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Determination of Structural Features of the Water-Insoluble Dietary Fiber from Oats by the Reductive-Cleavage Method

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ABSTRACT

The water-insoluble polysaccharides of the dietary fiber complex from oat grain and oat bran were isolated after enzymatic treatment for starch and protein digestion. Extraction of the alkali-soluble hemicellulose fractions was carried out using a range of aqueous alkali concentrations. The structural features of the hemicellulose fractions were studied by the reductive-cleavage method after ultrafiltration and freeze-drying. The alkali-soluble L*-arabino-*D*-xylans are highly branched. The alkali-soluble β-glucans contain a linear chain with (1→3) and (1→4) linkages. The analysis of the alkali-insoluble residue indicates the presence of cellulose. Knowledge of the structure of dietary fiber may help to explain their physiological effects in the digestive tract.*

INTRODUCTION

The water-insoluble polysaccharides from oats are used as dietary fiber. The importance of dietary fiber in human nutrition had been discussed extensively (Burkitt & Trowell, 1975; Schweizer & Würsch, 1984/85; Englyst & Cummings, 1985). Structural investigations are necessary to explain their physiological effects and their digestibility by the intestinal microflora (DuPont & Selvendran, 1987; Stevens & Selvendran, 1988).

The structural investigation of the water-insoluble dietary fiber from oat grain and oat bran by the reductive cleavage method (Rolf & Gray, 1982) is described in this paper. The reductive depolymerisation is well suited for the elucidation of linkage positions in pentose-containing

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carbohydrates (Heims *et al.*, 1989). The disadvantages of standard methylation analysis (Jansson *et al.*, 1976; Harris *et al.*, 1984), especially the critical acid hydrolysis step, could be circumvented.

The reductive depolymerisation method was therefore introduced into structural investigations of carbohydrates of both the dietary fiber complex and of cell-wall material from plant tissues.

EXPERIMENTAL

Materials

Oat grain and oat bran were donated by P. Kölln, Elmshorn (Germany). The material was milled with a Retsch mill to a particle size $< 0{\cdot}2$ mm and defatted with hexane. All chemicals were of highest available purity.

Isolation of water-insoluble dietary fiber

The water-insoluble dietary fiber was obtained after enzymatic treatment according to the method of Arrigoni *et al.* (1984) to remove soluble sugars, starch and protein.

Enzyme preparations purchased from Serva were: Protease from bacillus subtilis (0·3 DMC-U/mg) and amyloglucosidase rohalase from aspergillus niger (9 U/mg). Termamyl 120L was obtained from Tecator.

Extraction with alkali

Water-insoluble dietary fiber from oat grain and oat bran (1 g) was treated with aqueous 0·1 N sodium hydroxide (13 ml) for 2 h under nitrogen. The liquid phase was removed after centrifugation of the suspension for 15 min (2000 *g*). The extraction of the residue was completed by washing twice with 5 ml 0·1 N sodium hydroxide. The liquid phases were combined. Finally the residue was washed thoroughly with water to remove alkali and then vacuum dried at 40°C.

The extraction of the residue was repeated using 1·0 N and then 4·4 N sodium hydroxide as described. Three alkali-soluble fractions (ASF) and the alkali-insoluble residue (AIR) were obtained.

Molecular mass separation of ASF

The ASF were first neutralised with hydrochloric acid (pH 7·0) and then subjected to size-exclusion column chromatography (500×16 mm)

using Sephadex G-25 medium from Pharmacia as the stationary phase. Deionised and degrassed water was used as the mobile phase at a flow rate of 8 ml/min. A differential refractometer was used as detector. The high molecular fraction was collected, filtered through Sartorius ultrafiltration membranes with a molecular mass (MM) cut off of 10^5 daltons (d). The fractions $> 10^5$ d and $< 10^5$ d were lyophilised and subjected to reductive depolymerisation. Figure 1 shows the overall procedure.

Characterisation of ASF and AIR

The polysaccharides in the ASF and AIR were methylated using a slightly modified method introduced by Ciucanu and Kerek (1984).

Reductive depolymerisation and acetylation of the permethylated polysaccharides was conducted by the procedures of Jun and Gray (1987) and Heims *et al.* (1989). Reductive depolymerisation was performed in the presence of 5 equivalents/acetal bond each of triethylsilane and trimethylsilyl trifluoro-methanesulfonate, followed by in-situ acetylation to form the resulting anhydroalditol derivatives. Anhydroalditol derivatives were analyzed by g.l.c. and g.l.c.-mass spectrometry. The integral values of all peaks in g.l.c. were corrected by the effective-carbon-response (e.c.r.) method of Sweet *et al.* (1975).

