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Experimental evidence for a semi-flexible conformation for arabinoxylans☆

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Abstract

Purified water-soluble arabinoxylans from wheat flour were deferuloylated and fractionated into six fractions by graded ethanol precipitation. Further fractionation by HPSEC on Sephacryl S500 resulted in 48 subfractions with low polydispersity index. Conformational characteristics (persistence length q, hydrodynamic parameter v and Mark–Houwink exponent a) were similar among all subfractions and fitted with a semi-flexible conformation, whatever their structural characteristics. Substitution degree of the xylan backbone by arabinose residues has no influence on the conformation of arabinoxylans. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Arabinoxylans conformation; Ferulic acid ester; Macromolecular characteristics; Laser light scattering; NMR spectroscopy; Viscosimetry

1. Introduction

Xylans as well as arabinoxylans (AX) are known from X-ray fiber diffraction data, as extended chains involving twisted ribbon like strands, with threefold symmetry.[1–3] Xylans conformation is often compared to that of β-(1→4)-linked polysaccharides such as cellulose and mannans.[4] The β-(1→4)-D-xylan chain is however more flexible than the two-fold helix of β-(1→4)-cellulose, since there is only one hydrogen bond between adjacent xylosyl residues[2] as compared to two hydrogen bonds between adjacent glucosyl residues in cellulose.[5] The absence of a primary alcohol functional group external to the pyranoside ring as in cellulose and mannan, has a dramatic effect on the intra- and inter-chain hydrogen bonding interactions.

In cereal grains, AX are non starch polysaccharides from cell walls.[6] Arabinoxylans from endosperm are partly water-soluble and result in highly viscous aqueous solutions. This high viscosity of cereal grain water extract has a positive effect in some technological processes (bread-making)[7] but is generally considered as a negative parameter for the use of cereal grains in animal feeding and brewing. AX are constituted of a linear backbone of β-(1→4)-linked D-xylopyranosyl units to which α-L-arabinofuranosyl substituents are attached through O-2 and/or O-3; some ferulic acid

Abbreviations: a, Mark–Houwink exponent; AX, arabinoxylans; Ara, arabinose; HPSEC, high performance size exclusion chromatography; I, polydispersity index; MALLS, multi-angle laser light scattering; Mw, weight-average molar mass; MwXyl, weight-average molar mass of the xylan backbone; Mn, number-average molar mass; $[\eta]$, intrinsic viscosity; R_G, radius of gyration; SEC, size exclusion chromatography; q, persistence length; v, hydrodynamic parameter.

☆ Arabinoxylans conformation, Part 1.

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may esterify arabinofuranoyl residues at O-5. On average, an arabinose to xylose ratio of 0.6 is usually found in wheat water-soluble AX[8,9] but high natural variations are observed.[10,11] Previous authors[12] have ascribed the high viscosifying effect of AX to an extended rod-like conformation. Furthermore, it was generally assumed that variations in Ara/Xyl ratio affect the behavior of AX, the increase in arabinose content leading to a more 'stiffened' and extended conformation.[12,13]

In a previous paper,[14] we have shown that water-soluble feruloylated AX behaved as random coil in solution and that the substitution degree of the xylan backbone by arabinose residues apparently did not affect the conformation of the polysaccharide. However, ferulic ester bridges have been detected in these fractions which indicate that the behavior of the polysaccharide in solution do not only depend on the degree of polymerization (DP) of the xylan backbone and on its degree of substitution but also on the number of cross-links. In this report, AX conformation has been studied with regard only to the influence of Ara/Xyl ratio, after removal of ferulic acid ester constituents using laser light scattering and viscosimetry.

2. Experimental

Isolation and deesterification of arabinoxylans.—Water-soluble purified feruloylated arabinoxylans from wheat flour were prepared as previously described[14,15] and treated at rt for 2 h with 1 N NaOH. The deesterification was monitored by UV spectroscopy: at pH 10, ester linked ferulic acid has a maximum of absorption at 375 nm and free ferulic acid at 342 nm. The proportion of free and esterified ferulic acid was measured as previously described.[16] After complete deesterification, the mixture was neutralized with 12 N HCl, then precipitated with 4:1 EtOH–water and allowed to stand at 4 °C overnight. The precipitate was recovered by centrifugation (13,000*g*, 20 min), washed twice with EtOH 80%, ground in EtOH 100%, washed with acetone, and then dried in an oven at 40 °C overnight resulting in 30 g of pure and non feruloylated AX.

