosperm and aleurone layer, respectively (Fincher & Stone, 1986). They consist of a backbone of (1–4)-linked β-D-xylopyranosyl residues. Some of these residues are substituted at O-2, O-3 or at both O-2 and O-3 with α-l-arabinofuranosyl residues. Water-soluble and -insoluble barley arabinoxylans have been studied (Oscarsson, Andersson, Salomonsson & Åman, 1996; Viëtor, Angelino & Voragen, 1992; Viëtor, Angelino, Voragen & Kormelink, 1994a) but, little attention has been paid to barley malt water-soluble ones (Debyser, Schooneveld-Bergmans, Derdelinckx, Grobet & Delcour, 1997). However, as they exhibit a high viscosity in solution, they might be involved in brewing problems. A better knowledge of their structure is of importance to understand and to predict their degradation by endogenous or exogenous enzymes during malting and brewing processes.

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Abbreviations: AX: arabinoxylans; AG: arabinogalactans; Ara: arabinose; Xyl: xylose; Glc: glucose; Gal: galactose; Man: mannose; Araf: arabinofuranose; Xyl p: xylopyranose; Ara/Xyl: arabinose to xylose ratio; BSA: bovine serum albumin; DP: polymerization degree; GLC: gas liquid chromatography; HPLC: high performance liquid chromatography; HPSEC: high performance size-exclusion chromatography; $[\eta]$: intrinsic viscosity; $\bar{M}_w$: molar mass; MALLS: multi-angle laser light scattering; NMR: nuclear magnetic resonance; R_G: radius of gyration; SEC: size-exclusion chromatography; 2-Xyl: 2-O-substituted xylose; 3-Xyl: 3-O-substituted xylose; 2,3-Xyl: 2-O, 3-O-substituted xylose; u-Xyl: un-substituted xylose.

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The aim of this work was to isolate high molar mass water-soluble arabinoxylans from barley and barley malt, which are supposed to be responsible for the viscosity of wort and to study their structure. Water-soluble arabinoxylans from barley and barley malt, extracted at a pilot scale, have been fractionated by size-exclusion chromatography. Arabinoxylans with high molar mass have been isolated. These fractions have been characterized by a variety of methods, including NMR and methylation analysis, laser light scattering, viscosity measurements and enzymic degradations.

2. Experimental

2.1. Material

Water-soluble arabinoxylans from barley (AXb) and barley malt (AXm) (Alexis, harvest 1994) were isolated at a pilot scale according to a procedure previously described (Faurot, Saulnier, Berot, Popineau, Petit, & Rouau et al., 1995).

The following enzymes were used: Lichenase (EC 3.2.1.73. from Megazyme, 1000 U/ml from *Bacillus subtilis*), β-glucosidase (EC 3.2.1.21. from Megazyme, 40 U/ml from *Aspergillus niger*), Arabinofuranosidase (EC 3.2.1.21. from Megazyme, 250 U/mg, from *A. niger*), and different xylanases (EC 3.2.1.8.): one from Megazyme (xylanase 1) (157 U/mg, from *Trichoderma viride*) and two from Novo, (xylanase 2 and xylanase 3) (150 and 190 U/mg, respectively cloned monocomponent enzymes from *Aspergillus aculeatus* expressed in *Aspergillus oryzae*).

Lichenase and β-glucosidase were shown to be free of contaminating endoxylanase activities, since the viscosity (Ostwald viscometer) of solutions of AXb and AXm (2 mg/ml) was not decreased by adding 10 U of the enzymes.

Xylosidase and arabinofuranosidase activities were tested in the xylanases using 4-nitrophenyl-β-D-xylopyranoside and 4-nitrophenyl-α-L-arabinofuranoside as substrates. No contaminations with xylosidases and arabinofuranosidases were found in the commercial mixtures (data not shown).

2.2. Removal of β-glucans

Water-soluble polysaccharides from barley were solubilized in distilled water (1 g in 100 ml), solution was incubated at 40°C with 200 U of Lichenase for 1 h, pH 6.5, and then with 4 U of β-glucosidase for 30 min at pH 4.5. The enzymes were inactivated by heat (100°C, 10 min) and the polysaccharides were precipitated with ethanol 80% (1 night, 4°C). The precipitate was redissolved in water and freeze-dried: it represents AXb.