RESULTS AND DISCUSSION

Ground and defatted oat grain and oat bran (6 g) was subjected to enzymatic digestion in order to remove soluble sugars, starch and protein. The water-insoluble dietary fiber was obtained after centrifugation. Oat grain yielded 16% water-insoluble dietary fiber, oat bran 30%.

The water-insoluble residue (1 g) was extracted with 0·1 N, 1·0 N and 4·4 N sodium hydroxide. Oat grain gave 12% alkali-insoluble residue (AIR), oat bran 6%, after treatment with 4·4 N sodium hydroxide. The alkali-soluble fractions (ASF) were subjected to size-exclusion chromatography and then ultrafiltrated. The 0·1 N and 1·0 N ASF of grain and bran yielded $> 70\% > 10^5$ d, whereas the 4·4 N NaOH ASF yielded $60–70\% > 10^5$ d fraction. These results are summarised in Table 1.

The results of the structural investigations are listed in Table 2. The main polymers of fractions $> 10^5$ d from oat grain and oat bran are arabinoxylans which are highly branched as is indicated by the high proportion (40–25%) of 1,4-anhydro-2,3,5-tri-O-methyl-L-arabitol (2) from non-reducing L-arabino-furanosyl units. Only small amounts of 3-O-acetyl-1,4-anhydro-2,5-di-O-methyl-L-arabitol (5) and 5-O-acetyl-

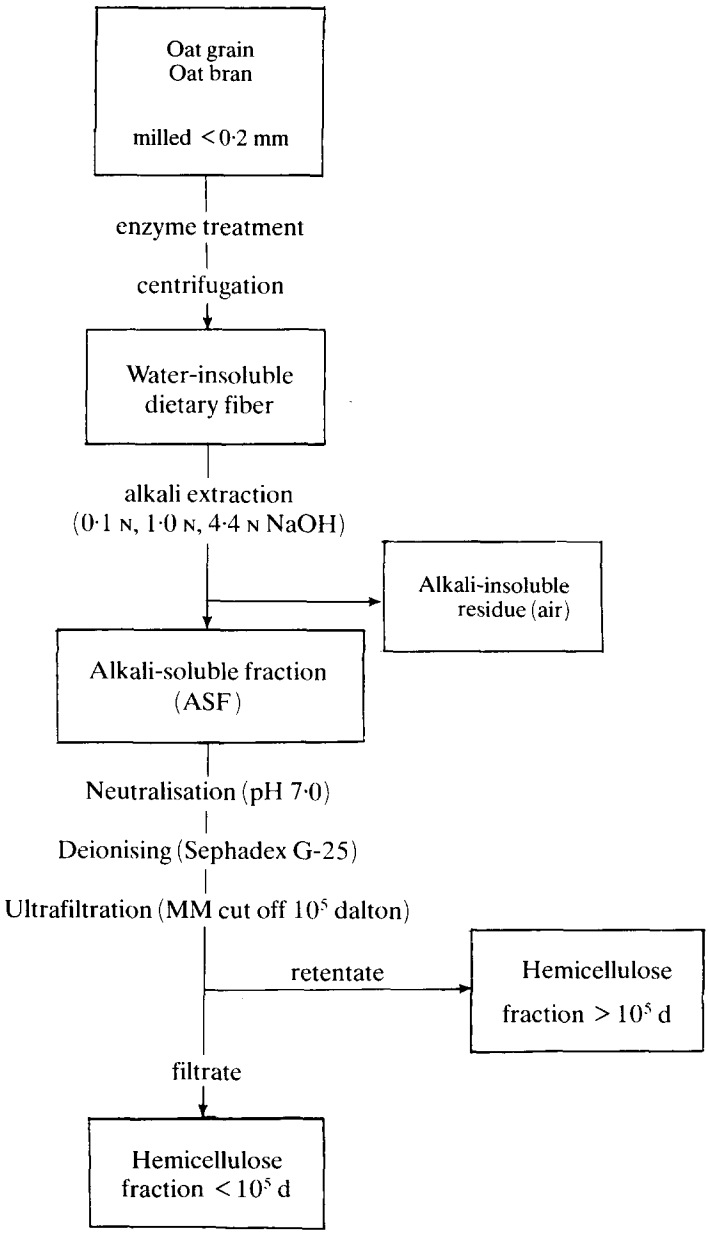

Fig. 1. Fractional extraction of water-insoluble dietary fiber from oat grain and oat bran.