Fractionation of arabinoxylans.—The pure AX were fractionated by graded ethanol precipitation as described previously.[14] A concentration of 0.5% in AX was used in order to avoid coprecipitation due to physical entanglement of arabinoxylan chains. Fractions obtained were designated F20, F30, F40, F50, F60 and F70 (the numbers refer to the saturation level of EtOH at which the material was collected).

The fractions obtained by EtOH precipitation, F30–F70, were solubilized (260 mg in 40 mL) in 0.1 M NaCl, filtered over a 0.45 μm membrane and eluted on a Sephacryl S500 HR column (100 × 5 cm) with 0.1 M NaCl at a flow rate of 300 mL/h at rt. Fractions (18 mL) were collected and analyzed for neutral sugar content by the automated orcinol method.[17] Tubes were pooled in 48 subfractions referenced as $F30_{1-10}$, $F40_{1-10}$, $F50_{1-10}$, $F60_{1-10}$ and $F70_{1-8}$.

Chemical analyses.—The monosaccharide composition was determined according to the method of Englyst and Cummings:[18] polysaccharides were hydrolyzed with 2 N sulfuric acid at 100 °C for 2 h. Individual sugars were then converted into alditol acetates and analyzed by gas-liquid chromatography. Analyses were made in duplicate (coefficients of variation $< 2\%$) and the arabinoxylan content was calculated from the sum of arabinose and xylose (except in purified water-soluble arabinoxylans and F70 where arabinose arising from arabinogalactans was estimated from galactose content assuming an arabinose to galactose ratio of 0.7).[19]

The absence of ferulic acid in AX was checked by HPLC.[20]

Physico-chemical determinations.—The purified polysaccharides were dissolved (5 mg/mL) for 2 h at 40 °C under magnetic stirring, filtered over 0.1 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 rt using a multi-angle laser light scattering (MALLS) detector (miniDawn®, Wyatt, USA, operating at three an-

gles: 41, 90 and 138°), a differential refractometer (ERC 7517 A) ($dn/dc = 0.146$ mL/g) and a differential viscometer (T-50A, Viscotek, USA). M_w and R_G were determined using ASTRA 1.4 software (Wyatt, USA) and $[\eta]$ using TRISEC software (Viscotek, USA).

The polydispersity index $I = M_w/M_n$ was calculated from SEC-MALLS determination.

Using intrinsic viscosity calculated from viscosimetric determination and molar mass calculated from SEC-MALLS determination for the different subfractions, the Mark–Houwink relation, $[\eta] = K \cdot M_w^a$, was obtained by plotting log[η] as a function of log M_w. Since arabinose residues are present only as side groups and do not contribute to chain elongation M_w of the xylan backbone (M_w/Xyl) were used in our calculations (i.e. molar mass was corrected from arabinose contribution).

Using values of R_G and M_w obtained from SEC-MALLS determinations for the different subfractions, relation $R_G = K' \cdot M_w^v$ was obtained by plotting log R_G as a function of log M_w. As described above, molar mass of the xylan backbone (M_wXyl) was used in our calculations.

Persistence length (q) representing the chain rigidity, was determined by the equations:[21] $q = (C_\infty + 1) \cdot I_0/2$ (where C_∞, the characteristic ratio is obtained from: $C_\infty = 6 \cdot R_G^2 \cdot M_0/I_0^2 \cdot M_w$, $I_0 = 0.54$ nm (length of a β-D-xylopyranose residue), $M_0 = 132$ g/mol (molar mass of a anhydro-xylose residue), and M_w the molar mass of the xylan backbone (M_wXyl) as described above.

