ELSEVIER

PII: S0144-8617(96)00032-X

Carbohydrate Polymers 31 (1996) 41–46

0144-8617/96/$15.00 + .00

Static and dynamic light scattering studies of heteroxylans from maize bran in aqueous solution

E.Chanliaud, P. Roger, L. Saulnier* & J.-F. Thibault

Laboratoire de Biochimie et Technologie des Glucides, INRA BP 1627, 44316 Nantes, Cedex 03, France

(Received 6 October 1995; accepted 1 December 1995)

Macromolecular features of four heteroxylan samples extracted from maize bran under different conditions of temperature, type and concentration of alkali were determined by light scattering measurements in 80 mM $NaNO_3$ after elimination of the aggregates by filtration through 0.2 μm pore size filters. The macromolecular parameters were similar for all four samples, with a slight molecular weight dependence on extraction conditions. Static and dynamic results were confirmed by SEC-MALLS measurements. The radii of gyration were $\approx$ 40 nm and the hydrodynamic radius $\approx$ 20 nm for a weight-average molecular weight of $\approx 2.8 \times 10^5$ g/mol and a polydispersity $\approx$ 2. The structural parameter ρ (= 1.6–2.1), calculated from these results, was characteristic of a branched rather rigid polymer. The hydrodynamic parameter ν_1 (= 0.3) indicated a compact structure, which could be due to the side chains or to a 'fringed micelle' organisation of the macromolecules. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The heteroxylans are cell wall polysaccharides which represent almost 40% of the maize bran. They mainly consist of a backbone of β-(1-4)-linked D-xylose residues highly substituted on *O*-2 or/and *O*-3 by short side chains. These branches are mainly composed of single arabinose residues, but longer chains of 2–3 sugar residues (containing arabinose, xylose and galactose) have also been reported (Whistler, 1989; Saulnier *et al.*, 1995a). Single glucuronic acid residues have also been reported as a side-chain component. The heteroxylans after alkaline extraction, could be used in industry as stabilizing or emulsifying agents (Watson, 1959). A previous study (Chanliaud *et al.*, 1995) showed that conditions of extraction and especially temperature of reaction, nature and concentration of alkali, greatly influenced the yield of heteroxylan extraction and that a very good extraction yield could be obtained. However, the chemical structure of all extracted heteroxylans as well as their intrinsic viscosities appeared to be very similar.

The solution properties of a hydrocolloid depend on a great number of parameters including its chemical structure, its molecular weight and the nature of the solvent. To have a better understanding of their extraction mechanisms and functional properties, static and dynamic light scattering methods have been employed to measure average molecular size and weight of four heteroxylan samples extracted from maize bran under different conditions. Size-exclusion chromatography combinated with refractometric and laser light scattering detectors allowed the determination of the distribution of molecular weight and size. From all these data, their size in the unperturbed state and their molecular conformation were obtained.

*To whom correspondence should be addressed.

EXPERIMENTAL

Sample preparation

Three samples of heteroxylans (HX-K100, HX-Ca, HX-K60) were extracted from commercial maize bran and provided by ULICE (Riom, France): 100 g of bran were stirred with one litre of alkaline solution in a thermostated reactor during 2 h. Extraction conditions (temperature, nature and concentration of alkali) are given in Table 1. The residue was separated by centrifugation (20 min; 10 000 **g**; 15°C), washed with 200 ml of distilled water and centrifuged again. The supernatants were pooled and concentrated.

A fourth sample (HX-N) was purified from the maize kernel lime-cooking extract *(Nejayote)* provided by PEPSICO (Pamplona, Spain). Polysaccharides were precipited at pH 3 by addition of 2 volumes of 95% ethanol at 4°C. Then they were redissolved in water and

Table 1. Laboratory conditions of the heteroxylan extraction

	Extractant	Alkali proportion (%w/v)[a]	Temperature (°C)
HX-K100	KOH	4.5	100
HX-Ca	$Ca(OH)_2$	1.0	100
HX-K60	KOH	2.8	60

[a]Weight of alkali/solution volume.

starchy material was eliminated by amyloglucosidase treatment (AMG NOVO 300L; 0.08% v/w; 2 h; 60°C).