1,4-anhydro-2,3-di-o-methyl-L-arabitol (6) were detected, indicating short chain branching. These results suggest that the main branches from the xylan backbone are terminal arabinofuranosyl residues as single units. Terminal arabinofuranosyl residues (40%) are present in the 0·1 N

TABLE 1
Amounts of Water-Insoluble Fiber, NaOH Extracts, and Relative Partition of ASF in Oat Bran and Oat Grain

Sample	*Oat bran*	*Oat grain*
Water-insoluble dietary fiber[a] (%)	29·9	15·8
NaOH extract (%)		
0·1 N	49·2	18·7
1·0 N	27·6	38·8
4·4 N	17·0	30·8
AIR[b]	6·3	11·8
Rel. partition of ASF[c] (%)		
0·1 N > 10^5 d	81·9	87·4
0·1 N < 10^5 d	18·1	12·6
1·0 N > 10^5 d	72·0	77·7
1·0 N < 10^5 d	28·0	22·3
4·4 N > 10^5 d	67·8	60·5
4·4 N < 10^5 d	32·2	39·5

[a]Of defatted dry matter.
[b]AIR = Alkali-insoluble residue.
[c]ASF = Alkali-soluble fractions.

NaOH extract, and about 25% in the 1·0 N and 4·4 N NaOH extract in oat grain as well as in oat bran.

Terminal non-reducing xylopyranosyl residues from the backbone were detected as 1,5-anhydro-2,3,4-tri-O-methyl-D-xylitol (1). 4-O-Acetyl-1,5-anhydro-2,3-di-O-methyl-D-xylitol (3) and its isomerisation product 5-O-acetyl-1,4-anhydro-2,3-di-O-methyl-D-xylitol (4) were obtained from the unbranched xylopyranosyl residues. The derived products of the branched units of the xylopyranosyl backbone are 3,4-di-O-acetyl-1,5-anhydro-2-O-methyl-D-xylitol (7), 2,4-di-O-acetyl-1,5-anhydro-3-O-methyl-D-xylitol (11) and 2,3,4-tri-O-acetyl-1,5-anhydro-D-xylitol (16) and the corresponding 1,4-anhydro isomerisation products (8, 12, 17). The main branching position is C-3 of the xylopyranosyl units. Branching, however, occurs also at position C-2 and C-2 together with C-3.

Only traces of the derivative from D-glucuronic acid or its 4-O-methyl ether (9), which are not distinguishable from one another by the described method, were detected.

The general structure for the arabinoxylan, which can be deduced from these results, is in good agreement with the proposed structure from Aspinall and Carpenter (1984). The xylan from oat spelts is a

TABLE 2

1,4- and 1,5-Anhydroalditol Derivatives Obtained from o-methylated Dietary Fiber From Oat Grain (OG) and Oat Bran (OB) by the Reductive-Cleavage Method with TMS-O-Triflat as Catalyst