NMR spectroscopy.—^{1}H NMR spectra (400 MHz) were recorded at 60 °C on a Brücker ARX spectrometer with 128 pulses collection and a pulse repetition time of 4 s and a pulse angle of 6 μs. Arabinoxylans were dissolved in D_2O (10 mg/mL).

3. Results and discussion

Chemical, physico-chemical and structural characterization of the fractions.—Composition of the purified water-soluble AX is given in Table 1. Purified AX contained mainly arabinose and xylose as neutral sugars. Galactose and proteins from arabinogalactan-proteins were also present.[6] Traces of glucose were still observed. Purified AX were free from any ferulic acid constituent. The substitution degree (0.55) of the xylan backbone with arabinose residues is in the range usually observed for wheat endosperm AX (0.53–0.70).[8,9,14,22] Macromolecular properties are reported in Table 2. Molar mass value of 280,000 g/mol is higher than that obtained by other authors using sedimentation techniques[12,23] or gel filtration[10,22] and lower than that determined by gel filtration.[24] This molar mass, as well as radius of gyration and intrinsic viscosity were lower than those obtained for feruloylated AX.[14]

Table 1
Composition of the purified non-feruloylated arabinoxylans and of fractions resulting from ethanol precipitation

	Yields [a]	Sugar content [b] (weight %)					A/X	Proteins [b] (weight %)
		Glc	Xyl	Ara	Man	Gal		
Purified AX		0.7	28.9	25.9	nd	14.0	0.55 [c]	8.7
F20	2.3	[d]	[d]	[d]	[d]	[d]	[d]	[d]
F30	51.2	nd	66.9	26.7	nd	nd	0.39	1.5
F40	4.7	nd	55.9	32.3	0.1	nd	0.58	3.5
F50	19.3	nd	52.3	35.2	0.7	nd	0.67	3.1
F60	16.9	nd	47.1	38.7	nd	0.2	0.82	2.7
F70	5.5	0.5	28.0	32.4	0.4	13.2	0.83 [c]	8.7

[a] Based on total amount of material recovered.
[b] Results obtained from duplicates, coefficients of variation <2%.
[c] Corrected from the presence of arabinogalactans. nd, not detected.
[d] Not studied.

Table 2
Macromolecular characteristics of purified AX and of ethanol precipitated fractions

	A/X	$M_w \times 10^{-3}$ [a] (g/mol)	R_G [a] (nm)	I	$[\eta]$ [a] (mL/g)	q [b] (nm)
Purified AX	0.55	280	38	1.8	320	6.2
F30	0.39	215	38	1.3	320	7.1
F40	0.58	200	36	1.5	325	7.8
F50	0.67	223	39	1.6	320	8.6
F60	0.82	285	40	1.7	330	7.8
F70 [c]	0.83	243	37	1.7	300	7.8

[a] Coefficients of variation $<5\%$.
[b] Persistence length calculated on the basis of unbranched arabinoxylans.
[c] For F70, values were determined without the arabinogalactans contribution.

Ethanol precipitations.—Ethanol fractionation of the purified AX gave five fractions: F30, F40, F50, F60 and F70. Their yields and chemical compositions are given in Table 1. Based on the total amount recovered after ethanol fractionation, Fraction F30 was the major one. The ethanol precipitation curves (Fig. 1) of feruloylated AX are different,[14] indicating that the removal of ferulic acid substituents has changed the behavior of AX.

Fractions F30, F40, F50 and F60 were essentially composed of arabinose and xylose. Fraction F70 was contaminated with galactose arising from arabinogalactans which co-precipitate with AX. Proteins content was very low in Fractions F30–F60 whereas F70 presented a high content of proteins due to the presence of arabinogalactan-proteins in this fraction.[6] Although feruloylated and non-feruloylated AX behaved differently upon ethanol precipitation, similar trends were observed for Ara/Xyl ratios which increased from F30 to F70.[9,10,14,25]

A typical HPSEC–MALLS chromatogram is shown in Fig. 2. Macromolecular characteristics of AX from Fractions F30–F70 are reported in Table 2. Weight-average molar masses are around 200,000–283,000 g/mol, intrinsic viscosities in the range 300–325 mL/g and radii of gyration around 35–40 nm. Values determined from feruloylated AX obtained at the same ethanol concentrations were higher ($M_w = 300{,}000$–$400{,}000$ g/mol and $R_G = 45$ nm).[14] Deesterification of AX has allowed to get rid of ferulic acid dimers which cross-linked their chains. All the fractions exhibited a relatively high-index of polydispersity (1.3–1.7).