All the heteroxylans were finally precipitated with 95% ethanol (2 vol.; 16 h; 4°C), recovered by filtration (pore diameter < 15 μm), dried by solvent exchange and in an oven at 40°C.

Light scattering experiments were performed on dilute solutions of concentrations (≤ 3 mg/ml) lower than the critical concentration (≈ 9 mg/ml; Saulnier *et al.*, 1993). The heteroxylans were dissolved in 80 mM $NaNO_3$ solutions (with 0.02% NaN_3) and were filtered through 0.2 μm pore size ANOTOP, 0.45 or 5 μm pore size Millipore-durapore filters. Heteroxylan concentrations were measured by using automated orcinol (Tollier & Robin, 1979) and *m*-hydroxybiphenyl (Thibault, 1979) methods.

Light scattering methods

Both static and dynamic measurements were carried out on a Malvern K7025 instrument in combination with an ion argon laser operating with vertically polarized light with wavelength λ = 514.5 nm from Spectra-Physics. Measurements were made over the angular range 30–150° at the temperature of 20°C.

Benzene was used as a reference in the static light scattering (s.l.s) measurements with a value of 2.2×10^{-5} m^{-1} for the Rayleigh factor. The refractive index increment was extrapolated to 0.146 g/ml from values commonly determined for polysaccharides in aqueous solutions (Ring *et al.*, 1985; Roger & Colonna, 1992; Ousalem *et al.*, 1993). Data were analyzed by using the Zimm plot procedure.

The *z*-average hydrodynamic radius ($< R_h^2 >_z^{1/2}$) was obtained by dynamic light scattering (d.l.s) measurements performed at angles from 30 to 150°, for five different concentrations ranging from 0.6 to 3 mg/l and treated using the method of cumulants (Koppel, 1972). $< R_h^2 >_z^{1/2}$ was calculated from the Stokes–Einstein relationship:

$$< R_h^2 >_z^{1/2} = \frac{k \cdot T}{6\pi \cdot \eta_s \cdot D_t}$$

where D_t is the translational diffusion coefficient extrapolated to zero concentration and zero angle, T the temperature (293 K) and η_s the viscosity of the solvent (0.998 mPass). The determination of the relaxation time (τ) distribution over a large time range was done by using a ALV-5000 correlator (ALV, Langeb, Germany). The auto-correlation curves were analysed by taking the inverse Laplace transform and employing the routine REPES (Johnsen & Brown, 1992) with a probability to reject of 0.5. The distributions are given, in the form of $\tau \cdot A(\tau)$ vs log t, with $A(\tau)$ equal to the signal amplitude.

SEC-MALLS

The distributions of molecular weight and radius of gyration were determined by steric exclusion chromatography; the high pressure system consisted of a 3 Shodex OH-pack columns (KB-806,-805 and -804) with an on line refractometric and a multi-angle laser-light scattering (MALLS) detectors (laser wavelength = 632.8 nm) (Dawn-F, Wyatt Technology Corporation, Santa Barbara, CA) as previously described (Roger & Colonna, 1993). The chromatographic system was eluted with 80 mN $NaNO_3$ at a flow-rate of 1 ml/min. Number-average ($<M>_n$), weight-average ($<M>_w$), z-average ($<M>_z$) molecular weights, $<R_g^2>_z^{1/2}$ and polydispersity index ($P = <M>_w<M>_n$) were established with ASTRA software (v. 2.02) (Wyatt Technology) using a Zimm extrapolation (Kc/Rθ). Calibration of the photodiodes and interdetector delay volume (0.173 μl) was obtained using pullulan standards with $<M>_w$ ranging from 5000 to 50 000 g/mol (Showa Denko, Japan).