2.3. Fractionation of polysaccharides

AXb and AXm were solubilised (250 mg in 5 ml of 0.05 M NaCl) and injected on a Sephacryl S500 HR column (100 × 2.6 cm) and eluted with 0.05 M NaCl at a flow-rate of 100 ml/h at room temperature. Fractions (6 ml) were collected and analysed for neutral sugar content by automated orcinol method (Tollier & Robin, 1979). To obtain sufficient material, the chromatography was repeated 3 times and 11 times, for barley and malt, respectively.

For both material, five fractions were collected and were referred to as AXb_1, AXb_2, AXb_3, AXb_4, AXb_5 and AXm_1, AXm_2, AXm_3, AXm_4, AXm_5 for barley and malt fractions, respectively.

2.4. Chemical analyses

Neutral sugars were determined by hydrolysis (Englyst & Cummings, 1988) of the polysaccharides with 2N sulphuric acid at 100°C for 2 h. Individual sugars were then converted into aldidol acetates and analysed by GLC. Analyses were made in duplicate and arabinoxylan content was calculated from the sum of arabinose and xylose, after correction from the presence of arabinogalactans assuming an arabinose to galactose ratio of 0.7 (Loosveld, Maes, Van Casteren, Schols, Grobet & Delcour, 1998).

The phenolics were analysed as previously described (Saulnier, Crepeau, Lahaye, Thibault, Garcia-Conesa, & Kroon, 1999) after deesterification in 2N NaOH for 30 min at 35°C in the dark.

Protein contents were determined according to Bradford (1976), using BSA as standard.

2.5. Macromolecular parameters

AX were dissolved (5 mg/ml) for 2 h at 40°C under magnetic stirring, filtered over 0.45 μm membrane and injected at 25°C on a high performance size-exclusion chromatography (HPSEC) system constituted of two Shodex OH-pack SB HQ 804 and 805 columns eluted at 0.7 ml/min with 50 mM $NaNO_3$, containing 0.02% NaN_3. On-line molar mass and intrinsic viscosity determinations were performed at room temperature using a multi-angle laser light scattering (MALLS) detector (mini-Dawn^R, Wyatt, Santa Barbara, operating at 3 angles: 41, 90 and 138°), a differential refractometer (ERC 7517 A) ($dn/dc = 0.146$ ml/g), an UV detector ($\lambda = 280$ nm) and a differential viscometer (T-50A, Viscotek). $\bar{M}_w$ and R_G were determined using Astra 1.4 software (Wyatt) and intrinsic viscosity $[\eta]$ using Trisec software (Viscotek).

2.6. NMR

^{1}H-NMR spectra (400 MHz) were recorded at 60°C on a Brücker ARX spectrometer. Arabinoxylans were dissolved in D_2O (10 mg/ml). D_2O was used as reference. 128 pulses were collected with a pulse repetition time of 4 s and pulse angle of 6 μs.

2.7. Methylation analysis

The polysaccharides were methylated (Hakomori, 1964) and hydrolysed with 2N trifluoroacetic acid (120°C, 1.25 h). The partially methylated sugars were converted into their alditol acetates and analysed by GLC as previously described (Saulnier, Mestres, Doublier, Roger & Thibault, 1993). Identification of the peaks was based on relative retention times.

2.8. Enzymic degradation

To AXm_1 solutions (0.1% in acetate buffer pH 6) 2 U of the xylanases were added. Enzymic degradation was followed by viscosimetry at 40°C using an Ostwald capillary tube and by HPSEC. Intrinsic viscosities were calculated using Huggins and Kraemer equations.

AXm_1 solutions (0.5% in acetate buffer pH 4.5) were incubated with arabinofuranosidase (12 U, 40°C, 1 h). Liberated arabinose was measured by the method of Melrose and Sturgeon (1983). The oligomers were analysed on a DIONEX bioLC system equipped with a Carbopac PA I (250 × 4 mm) column and a pulse amperometric detector (PAD) with a gold electrode. Gradient elution was performed using water (A), 0.1 M NaOH (B) and 0.4 M NaOAc/0.1 M NaOH (C) solvents at 1 ml/min at 25°C; applied gradient was 80% A/20% B for 20 min, 100% B from 21 to 50 min, 100% C from 51 to 60 min and 80% A/20% B from 61 to 75 min.