Compound		*Relative mol%*													
		ASF > 10^5 d						*ASF < 10^5 d*						*AIR*	
		OG[a]	*OB*	*OG*	*OB*	*OG*	*OB*	*OG*	*OB*	*OG*	*OB*	*OG*	*OB*	*OG*	*OB*
		0·1 M[b]		*1·0 M*		*4·4 M*		*0·1 M*		*1·0 M*		*4·4 M*			
1	t-Xyl(p)	2·8	2·8	1·5	1·1	1·6	1·8	1·0	1·5	2·1	0·5	3·2	3·9	1·0	0·9
2	t-Ara(f)	37·4	37·9	26·9	25·1	31·4	26·4	22·1	19·7	14·9	18·0	19·4	18·2	16·0	13·0
3 + 4	4-Xyl(p) + 5-Xyl(f)	18·7	22·0	23·4	22·2	22·8	24·0	15·0	15·4	20·7	22·4	27·2	23·2	10·4	7·7
5	3-Ara(f)	2·0	2·3	1·4	1·4	1·7	2·0	3·6	2·4	2·2	3·0	5·3	4·0	n.d.	n.d.
6	5-Ara(f)	2·4	2·5	1·0	1·1	0·9	4·1	1·2	2·1	2·2	5·7	3·5	tr.	2·7	1·2
7 + 8	3,4-Xyl(p) + 2,5-Xyl(f)	19·6	17·9	20·0	18·5	22·6	19·3	12·8	10·7	12·3	14·5	14·7	13·7	10·3	7·3
9	Glc(p)A	1·8	1·0	1·2	0·9	1·0	1·5	0·5	tr.	0·3	tr.	1·2	tr.	1·0	0·5
10	t-Glc(p)	tr.	tr.	tr.	tr.	tr.	tr.	4·6	3·8	1·8	tr.	tr.	2·0	0·8	0·7
11 + 12	2,4-Xyl(p) + 2,5-Xyl(f)	4·0	3·7	2·8	2·5	4·7	3·7	0·7	1·1	1·1	1·8	2·3	5·2	2·2	1·8
13 + 14	4-Glc(p) + 5-Glc(f)	5·2	4·1	15·8	17·7	7·8	11·6	24·8	29·6	31·6	23·9	17·9	22·4	51·8	61·4
15	3-Glc(p)	n.d.	n.d.	4·0	7·0	2·8	3·1	10·7	10·5	9·6	8·7	4·6	7·4	1·3	2·1
16 + 17	2,3,4-Xyl(p) + 2,3,5-Xyl(f)	6·1	5·8	2·0	2·5	2·7	2·5	3·0	3·2	1·2	1·5	0·7	tr.	1·4	1·4
18	4,6-Glc(p)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1·1	2·0

[a]Sample.

[b]Alkali-treatment.

For identification of compound numbers see text. The numbers of the compounds abbreviated indicate the position of carbons bearing acetoxy groups. In addition (p) indicates 1,5-anhydroalditol derivatives, (f) 1,4-anhydroalditol derivatives.

ASF = Alkali-soluble Fraction; AIR = Alkali-insoluble Residue; n.d. = not detected; tr. = traces.

lightly branched arabinoglucuronoxylan (Heims *et al.*, 1989). From other cereals like wheat or rye well defined anatomical portions of the plant had been investigated. The structure of the oat arabinoxylan now reported is also of the highly branched type similar to wheat bran (Brillouet & Joseleau, 1987; DuPont & Selvendran, 1987) and rye bran (Hromadkova *et al.*, 1987).

β-D-Glucans are also observed in the ASF $> 10^5$ d indicated by the formation of 4-O-acetyl-1,5-anhydro-2,3,6-tri-O-methyl-D-glucitol (13) and its isomerisation product 5-O-acetyl-1,4-anhydro-2,3,6-tri-O-methyl-D-glucitol (14), as well as 3-O-acetyl-1,5-anhydro-2,4,6-tri-O-methyl-D-glucitol (15), which are derived from 4- and 3-linked D-glucopyranosyl units. 1,5-Anhydro-2,3,4,6-tetra-O-methyl-D-glucitol (10) arises from terminal non-reducing D-glucopyranosyl residues. The β-D-glucan of oat contained unbranched chains of 4- and 3-linked β-D-glucopyranosyl residues in the ratio of 3:1. These results agree well with previous findings (Wilkie, 1979; Aspinall & Carpenter, 1984). These β-D-glucans are widely distributed and had been isolated from rye endosperm (Smith & Stone, 1973) and wheat stems (Kivilaan *et al.*, 1971).

Arabinoxylans and β-D-glucans are also found in the ASF $< 10^5$ d of oat grain and oat bran. There is a smaller degree of branching of the arabinoxylan in these fractions as compared to that in ASF $> 10^5$ d. A higher content of β-D-glucan was observed in comparison to ASF $> 10^5$ d.

4-O-Acetyl-1,5-anhydro-2,3,6-tri-O-methyl-D-glucitol (13) and its 1,4-anhydro isomerisation derivative (14) are the major products (60%) in the AIR, obtained after treatment with 4·4 N NaOH. These derivatives originate from the alkali-insoluble cellulose. Only small amounts of 4,6-di-O-acetyl-1,5-anhydro-2,3-di-O-methyl-D-glucitol (18) are detected. The AIR also contained arabinoxylans, but these were not completely extracted under these conditions.

The results presented here indicate that water-insoluble dietary fiber from both oat grain and oat bran are chemically and structurally very similar, allowing one to conclude that the water-insoluble dietary fiber is concentrated in the outer cells of the cereal grain. Furthermore, these results demonstrate the suitability of the reductive-cleavage method for structural investigations of polysaccharides.

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