RESULTS

Samples purification

Dynamic light scattering measurements of all the heteroxylan solutions filtered on 5 μm membranes (the recoveries of polysaccharides after filtration were ~100%) gave relaxation time spectra (Fig. 1) consisting of two peaks. The large peak at the higher relaxation time (log τ ≈ 3.5) was due to a strong scattering of a minor population, probably aggregates, and the small one to the scattering of a majority of single chains of heteroxylans (log τ ≈ 2.5).

In order to obtain solutions without aggregate contamination, several methods were tried (heating, enzymic starch elimination) without success. Effects of filtration on light scattering of HX-K100 were studied with three different porosities (Fig. 1). A small reduction in the aggregate content was observed when the sample was filtered through on a 0.45 μm membrane. In contrast, a filtration through a 0.2 μm membrane eliminated the minor population and kept the population with low relaxation times. At the same time, 15% of the polysaccharides were lost, except for HX-K60 where 60% of the material was removed by the filter. Unless stated otherwise filtration through a 0.2 μm membrane was used to purified solutions for further experiments.

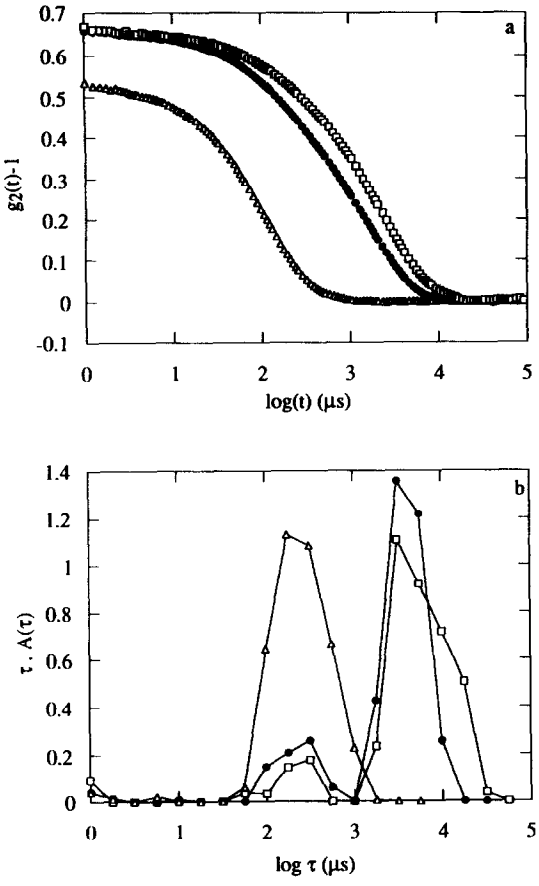

Fig. 1. Normalised autocorrelation functions (a) and distributions of relaxation time corrected by its amplitude (b) for HX-K100 filtered through 5 (□), 0.45 (●) and 0.2 μm (△) membranes.

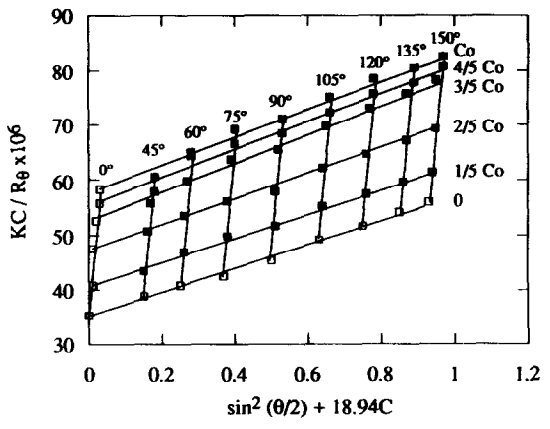

Fig. 2. Zimm plot obtained for HX-Ca in 80 mM $NaNO_3$ $C_0 = 1.76$ mg/ml.