3. Results and discussion

3.1. Extraction

Extraction on a large scale of barley and barley malt gave from 50 kg of both materials, 604 and 217 g of water-soluble polysaccharides, respectively.

However, water-soluble polysaccharides from barley were highly contaminated with glucose arising from β-glucans. β-glucans were not found in malt because they have been degraded by endogenous barley β-glucanases during malting (Viëtor et al., 1994a). Attempts to fully separate β-glucans from water-soluble barley arabinoxylans using stepwise fractionation by EtOH or $(NH_4)_2SO_4$ failed. An enzymic treatment was therefore applied with Lichenase and β-glucosidase. Water-soluble arabinoxylans from barley and barley malt were referred to as AXb and AXm, respectively.

Chemical compositions of the two starting materials are given in Table 1. After correction from the presence of arabinogalactans, arabinoxylans represent 75 and 85.7 mol% of neutral sugars from AXb and AXm, respectively. Removal of β-glucans from barley water-soluble polysaccharides was efficient since AXb contains only 5.8 mol% of residual glucose. Water-soluble arabinoxylans represent 0.1% (w/w) of barley flour, whereas they represent 0.2% (w/w) of malt flour: water-soluble arabinoxylans are therefore in larger amount in malt than in barley. Previous result (Vermeylen, 1962) indicated a 4.5 higher amount of water-soluble arabinoxylans in malt than in barley. This increase of arabinoxylans proportion after malting attests that some are solubilised during this step. In contrast, Debyser et al. (1997) have obtained comparable levels of water-soluble arabinoxylans in barley and in barley malt.

Arabinose to xylose ratios of the arabinoxylans from barley and malt were close to 0.65, in agreement with Debyser et al. (1997).

Galactose arising from arabinogalactans was also present: assuming an arabinose to galactose ratio of 0.7 (Loosveld et al., 1998), they represent 16.6 and 11.8 mol% of neutral sugars in AXb and Axm, respectively.

Table 1
Composition of the initial samples and of their fractions

Samples	Yields[a]	Sugar composition (mol%)[b]					AX[c](mol%)	AG(mol%)	Ara/Xyl[c]	Proteins[b](g/100 g AX)	Ferulic acid[d](g/100 g AX)
		Ara	Xyl	Man	Gal	Glc					
Axb	–	36.8	45.7	2.7	9.0	5.8	74.9	16.6	0.64	29	0.62
Axm	–	38.8	52.3	0.1	6.4	2.4	85.7	11.8	0.64	23	0.95
Axb_1	7.3	45.9	47.7	0	2.5	3.9	91.5	4.6	0.92	Nd	1.10
Axb_2	5.7	41.8	51.9	0.9	4.0	1.4	90.3	7.4	0.74	13	0.91
AXb_3	10.8	37.1	48.0	0.6	11.0	3.3	75.8	20.3	0.58	9	0.50
AXb_4	34.4	33.6	41.1	1.3	17.9	6.1	59.6	33.0	0.45	12	0.64
AXb_5	41.8	28.2	30.6	3.6	19.0	18.6	42.8	35.0	0.40	41	0.91
AXm_1	2.6	43.1	48.6	0.7	0.9	6.7	90.8	1.7	0.87	1	0.29
AXm_2	7.1	41.7	51.5	0.5	3.7	2.6	90.1	6.8	0.75	4	0.66
AXm_3	22.4	38.3	50.0	0.9	5.1	5.7	84.0	9.4	0.68	12	0.76
AXm_4	39.3	37.9	47.0	0.9	8.8	5.4	77.5	16.2	0.65	25	1.05
Axm_5	28.6	37.2	46.4	0.8	9.5	6.1	75.6	17.5	0.63	31	1.05

[a] Based on total amount recovered after fractionation.
[b] Results obtained from duplicates, coefficients of variation <4%.
[c] Corrected from the presence of arabinogalactans.
[d] Results obtained from duplicates, coefficients of variation <2% AX = %Xyl + (%Gal × 0.7 × 180/150)) and AG = %Gal + (%Gal × 0.7 × 180/150)).

Some proteins were also co-extracted; they represented approximately the same proportion of the samples in AXb and in AXm.