Gel filtration chromatography on Sephacryl S500.—In order to get populations of lower polydispersity index, Fractions F30–F70 were passed over Sephacryl S500 column resulting

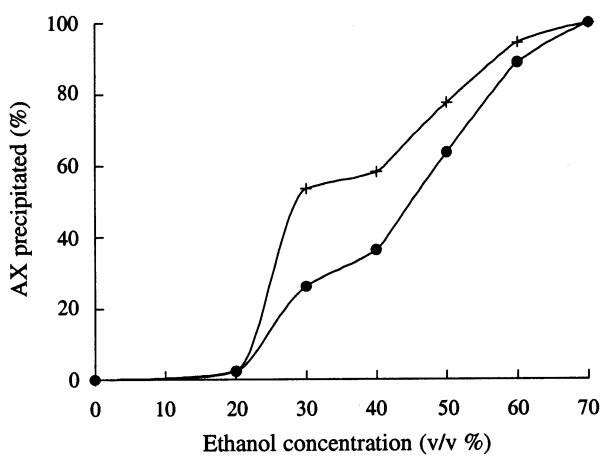

Fig. 1. Cumulative precipitation plot of AX with increasing ethanol concentration. (●) feruloylated AX; (+) non-feruloylated AX.

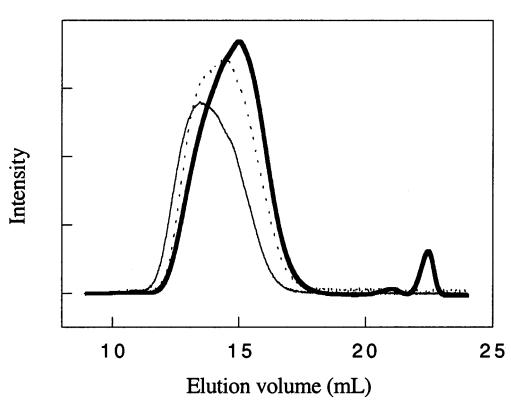

Fig. 2. Elution profile of Fraction F30 obtained by chromatography on Shodex OH-pak 804 and 805 columns. —, refractometric signal; —, laser light scattering signal; ---, viscosimetric signal.

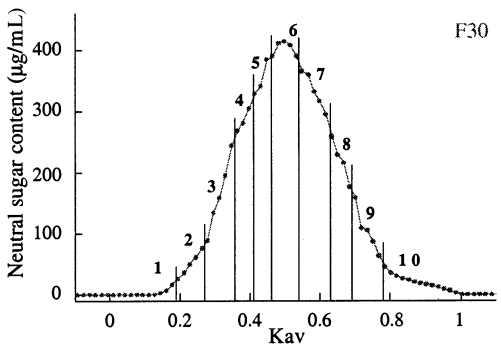

Fig. 3. Elution profile obtained on a gel permeation Sephacryl S500 column for Fraction F30.

in 48 subfractions which have been pooled as indicated (Fig. 3). Yields were 84, 93, 85, 97 and 75% for F30, F40, F50, F60 and F70, respectively.