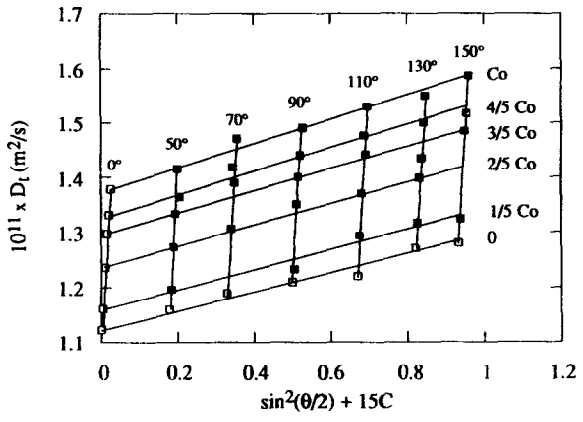

Fig. 3. Zimm-dynamic plot obtained for HX-N in 80 mM $NaNO_3$ C_0 = 1.79 mg/ml.

S.l.s. and d.l.s. results

The results obtained by s.l.s. and d.l.s. are given in Table 2. Examples of Zimm and dynamic-Zimm plots are shown in Figs 2 and 3, respectively. The linearity of extrapolations to zero angle and concentration confirmed that there was only one population in solution.

The weight-average molecular weights ($<M>_w$) were in the same range and varied from 2.4 (for HX-N) to 3.1 10^5 g/mol (for HX-K100); the sample with the highest $<M>_w$ was HX-K100 which was extracted with the highest temperature and alkali concentration.

The z-average radii of gyration ($<R_g^2>_z^{1/2}$) were all around 38 nm whereas hydrodynamic radii were close to 20 nm, whatever the samples. In all cases, the second virial coefficient (A_2) was positive (~6–7 × 10^{-4} mol/cm^3/g^2) indicating that the solutions were good polymer-solvent systems.

The structural ratios $\rho\,(<R_g^2>_z^{1/2}/(<R_h^2>_z^{1/2})$ were calculated from the light scattering results and ranged from 1.6 to 2.1. These values were empirically attributed to a random coil behaviour in good solvent for linear or branched polydisperse ($<M>_w/<M>_n = 2$) polymers (Burchard, 1992).

Table 2. Static and dynamic results of light scattering measurements on heteroxylan samples

	$<M>_w \times 10^{-5}$	$A_2 \times 10^4$ (mol/cm^3/g^2)	$<R_g^2>_z^{1/2}$ (nm)	$<R_h^2>_z^{1/2}$ (nm)
HX-K100	3.1	6.8	37	23
HX-Ca	2.8	6.1	41	19
HX-K60	2.7	6.9	36	21
HX-N	2.4	7.7	38	18

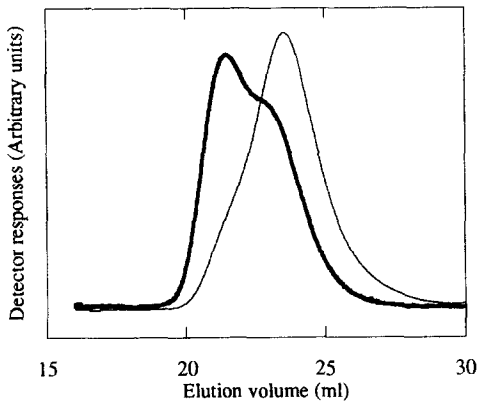

Fig. 4. Steric-exclusion chromatography of HX-K100. Refractometer (fine line) and light scattering at 90° (bold line) responses.

SEC-MALLS results

SEC-MALLS chromatograms were similar for all samples and an example is shown in Fig. 4. The refractometric detector showed a single roughly symetric peak with a shoulder at lower elution volumes. This shoulder gave an intense signal by light scattering but represented less than 2% of total polymers measured from the refractometric response.