Ferulic acid contents (0.62 and 0.95 g/100 g AX) were in both cases higher than those found by Izydorzyk, Biliaderis and Bushuk (1991) and Dervilly, Saulnier, Roger and Thibault (2000) (0.1–0.2% (w/w)) for wheat water-soluble arabinoxylans. These results confirm that ferulic acid esterified not only water-insoluble barley arabinoxylans but also water-soluble barley arabinoxylans (Ahluwalia & Fry, 1986). Furthermore, ferulic acid dimers: 8-O-4′, 8-5′ and 5-5′ forms, were also present in AXb and AXm (Table 2), suggesting that some of the water-soluble arabinoxylans might be cross-linked. Similar results have been observed for barley spent grain (Bartolomé, Faulds, Kroon, Waldron, Gilbert & Hazlewood, 1997; Bartolomé & Gomez-Cordovés, 1999) and for wheat endosperm water-soluble arabinoxylans (Dervilly et al., 2000). AXm had higher levels of dimers than AXb, suggesting that coupling of ferulic acid might have occurred during malting or during extraction, due to the presence of peroxidases and polyphenol oxidases (Cochrane, 1994; Rasmussen, Henriksen, Abelskov, Jensen, Hejgaard & Welinder, 1997).

Physicochemical characteristics are given in Table 3. Molar mass of AXb (340 g/mol) was higher than that of AXm (250 g/mol). Few data are available in literature on molar mass determination of arabinoxylans from barley or barley malt. Very high molar mass (1 000 000 g/mol) was estimated by gel filtration (Forrest & Wainwright, 1977), whereas considerably lower value (58 800 g/mol) was found by sedimentation velocity techniques (Podrazky, 1964). Higher value for barley than barley malt indicated a degradation during malting (Viëtor et al., 1992). Intrinsic viscosities were low for both materials, especially when compared to values determined for water-soluble non-starch polysaccharides from barley (450 ml/g) (Girhammar & Nair, 1992). However, their sample was mainly composed of β-glucans (48%), arabinoxylans representing only 14%.

3.2. Arabinoxylans fractionation

AXb and AXm have been fractionated on size-exclusion chromatography on Sephacryl S500 (Fig. 1). Chromatographic yields (in neutral sugars) were 77% for AXb and 80% for AXm. The elution profile showed, for AXb and AXm, that most of the arabinoxylans ($\approx 70\%$) had low hydrodynamic volumes (Kav > 0.8). In both cases, five fractions were pooled as indicated. Yields of fractions based on total amount recovered after fractionation are shown on Table 1.

Table 2
Ferulic acid dimers content (Nd: not detected; results obtained from duplicates, coefficients of variation <6%)

	Ferulic acid dimers (μg/g AX)			
	5-5′	8-O-4′	8-5′	8-8′
Axb	35	67	13	Nd
Axm	391	652	600	Nd

Table 3
Macromolecular characteristics ($\bar{M}_w$: molar mass; R_G radius of gyration; I: polydispersity index; $[\eta]$: intrinsic viscosity)

Arabinoxylan	$\bar{M}_w \times 10^{-3}$ (g/mol)	R_G (nm)	I	$[\eta]$ (ml/g)
Axb	340	38	5.1	60
Axm	250	19	4.6	52
AXb_1	–[a]	–	–	Nd
AXb_2	370	42	2.6	165
AXb_3	315	43	2.6	71
AXb_4	280	35	2.9	53
AXb_5	215	33	2.9	20
Axm_1	–[a]	–	–	240
Axm_2	340	36	3.8	160
Axm_3	94	35	2.2	80
Axm_4	54	33	1.4	31
Axm_5	47	30	1.4	31

[a] data not given (overestimated values due to aggregation phenomena).

3.3. Chemical composition of barley and malt fractions

Barley fractions were mainly composed of arabinoxylans (Table 1). Fractions AXb_3, AXb_4 and AXb_5 were contaminated with galactose arising from arabinogalactans. Glucose concentrated in AXb_5 but cannot be ascribed to the presence of β-glucans or starch, specific determination of these two compounds being negative. Arabinose to xylose ratios decreased from 0.92 to 0.40 when fractions were obtained at higher elution volumes, indicating that fractions exhibiting different structures have been obtained. Purity of the fractions decreased from AXb_1 to AXb_5: they contained more arabinogalactans and proteins when increasing elution volume.

