Carbohydrate Research, 143 (1985) 271–274
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Effects of delignification and of *N*-methylmorpholine *N*-oxide treatments on properties of wheat-bran arabinoxylans

WARREN D. HOLLOWAY*,
Applied Biochemistry Division, DSIR and Department of Food Technology, Massey University, Palmerston North (New Zealand)

JOHN LELIEVRE, AND EDWARD L. RICHARDS
Department of Food Technology, Massey University, Palmerston North (New Zealand)
(Received August 20th, 1984; accepted for publication in revised form, April 7th, 1985)

Many investigators have studied methods of extracting polymeric constituents from plant cell-walls[1–3]. However one of the principal difficulties that still remains is the isolation of the water-insoluble polysaccharides, particularly the arabinoxylans. Classical fractionation methods employ differential solubilisation and precipitation procedures[4]. While these methods represent the best procedures currently available, they do not appear to be ideal, as degradation of the polymers may occur.

Many of the earlier studies on the isolation of arabinoxylans were performed on woody tissues[5]. These studies showed that, with mature tissue, delignification is required to obtain appreciable yields of polysaccharide[6]. Although depolymerisation of polysaccharides may occur with delignification[7], this procedure is still widely used in the isolation of arabinoxylans from non-woody tissues[8,9].

The first part of this study was undertaken to determine the effect of delignification on arabinoxylans isolated from wheat bran. This material was selected for investigation as it is an important component of both foods and feeds and is also a significant source of dietary fibre.

The second part is concerned with the use of a new solvent, *N*-methylmorpholine *N*-oxide (NMMNO), which has been reported to dissolve cellulose nondegradatively[10]. NMMNO has also been used to dissolve entire cell-walls[11]. The possibility of utilising NMMNO as a nondegradative solvent for arabinoxylans was therefore investigated.

RESULTS AND DISCUSSION

Table I shows that a significant amount of arabinoxylan can be extracted from wheat bran that has not been delignified. Table I also shows that the compositions

*Current address: Chemistry Department, Australian National University, Canberra, Australia.

0008-6215/85/$ 03.30 © 1985 Elsevier Science Publishers B.V.

TABLE I

YIELD AND COMPOSITION OF WHEAT-BRAN HEMICELLULOSE EXTRACTED IN ALKALI BEFORE (A) AND AFTER SUBSEQUENT CHLORITE DELIGNIFICATION (B)

	% Yield	*Rhamnose*	*Arabinose*	*Xylose*	*Mannose*	*Galactose*	*Glucose*	*Uronic acid*	*Methoxyl*	*Acetyl*	*Protein*
A	7.9	0	31.9	60.1	0.3	12.2	4.0	0.7	0.1	0.1	0.1
B	3.8	0	29.4	61.8	0	0.8	5.7	1.2	0 1	0.5	0.5

TABLE II

VISCOSITY VALUES OF HEMICELLULOSES EXTRACTED IN ALKALI BEFORE AND AFTER SUBSEQUENT DELIGNIFICATION AND AFTER RECOVERY FROM SOLUBILISATION IN *N*-METHYLMORPHOLINE *N*-OXIDE (NMMNO)

Treatment of hemicellulose	*Viscosity number (mL/g)*
Alkali extraction	220.6
Alkali extraction then delignification	68.3
Alkali extraction after delifnification	74.2
NMMNO control[a]	81.9
NMMNO solubilised	6.3

[a]Control sample of nondelignified arabinoxylan was treated the same way as the NMMNO-solubilised sample, except that water was substituted for NMMNO.

of the arabinoxylans, whether extracted before or after delignification, are similar. However, as is evident from Table II, the arabinoxylans differ considerably in their viscosities. As the composition of the two polymers is similar, it is possible that the arabinoxylan extracted after chlorite delignification has been degraded. To confirm this, a sample of arabinoxylan extracted prior to delignification was subjected to the chlorite treatment used for delignification. As Table II shows, this treatment caused extensive depolymerisation, as indicated by the decrease in limiting-viscosity number from 220.6 to 68.3 mL/g.

It may also be seen in Table II that the limiting-viscosity number of the alkali-extracted arabinoxylan that had been solubilised and then recovered from NMMNO was only 6.3 mL/g, as compared with the control sample of 81.9 mL/g. Some degradation of the control sample occurred, possibly because of the elevated temperature (120° for 30 min) that solubilisation with NMMNO required[10]. This suggests that when NMMNO is used to solubilise cell walls, extensive degradation of arabinoxylans occurs.