Mean macromolecular features are given in Table 3. $<M>_w$ were similar to those found by s.l.s. measurements: the differences between the two measurements were lower than 4%. The $<M>_w$ of HX-N was slightly lower than those of the samples extracted in the laboratory. Comparison of the different mean molecular weights indicated that polydispersity was rather narrow [polydispersity index (P) < 2.1], excepted for HX-K60 which had polydispersities equal to 2 after 0.45 μm filtration, and 5.1 after 0.2 μm filtration. Radii of gyration were rather closer to those found with s.l.s. (difference < 15%) and ranged from 34 (for HX-N) to 42 nm. $<R_g^2>_z^{1/2}$ was slightly smaller for HX-N and this confirmed the lower $<M>_w$ value.

Similar chromatograms but with loss of intensity and macromolecular features were obtained after filtration of the solutions on 0.45 μm membranes (Fig. 5), except for HX-K60. This fact indicates again that the minor population observed by d.l.s. were aggregates since the shear stress caused by high pressure system apparently

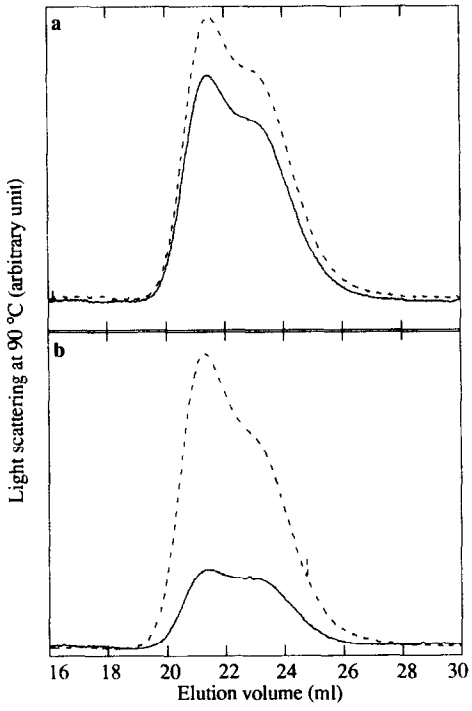

Fig. 5. Light scattering responses at 90° for HX-Ca (a) and HX-K60 (b) filtered through 0.45 (dotted line) and 0.2 (plain line) μm membranes.

disrupted the weak bonds which aggregated macromolecules. HX-K60 lost a large proportion of its scattering signal after filtration on 0.2 μm membranes and the loss occurred preferably on high elution volumes. The shear stress did not eliminate the larger population. This could indicate that in HX-K60 there were some bonds which were not destroyed by its mild extraction conditions but were destroyed by the stronger conditions used for the other samples.

Figure 6 shows the dependence of size upon mass and the mass distribution for HX-N. This behaviour was similar for the other samples. It was possible to fit two linear relations of the logarithmic form of:

$$R_g = K M^{\nu}$$

The first hydrodynamic parameter ν_1 which was obtained for molecular weights higher than 1.3–2.5 × 10^5 g/mol, was ≈0.3 for all samples. This value, corresponding to up to 60% of the total population, indicates

Table 3. Macromolecular features of heteroxylans determined by SEC-MALLS (filtration on 0.2 μm membranes)

	$<M>_n \times 10^{-5}$ (g/mol)	$<M>_w \times 10^{-5}$ (g/mol)	$<M>_z \times 10^{-5}$ (g/mol)	$<R_g^2>_z^{1/2}$ (nm)	P^a
HX-K100	1.6	3.0	8.0	40	1.9
HX-Ca	1.3	2.9	7.4	41	2.1
HX-K60	0.6	2.8	6.8	42	5.1
HX-N	1.2	2.5	5.6	34	2.1

[a]Polydispersity index = $<M>_w/<M>_n$.