Each subfraction was characterized using ^{1}H NMR spectroscopy and the relative proportion of unsubstituted, monosubstituted and disubstituted xyloses are presented in Fig. 4. Overall, monosubstitution levels remained rather low ($< 20\%$) and constant within all subfractions. Disubstitution level increased as the ethanol concentration increased whereas unsubstituted xylose level decreased. Within each ethanol fraction, subfractions with high hydrodynamic volumes were more disubstituted and less unsubstituted than those with lower hydrodynamic volumes. As already re-

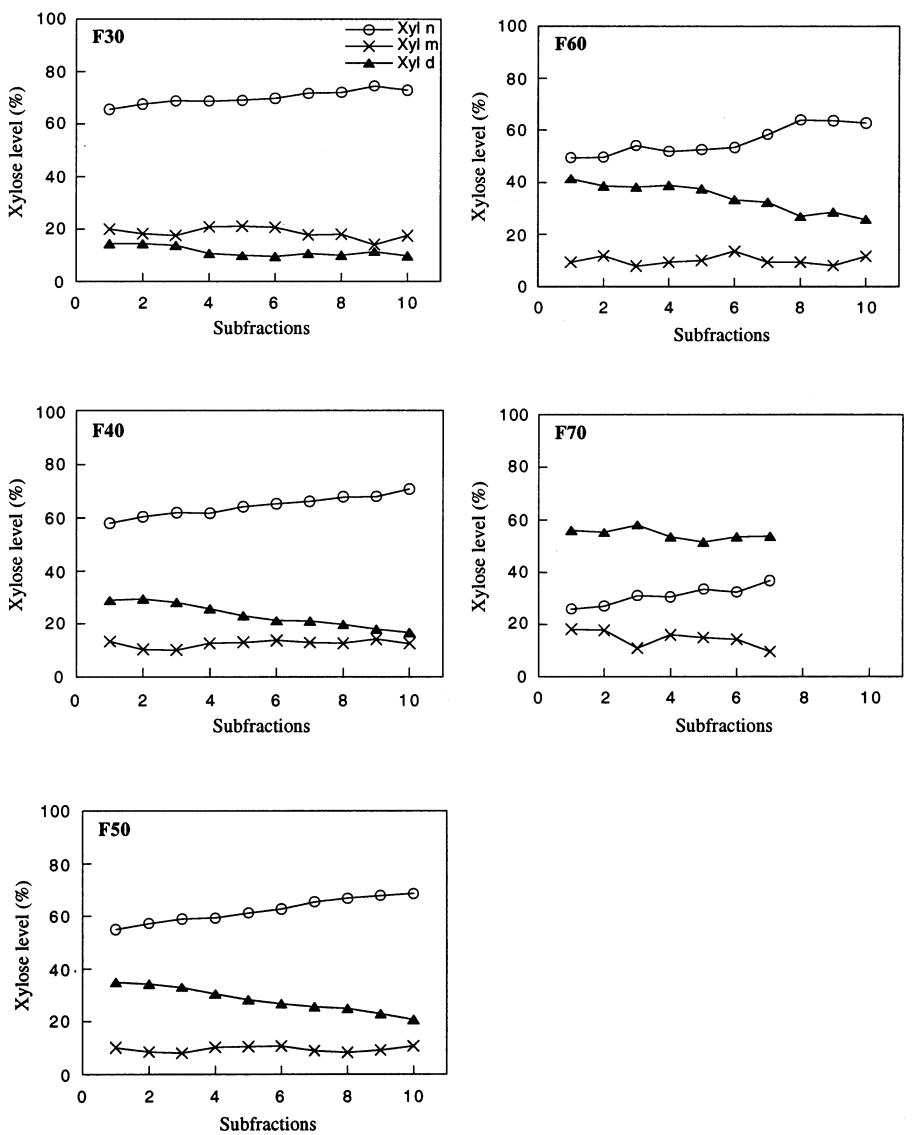

Fig. 4. Relative percentage of the various substitution patterns for each subfraction. Xyl n, m, d, xylose residues, respectively, non-substituted, monosubstituted via O-3 and disubstituted via O-2 and O-3.