Chemical composition of malt fractions showed the same

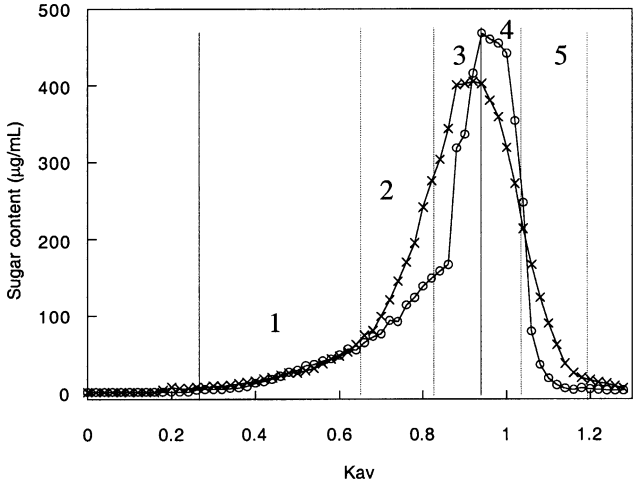

Fig. 1. Elution profile of AXb (x) and AXm (o) obtained by chromatography on a gel permeation Sephacryl S500 column eluted with 0.05 M NaCl (orcinol detection).

trends: arabinose to xylose ratios decreasing from AXm_1 to AXm_5. Galactose and protein content increased from AXm_1 to AXm_5 suggesting that proteins were mainly bound to contaminating arabinogalactans.

Ferulic acid content increased from AXm_1 to AXm_5 indicating that it increased when the substitution degree of the fractions decreased, as already seen for water-soluble wheat endosperm arabinoxylans (Dervilly et al., 2000), such a trend was not observed for the barley fractions.

3.4. Physico-chemical characteristics

Molar masses, radius of gyration and intrinsic viscosities of barley and malt fractions decreased as elution volume increased. In both cases, AXb_1 and AXm_1 exhibited high hydrodynamic volume and high intrinsic viscosities (Fig. 2). However, their molar masses are not indicated in Table 3, since they were overestimated because of aggregating phenomena. Aggregation is observed by the occurrence at the void elution volume of a laser light scattering signal without any refractometric signal. In malt, arabinoxylans obtained at high elution volumes (from AXm_3 to AXm_5) have lower molar masses than their counterparts in barley, giving evidence of arabinoxylans degradation during malting (Debyser et al., 1997).

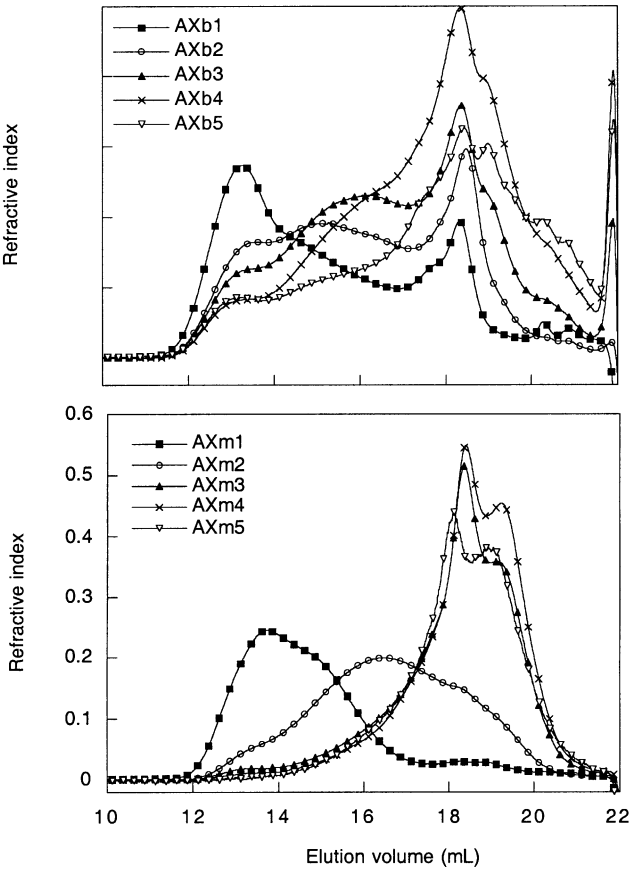

Fig. 2. Refractometric profiles of barley and barley malt fractions (5 mg/ml in $NaNO_3$ 50 mM) on Shodex OH-pack 804 and 805 columns.