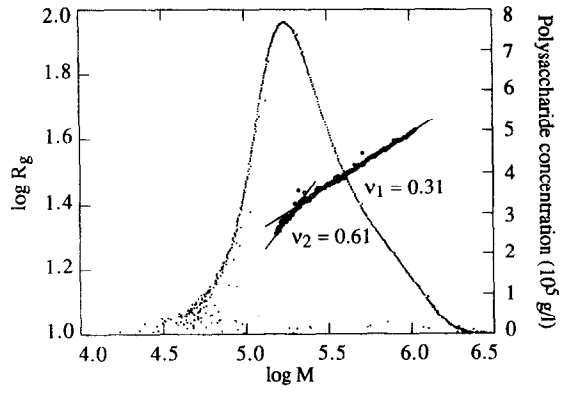

Fig. 6. log R_g as a function of log M and molecular weight distribution obtained for HX-N.

that molecular dimensions increased only slowly with the molecular weight and thus the molecules have a compact structure. For the lower molecular weights, a second hydrodynamic parameter was calculated equal to 0.5–0.6, which is characteristic of more highly expanded macromolecules.

DISCUSSION

The different light scattering methods gave complementary results which allow us to draw some conclusions about the solution behaviour of the heteroxylans from maize bran.

Light scattering measurements showed the presence of a small amount of aggregates in native solution of heteroxylans; in most of the samples, the bulk of the population was homogeneous and slightly polydisperse. The four samples had roughly the same macromolecular features but some small differences appeared.

The studies carried out on the four samples of heteroxylans showed that the dimensions of the isolated macromolecules did not depend on their conditions of extraction. However, it seemed that mild extraction conditions allowed the presence of intermolecular bonds. The molecular weight of the isolated macromolecules seemed to increase slightly with the severity of the extraction conditions (from 2.7 to 3.1 10^5 g/mol for HX-K100 and HX-K60, respectively). This hypothesis is confirmed by the lower value ($<M>_w = 2.310^5$g/mol) found by Saulnier *et al.* (1993) with SEC-MALLS for heteroxylans extracted from maize bran with 1% $Ca(OH)_2$ at 100°C during 40 min (extraction yield = 25%). This information could be related to the independence of chemical properties of heteroxylans and the slight dependence on their intrinsic viscosities (from 185 to 171 ml/g for HX-K100 and HX-K60, respectively) on extraction conditions, as previously described (Chanliaud *et al.*, 1995). It has been suggested that heteroxylans might be linked to cell-wall proteins and through diferulic acid ester linkages in the cell-wall (Saulnier *et al.*, 1995b). Alkaline treatment would be able to cleave these linkages in a more or less effective way depending on conditions (temperature and alkali concentration), releasing a polysaccharide population exhibiting essentially similar structural and conformational features.

The lower values obtained for the dimensions and molecular weight of HX-N could be due to the different variety or source of maize as well as to different extraction conditions used. Further studies should be carried out to understand the effect of variety.

The $<M>_w$ values found in this study were significantly larger than those determined previously (Chanliaud *et al.*, 1995) from SEC and the called 'universal' calibration (from 1.6 to 2.3 10^5 g/mol for HX-N and HX-K100, -KCa, respectively). This would imply that calibration was not valid for the pair heteroxylan-pullulan over the molecular weight range studied. The compact local structure indicated by the hydrodynamic parameters ν_1 (=0.3) could explain this discrepancy (Dubin & Principi, 1989). In the same way, the intrinsic viscosities, much smaller than those of alginates of the same molecular weight [495 ml/g in aqueous solvent (Smidsrod, 1970)], indicated that the molecule took up a smaller volume in solution. However, the structural ratios ρ and ν_2 indicated a random coil conformation for the heteroxylans. This could be explained by the presence in the heteroxylans of numerous short lateral chains which might cause a local dense structure around the xylan backbone. The macromolecular structure could be of the 'fringed micelle' type (Burchard, 1993) which corresponded to the succeeding of lateral intermolecular alignments and free chain portions and which gave a compact structure but allowed significant flexibility.