Table 3
Macromolecular characteristics of the 48 subfractions

Fractions	Ara/Xyl	$M_w \times 10^{-3}$ (g/mol)	$[\eta]$ (mL/g)	R_G (nm)	I	q (nm)
F30.1	0.49	460	630	55	1.1	7.5
F30.2	0.47	405	625	50	1.1	6.9
F30.3	0.45	300	550	44	1.1	7.1
F30.4	0.42	230	460	39	1.1	7.2
F30.5	0.41	175	380	34	1.2	7.1
F30.6	0.40	150	345	32	1.3	7.3
F30.7	0.39	140	330	31	1.3	7.3
F30.8	0.38	125	285	31	1.3	8.1
F30.9	0.37	115	280	28	1.4	7.1
F30.10	0.37	115	280	28	1.4	7.1
F40.1	0.71	535	680	58	1.1	8.2
F40.2	0.69	470	625	52	1.0	7.4
F40.3	0.66	260	510	40	1.0	7.8
F40.4	0.64	195	425	32	1.1	6.6
F40.5	0.59	170	330	33	1.2	7.7
F40.6	0.56	125	200	32	1.3	9.6
F40.7	0.55	120	260	31	1.4	9.4
F40.8	0.52	120	240	29	1.4	8.1
F40.9	0.50	100	250	nd	1.4	nd
F40.10	0.46	90	165	d	1.4	nd
F50.1	0.80	530	740	57	1.1	8.4
F50.2	0.77	410	675	50	1.1	8.2
F50.3	0.74	300	560	41	1.1	7.4
F50.4	0.71	235	455	37	1.1	7.6
F50.5	0.67	175	375	32	1.1	7.4
F50.6	0.64	150	305	30	1.2	7.5
F50.7	0.60	130	275	28	1.2	7.3
F50.8	0.58	105	185	26	1.3	7.7
F50.9	0.55	94	185	25	1.4	7.8
F50.10	0.52	90	185	nd	1.4	nd
F60.1	0.92	660	620	1	1.1	8.2
F60.2	0.89	590	600	57	1.1	7.9
F60.3	0.84	465	480	50	1.1	7.5
F60.4	0.87	330	400	45	1.1	8.7
F60.5	0.85	230	300	37	1.2	8.3
F60.6	0.80	185	260	32	1.2	7.6
F60.7	0.74	130	200	30	1.2	9.1
F60.8	0.63	125	220	27	1.3	7.2
F60.9	0.65	75	140	nd	1.3	nd
F60.10	0.63	70	130	nd	1.4	nd
F70.1	1.30	655	540	59	1.1	9.2
F70.2	1.28	535	520	51	1.1	8.4
F70.3	1.27	400	425	45	1.1	8.7
F70.4	1.23	240	310	33	1.1	7.7
F70.5	1.18	155	220	25	1.0	6.7
F70.6	1.21	115	160	nd	1.2	nd
F70.7	1.17	85	110	nd	1.2	nd
F70.8	0.84	45	40	nd	1.4	nd

ported,[14] the arabinose to xylose ratio reflects the level of disubstituted xyloses.

A large chemical heterogeneity was observed within each ethanol precipitated fraction as shown from the decrease of arabinose to xylose ratio when the elution volume increased (Table 3). Isolated subfractions exhibited a low polydispersity index, especially when obtained at low elution volumes. According to size exclusion mechanisms, molar

masses, intrinsic viscosities and radii of gyration decreased as the elution volume increased.

Conformationnal results — influence of substitution on arabinoxylans conformation.— In order to study the influence of the substitution on the xylan chain conformation, the molar mass of the xylan backbone (M_wXyl) was used since arabinose residues are present as side groups and do not contribute to chain elongation.

Persistence lengths determined for the different fractions (Tables 2 and 3) were similar confirming that chain rigidity was not affected by the degree of substitution. Furthermore, values calculated ($q = 6–8$ nm) indicated that arabinoxylan chains are semi-flexible, in comparison with very flexible polysaccharides ($q = 1.7$ nm) such as amylose or pullulans,[21] or very stiff polymer such as xanthan which exhibit a persistence length of about 37 nm for single chain[26] and up to 100–150 nm for double stranded chains.[26,27] Galactomannans which exhibit a linear backbone of β-(1→4)-linked D-mannose units with single α-(1→6)-linked D-galactose substituents are close to AX according to their structures. Molecular modeling and experimental studies, using SEC combined with a MALLS detector, have respectively predicted and determined their persistence lengths: 9.6 and 9.3 nm,[28] which indicate that galactomannans behave as semi-flexible polymers.