3.5. ^{1}H-NMR and methylation analysis

Methylation results are shown in Table 4. All the fractions are mainly constituted of substituted xylose residues ($\approx$ 50–65%), as previously observed by Oscarsson, Andersson, Salomonsson and Åman (1996) and Debyser et al. (1997). Fractions of high molar mass (AXb_1, AXm_1) are more di-substituted than fractions of low molar mass. This high level of di-substituted xylose residues was also evident from NMR results (Fig. 3), where anomeric protons of *Araf* linked to O-3 and O-2 of the same *Xylp* were responsible for peaks at 5.23 and 5.28 ppm. Proportions of 2-Xyl and 3-Xyl determined were in the lower range of values reported by Oscarsson et al. (1996) (6–15% and 11–20%, respectively) for barley water-soluble arabinoxylans.

For barley and barley malt, fractions recovered from SEC at the same elution volume were structurally similar.

So, AXb_1 and AXm_1 represent high molar mass arabinoxylans exhibiting a high intrinsic viscosity and an original structure, most of the arabinose residues being present as di-substitution.

3.6. Enzymic degradations

The highly di-substituted structure determined for AXm_1 allowed to envisage a rather difficult enzymic degradation of the polymer. Indeed, study on xylanases (Kormelink, Gruppen & Voragen, 1993) indicates that xylanases are sensitive to the arabinose distribution along the xylan backbone and that high level of di-substitution might restrict their efficiency. An increase of the arabinose to xylose ratio is known to decrease the xylanases activity, endo-xylanases cleaving mainly un-branched parts of the backbone. Moreover, 2-Xyl pattern of substitution is also reported to restrict *endo*-xylanases activity (Oscarsson et al., 1996; Viëtor, Angelino, Voragen, Hoffman, Kamerling & Vliegenthart, 1994b).

Action of different xylanases has been followed by viscosity measurements (Fig. 4). A decrease of specific viscosity is observed whatever the xylanase used. Xylanase 1 (from *Trichoderma*) hydrolysed the polymer faster than xylanases 2 and 3 (from *Aspergillus*), but to a lesser extent.

Table 4
Proportions (%) of un-, mono- and di-substituted xylose residues calculated from methylation analysis

Arabinoxylans	2-Xyl	3-Xyl	2,3-Xyl	u-Xyl
Axb	5.0	9.0	22.0	64.0
Axm	6.0	9.0	25.0	60.0
AXb_1	7.0	4.0	26.0	57.0
AXb_2	6.0	5.0	21.0	65.0
AXb_3	6.5	5.5	22.0	64.0
AXm_1	8.5	3.5	31.0	50.0
AXm_2	8.0	4.6	24.0	60.0
AXm_3	8.0	6.0	23.0	60.0

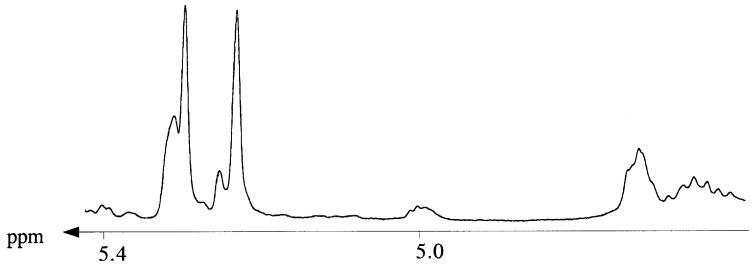

Fig. 3. NMR spectrum of the anomeric proton of arabinose residue from fraction AXm1.

Intrinsic viscosity decreased from 240 to 70 ml/g with xylanase 1 and to 45 ml/g with xylanases 2 and 3. Refractometric profile (Fig. 5) gives evidence that a population of arabinoxylans (approximately 10% w/w of AXm1, calculated according to the area under the refractometric profile) resists xylanases activity.

HPLC analysis of the reaction mixture showed the absence of short oligomers (DP < 6), suggesting that the substitutions are rather evenly distributed along the xylan backbone and that long un-substituted regions do not exist on the xylan backbone.