EXPERIMENTAL

Preparation of starting material. — Wheat bran was boiled under reflux in ethanol to remove pigments and lipids, washed with cold water, and then boiled in a large volume of water for 30 min. When cool, amylase was added to remove starch. The preparation was treated with ammonium oxalate, washed in water, and then dried. This preparation (residue A) was the starting material for all further extractions.

Extraction with alkali. — To obtain without delignification, residue A was treated with sodium hydroxide solution (10% w/v) for 1 h at room temperature (20°). The suspension was mixed continuously and kept under nitrogen during this process. The suspension was then centrifuged to give a supernatant solution and an alkali-insoluble material (residue B). The supernatant solution was filtered and added to three volumes of ethanol. The resultant precipitate of arabinoxylan was

washed repeatedly with ethanol and then freeze-dried. To obtain a sample of arabinoxylan after delignification, residue B was treated with water (100 mL) plus acetic acid (0.5 mL) and heated to 75°. To this suspension sodium chlorite (4.0 g) was added slowly over a period of 100 min. After a further 90 min, the suspension was cooled and decanted. The resultant material was finally re-extracted with alkali by the process already described.

N-Methylmorpholine N-oxide (NMMNO) treatment. — The suitability of NMMNO as a nondegradative solvent for arabinoxylans was investigated by adding NMMNO (12.5 g) to the polymer (0.5 g); water (12.5 g) replaced NMMNO in the control. Both samples were heated (120°) under nitrogen for 30 min. Dimethyl sulphoxide (50 mL) was then added to each sample. After cooling to room temperature, both solutions were dialysed separately against distilled water for 48 h. The resultant samples were then freeze-dried.

Analytical methods. — The compositions of the dried polymers were determined by measuring the individual sugars, after hydrolysis by trifluoroacetic acid, by g.l.c.[12] of the alditol acetates on a column of 3% ECNSS-M. The uronic acids were measured using *m*-hydroxydiphenol[13], acetyl groups by the hydroxylamine procedure[14], methoxyl groups by the acetylacetone method[15], and protein using Folin–Ciocalteu reagent[16].

Viscosity. — The viscosity measurements were made at 25 ±0.05° using Cannon–Fenske viscometers. Solutions of arabinoxylans (250 mg in 1.0M NaOH, 25 mL) were stirred under nitrogen (10 min), filtered through No. 2 sintered-glass filters, and placed in the viscometers. The solutions were maintained under an atmosphere of nitrogen at all times. The small amount of undissolved material obtained on filtration was washed twice with ethanol and dried at 105°. This weight was subtracted from the starting weight in order that the final concentration could be calculated. The results were expressed as viscosity number (η_{sp}/c), with c in g/mL.

REFERENCES

1 R. L. WHISTLER AND C L. SMART, *Polysaccharide Chemistry*, Academic Press, New York, 1953, pp. 112–123.
2 G. O. ASPINALL, *The Polysaccharides*, Vol. 1, Academic Press, New York, 1982.
3 S. G. RING AND R. SELVENDRAN, *Phytochemistry*, 19 (1980) 1723–1730.
4 S. WADA AND P. RAY, *Phytochemistry*, 17 (1978) 923–931.
5 R. L. WHISTLER AND J. N. BEMILLER, *Methods Carbohydr. Chem.*, 3 (1963) 21–22.
6 T. E. TIMELL, *Adv. Carbohydr. Chem.*, 19 (1964) 249.
7 C. P. GLAUDEMANS AND T. E. TIMELL, *Svensk Papperstidn.*, 60 (1957) 869–871.
8 G. O. ASPINALL AND D. MCGRATH, *J. Chem. Soc. C*, (1966) 2133–2136
9 J. BRILLONET, J.-P. JOSELEAU, J. UTILLE, AND D. LELIEVRE, *J. Agric. Food Chem.*, 30 (1982) 488–494.
10 J.-P. JOSELEAU, G. CHAMBAT, AND B. CHUMPITAZI-HERMOSA, *Carbohydr. Res.*, 90 (1981) 339–344.
11 H. CHANZY, M. DUBE, AND R. H. MARCHESSAULT, *J. Polym. Sci., Polym. Lett.*, 18 (1979) 13–25
12 T. M. JONES AND P. ALBERSHEIM, *Plant Physiol.*, 49 (1972) 926–936.
13 N. BLUMENKRANTZ AND J. ASBOE-HANSEN, *Anal. Biochem.*, 54 (1973) 484–489.
14 E. A. MCCOMB AND R. M. MCCREADY, *Anal. Chem.*, 29 (1957) 819–821.
15 P. J WOOD AND I. R. SIDDIQUI, *Anal. Biochem.*, 39 (1971) 418–428.
16 G. R SCHACTERLE AND R. L. POLLACK, *Anal. Biochem.*, 51 (1973) 654–655