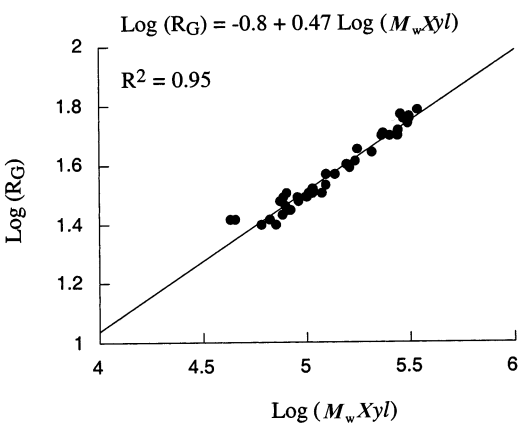

Fig. 5. Plot of log(R_G) against log(M_wXyl). Calculation of the hydrodynamic parameter v for arabinoxylans.

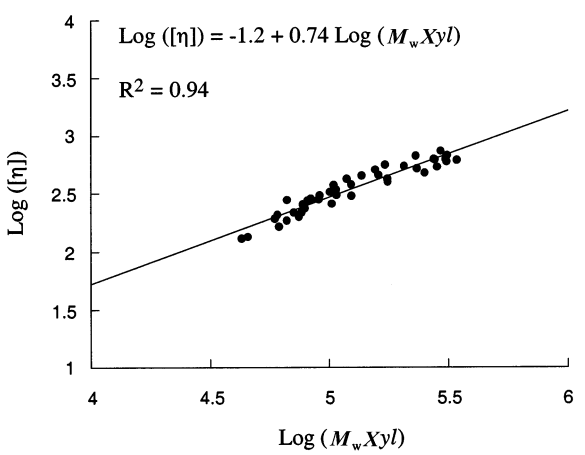

Fig. 6. Plot of log([η]) against log(M_wXyl): Mark–Houwink relationship for arabinoxylans.

Relation obtained between M_wXyl and R_G with values from SEC-MALLS determinations (Fig. 5) gives a slope, v, equal to 0.47, typical of a random coil conformation.

The Mark–Houwink equation $[\eta] = K \cdot M_w^a$, where both K and a are constants for a given well-defined polysaccharide–solvent system has been established. In Fig. 6, interception on the Y-axis gives $\log K$ (0.06) and the slope gives parameter a, related to chain conformation. The value determined for a (0.74) is characteristic of a random coil. Same trends have been obtained on rye water-soluble arabinoxylans[29] with a Mark–Houwink parameter close to 0.9 for molecules with molar masses in the range 50,000–30,0000 g/mol. Mark–Houwink relations with $a = 0.72$ have been established for galactomannans.[30,31] The authors have also concluded to a random coil conformation in solution, for polymers with molar masses in the range 50,000–1,000,000 g/mol.

4. Conclusions

Homogeneous fractions have been obtained from pure non feruloylated wheat water-soluble arabinoxylans. It has been shown that non-feruloylated AX behave differently as compared to feruloylated ones when submitted to ethanol fractionation. This can be explained by differences in macromolecular arrangement for both populations, feruloylated polysaccharides being partially bridged with ferulic acid resulting in a larger size for the molecule.

Our data indicate that arabinoxylans behave as semi-flexible random-coils, a result which is in conflict with previous results which assign them an extended rod-like conformation.[12] Previous results on arabinoxylans conformation[12] were obtained using sedimentation equilibrium ultracentrifugation technique on very polydisperse samples and the molar mass of AX samples were presumably underestimated (M_w: 65,000 g/mol for an intrinsic viscosity [η] of 620 mL/g) so that an inaccurate extended conformation has been deduced.

Conformational characteristics were similar for all subfractions whatever their structure, leading to the conclusion that structural characteristics have no influence on macromolecular properties of AX. Similar conclusions have already been drawn on feruloylated wheat water-soluble AX.[14]

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