In solution, the dimensions of a macromolecule depend on segment–segment and segment–solvent interactions which are expressed through α, the expansion factor. In order to eliminate solvent effect, the dimensions of the heteroxylans in the unperturbed state were calculated from the light scattering measurements. The z-average radius of gyration in the unperturbed state ($<R_{g\theta}^2>_z^{1/2}$) could be determined by the relation:

$$<R_g^2>_z = \alpha^2 <R_{g\theta}^2>_z$$

where α can be calculated from the Flory-Orofino equation:

$$A_2 = \frac{16\pi}{(3)^{3/2}} \frac{N}{<M_z>^2} <R_g^2>_z^{3/2} \; ln\left[1 + \frac{\pi^{1/2}}{2}(\alpha^2 - 1)\right]$$

Table 4. Expansion factor and z-average radius of gyration in the unperturbed state

	α^2	$<R_{g\theta}^2>^{1/2}$ (nm)
HX-K100	3.83	20
HX-Ca	2.44	26
HX-K60	2.75	24
HX-N	2.29	22

Values of $<R_{g\theta}^2>_z^{1/2}$ and α^2 are shown in Table 4. The high values of α indicated an important excluded volume effect and the non-negligeable expansion of the heteroxylans in 80 mM $NaNO_3$. The radii of gyration in the unperturbed state were found to be similar and equal to 23 ± 3 nm. These values can be taken into account only if the short length of the substituents allowed the hypothesis that heteroxylans behave in solution as linear polymers.

In conclusion, heteroxylan molecules behave in solutions as rather flexible chains, well hydrated and with a relatively low polydispersity. Their dimensions were

$$<R_g^2>_z^{1/2} \approx 40\,\text{nm and} <R_h^2>_z^{1/2} \approx 20\,\text{nm}$$

for a weight-average molecular weight of $\approx 2.8 \times 10^5$ whatever the conditions used for their extraction. Their molecular weight and hydrodynamic radius values were similar to those of some heteroxylans from whole grains of wheat ($<M>_w = 2.54 \times 10^5$ g/mol; $<R_h^2>_z^{1/2} = 23.5$ nm; Girhammar & Nair, 1992) and those of some pectins ($<M>_w = 2.5 \times 10^5$ g/mol; $<R_h^2>_z^{1/2} = 25$ nm; Ousalem *et al.*, 1993). These similarity were confirmed by comparable values of their intrinsic viscosities: $[\eta]$ = 170–186, 160 and 185 ml/g for heteroxylans from maize bran (Chanliaud *et al.*, 1995), heteroxylans from whole grain of wheat and pectins (Mitchell, 1979), respectively. Both polysaccharides were composed of a backbone highly substituted by side chains and could be considered as a semi-flexible random coil. The values found in this study were different from those of heteroxylans from whole grain of rye ($<M>_w = 7.7 \times 10^5$ g/mol; $<R_h^2>_z^{1/2} = 36.2$ nm; Girhammar & Nair, 1992). But some fractions of these heteroxylans had a large tendency to aggregate because of the rather linear structure of the molecule (Ebringerova *et al.*, 1994).

ACKNOWLEDGEMENT

The authors thank ULICE from the LIMAGRAIN group, for financial support.

REFERENCES

Burchard, W. (1992). Static and dynamic light scattering approaches to structure determination of biopolymers. In *LaserLight Scattering in Biochemistry*, eds. S. E. Harding, D. B. Sattelle & V. A. Bloomfied. Royal Society of Chemistry, Cambridge, pp. 3–22.

Burchard, W. (1993). Solution properties of plant polysaccharides as a function of their chemical structure, In *Plant polymeric carbohydrates*, eds. F. Meuser, D. J. Manners & W. Seibel. Royal Society of Chemistry, Cambridge, pp. 215–232.