Treatment with arabinofuranosidase released arabinose (30% of total arabinose), and with the addition of xylanases 2 and 3 intrinsic viscosity decreased to 15 ml/g. Arabinofuranosidase have improved xylanase action, confirming that arabinose substitutions on the xylan backbone interfere with xylanases action.

4. Conclusion

Barley and barley malt water-soluble arabinoxylans have been fractionated according to their hydrodynamic volume by size-exclusion chromatography. In both cases, high molar mass fractions have been obtained. Their propensity to aggregate as well as their high viscosity could explain, at least partly, filtering problems in the beer industry. Arabinoxylans from these fractions have a very high Ara/Xyl ratio. 50% of their xylose residues are un-substituted, 31% are di-substituted, 3.5% are mono-substituted via O-3 and 8.5% via O-2. This particular di-substituted structure could be responsible for a limited enzymic degradation during mashing. Although xylanases activity leads to a fall in viscosity, no small oligomers are liberated during the hydrolysis.

The validation of the effect of each malt fractions on wort viscosity has been achieved in TEPRAL (Leclercq, Dervilly, Saulnier, Dallies, Zimmermann & Roue, 1999). Their functionality in wort has been studied by measuring the filtration capacity in wort. High molar mass AX showed an elevated viscosity and affected wort filtration, confirming their negative effect on wort filtration performances. Aggregating phenomena of higher molar mass fractions as observed on a laboratory scale would be likely to occur during brewing where extreme temperatures are used (high then low) enhancing aggregates formation and probably leading to plugged filters. It would be of great interest to follow the evolution of these high molar mass arabinoxylans during all the process. Furthermore, ferulic acid dimers were detected in rather high proportion in malt arabinoxylans, suggesting that water-soluble arabinoxylans

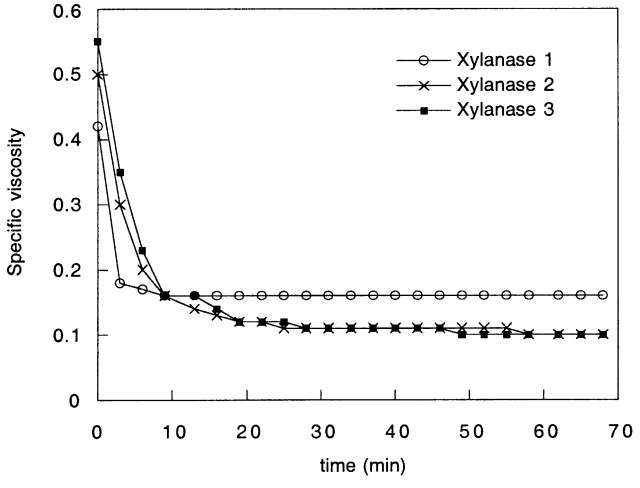

Fig. 4. Enzymic degradation of AXm1 by different xylanases followed by viscosimetry at 40°C using an Ostwald capillary tube.

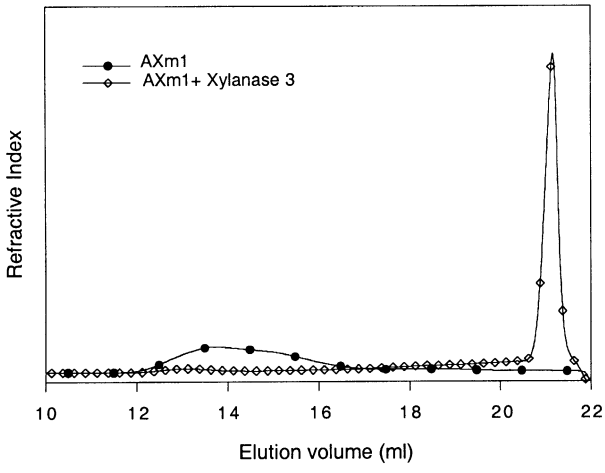

Fig. 5. Refractometric signal of AXm1 and AXm1 after enzymic degradation with xylanase 3 and chromatographied on a Shodex OH-pack 804 and 805 columns eluted with $NaNO_3$ 50 mM

might be partially cross-linked, affecting possibly the filtering problems.

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