Chanliaud, E., Saulnier, L. & Thibault, J.-F. (1995). Alkaline extraction and characterisation of heteroxylans from maize bran. *J. Cereal Sci.*, **21**, 195–203.

Dubin, P.L. & Principi, J.M. (1989). Failure of universal calibration for size exclusion chromatography of rodlike macromolecules versus random coils and globular proteins. *Macromolecules*, **22**, 1891–1896.

Ebringerova, A., Hromadkova, Z., Burchard, W., Dolega, R. & Vorwerg, W. (1994). Solution properties of water-insoluble rye-bran arabinoxylan. *Carbohydr. Polym.*, **24**, 161–169.

Girhammar, U. & Nair, B.M. (1992). Isolation, separation and characterization of water soluble non-starch polysaccharides from wheat and rye. *Food Hydrocoll.*, **6**, 285–299.

Johnsen, M. & Brown, W. (1992). An overview of current methods of analysing QLS data, In *Laser Light Scattering in Biochemistry*, Eds S. E. Harding, D. B. Sattelle & V. A. Bloomfied, Royal Society of Chemistry, Cambridge, pp. 75–91.

Koppel, D.E. (1972). Analysis of macromolecular polydispersity in intensity correlation spectroscopy: the method of cumulants. *J. Chem. Phys.*, **57**, 4814–4817.

Mitchell, J.R. (1979). Rheology of polysaccharide solutions and gels. In *Polysaccharides in Food*, Eds: J.M.V. Blanshard & J.R. Mitchell. Butterworths, London, pp. 51–72.

Ousalem, M., Bunsnel, J.P. & Nicolai, T. (1993). A static and dynamic light scattering study of sharp pectin fractions in aqueous solution. *Int. J. Biol. Marcromol.*, **15**, 209–213.

Ring, S.G., l'Anson, K.J. & Morris, V.J. (1985). Static and dynamic light scattering studies of amylose solutions. *Macromolecules*, **18**, 182–188.

Roger, P. & Colonna, P. (1992). The influence of chain length on the hydrodynamic behaviour of amylose. *Carbohydr. Res.*, **227**, 73–83.

Roger, P. & Colonna, P. (1993). Evidence of the presence of large aggregates contaminating amylose solutions. *Carbohydr. Polym*, **21**, 83–89.

Saulnier, L., Mestres, C., Doublier, J.-L., Roger, P. & Thibault, J.-F. (1993). Studies of polysaccharides solubilized during alkaline cooking of maize kernels. *J. Cereal Sci.*, **17**, 267–276.

Saulnier, L., Vigouroux, J., & Thibault, J.-F. (1995a). Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydr. Res.*, **272**, 241–253.

Saulnier, L., Marot, C., Chanliaud, E. & Thibault, J.-F. (1995b). Cell wall polysaccharide interactions in maize bran. *Carbohydr. Polym.*, **26**, 279–287.

Smidsrod, O. (1970). Solution properties of alginate. *Carbohydr. Res.*, **13**, 359–372.

Thibault, J.-F. (1979). Automatisation du dosage des substances pectiques par la méthode au méta-hydroxydiphényl. *Lebensm. Wiss. u. Technol.*, **12**, 247–251.

Tollier, M.T. & Robin, J.P. (1979). Adaptation de la méthode à l'oricnol sulfurique au dosage automatique des glucides neutres totaux: conditions d'application aux extraits d'origine végetale. *Ann. Technol. Agric.*, **28**, 1–15.

Watson, S.A. (1959). Corn hull gum. In *Industrial Gums*, ed. R.L. Whistler. Academic Press, New York, pp. 299–306.

Whistler, R.L. (1989). Forthcoming opportunities for hemicelluloses. In *Frontiers in Carbohydrate Research*, eds. R.P. Millane, J.N. BeMiller & R. Chandrasekaran. Elsevier Applied Science, London, pp. 289–296.