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Isolation and chemical characterization of water-soluble mixed-linked β-glucans and arabinoxylans in oat milling fractions

Eric Westerlund, Roger Andersson & Per Åman

Department of Food Science, Swedish University of Agricultural Sciences, Box 7051, S-750 07 Uppsala, Sweden

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Dehulled oats were separated into fractions of bran, outer starchy endosperm and inner starchy endosperm, after and without prior steam-flaking. The bran had significantly higher contents of protein, fat, ash and dietary fibre and lower content of starch than the endosperm fractions. An improved procedure was developed for the isolation of pure (92–99%) water-soluble mixed-linked (1–3), (1–4)-β-D-glucans, involving fat extraction, enzymatic removal of starch and protein, and subsequent precipitation of water-soluble polysaccharides with 60% aqueous ethanol and 20% (w/v) aqueous $(NH_4)_2SO_4$. High-field ^{13}C-NMR analysis of the isolated mixed-linked β-glucans showed the polysaccharide structure to be similar in all oat fractions. The proportion of isolated (1–3)-β-linkages in the polymer was on average 28·3% (SD 0·8) according to ^1H-NMR analysis. ^1H-NMR also showed that water-soluble oat arabinoxylans were composed of a main chain of (1–4)-linked β-D-xylopyranosyl residues, substituted by terminal α-L-arabinofuranosyl residues predominantly at O-3 but also at both O-2 and O-3. The proportion of xylose residues substituted solely at O-3 was 35% higher in bran than in endosperm arabinoxylans.

INTRODUCTION

Research on oats and oat products has been stimulated by the findings that oat bran possesses serum cholesterol-lowering properties (Kirby *et al.*, 1981; Ernst *et al.*, 1985; Hurt *et al.*, 1988). Mixed-linked (1–3), (1–4)-β-D-glucans are the predominant cell wall components of oat endosperm, and are believed to play an important role in lowering serum cholesterol. The content of mixed-linked β-glucans is highest in the subaleurone layer, which can be enriched in the bran fraction by milling (Wood *et al.*, 1991*a*). Commercial oat bran often contains 7–10% of mixed-linked β-glucans (Wood *et al.*, 1991*a*; Vollendorf & Marlett, 1991) of which a large part is water-soluble.

Structural studies have shown that soluble mixed-linked β-glucans in cereals are composed of cellotriosyl and cellotetraosyl units separated by single (1–3)-β-linkages (Dais & Perlin, 1982; Woodward *et al.*, 1983; Vårum & Smidsrød, 1988; Wood *et al.*, 1991*b*). The distribution of cellotriosyl and cellotetraosyl units may be described as random in barley (Staudte *et al.*, 1983). The occasional (1–3)-linkages cause irregularities in the molecular conformation compared to cellulose which is entirely built up of (1–4)-β-linkages, and as a result mixed-linked β-glucans are partly water-soluble (Clarke & Stone, 1963). Characteristically these polymers can have very high molecular weights, 1 000 000–40 000 000 (Forrest & Wainwright, 1977; Wood *et al.*, 1989, 1991*c*), and form highly viscous solutions. Viscosity has been suggested as one of the factors responsible for the hypocholesterolaemic effects of mixed-linked β-glucans (Andersson & Chen, 1986). It is therefore imperative to avoid breakdown of the molecular size, occurring for example by exposure to shear forces and endogeneous β-glucanases (Wood *et al.*, 1989), when the cholesterol-lowering effect of foods containing mixed-linked β-glucans is to be studied.

The other predominant cell wall polysaccharides in oats are arabinoxylans (MacArthur & D'Appolonia, 1980; Frölich & Nyman, 1988). Insoluble and soluble arabinoxylans in oat bran have been found to be composed of a main chain of (1–4)-linked β-D-

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xylopyranosyl residues that are substituted by terminal arabinofuranosyl residues, predominantly at O-3 but also to some extent both at O-2 and O-3 (Aspinall & Carpenter, 1984; Heims & Steinhart, 1991). In oat endosperm, soluble arabinoxylans are present in smaller amounts than mixed-linked β-glucans (Henry, 1987; Frölich & Nyman, 1988), but their contribution to cell wall structure and viscosity may nevertheless be important. In a recent investigation (Bengtsson *et al.*, 1992) it was shown that for rye arabinoxylans the content of xylose residues, substituted both at O-2 and O-3, was strongly correlated to the viscosity of flour slurries.

The main aim of the present paper was to develop a mild method for the isolation of highly purified water-soluble mixed-linked β-glucans from oat bran and starchy endosperm, in order to be able to study the chemical structure and other characteristics. Increased knowledge on this matter is of importance when serum cholesterol-lowering mechanisms and technological properties of oats are to be evaluated.

EXPERIMENTAL

Oat samples

Oat grains (cultivar Vital, harvested in 1987) were dehulled by Svenska Lantmännen (Vårgårda, Sweden) and the kernels then treated for 12 min at 80°C in a rotating drum and dried to 9% moisture content. Next, one batch (A) was sacked directly, whereas another batch (B) was steamed for 18 min to reach 85–90°C, rolled and the resulting 0·8-mm-thick flakes dried to 12% moisture content. Each batch (400–500 kg) was further processed in a pilot plant roller mill (Mariestad, Sweden) of the same type used in commercial wheat milling. Three milling fractions mainly composed of bran, outer starchy endosperm and inner starchy endosperm, respectively, were obtained in relative yields of 25%, 23% and 52% (batch A) and 26%, 29% and 45% (batch B). The distribution of particles with a size less than 0·5 mm was (in the order bran, outer, inner endosperm) 16%, 90% and 97% (batch A) and 19%, 96% and 94% (batch B). Each oat fraction was analysed by AB AnalyCen (Lidköping, Sweden) for the content of starch (Åman & Hesselman, 1984), crude protein (N × 6·25) according to AOAC (1980), crude fat (Anonymous, 1974), ash by heating at 600°C for 6 h, soluble (at 38°C) and insoluble mixed-linked β-glucans (Åman & Graham, 1987) and total dietary fibre (Asp *et al.*, 1983). The content and composition of water-soluble (at 96°C) and -insoluble non-starch polysaccharides and the amount of Klason lignin were determined in the authors' laboratory according to Theander and Westerlund (1986).

Isolation of mixed-linked β-glucans

Duplicate samples of the oat fractions (7·50 g bran or 30·00 g endosperm) were slurried with 30 ml boiling hot 2-propanol in 100-ml thick-walled glass tubes, and 45 ml room-tempered petroleum ether (bp 60–70°C) was added. The tubes were screw-capped, sonicated for 10 min, centrifuged (1000 ***g***, 10 min) and the supernatant decanted. The pellets were further extracted twice with 50 ml of a room-tempered mixture of 2-propanol and petroleum ether (2:3), followed by two similar extractions with 50 ml 90% (v/v) aqueous ethanol. The final pellets, while still wet, were combined for each oat fraction in a 1-litre Duran bottle. Water (300 ml, preheated at 96°C for 15 min) containing 84 mg $CaCl_2$ and 2·5 ml Termamyl 120 L (Novo Nordisk A/S, Copenhagen, Denmark) were then added, the bottle screw-capped and kept in a water-bath for 2 h at 96°C. Bottles were occasionally thoroughly shaken during the first 30 min and the internal pressure built up occasionally released. Next, the mixture was centrifuged (2300 ***g***, 30 min), the supernatant decanted and the pellet washed by centrifugation with two 25-ml portions of water (previously used for quantitative transfer of the slurry from the Duran bottle). The three supernatants were pooled back into the rinsed Duran bottle, and 60 mg NaN_3 and 30 mg pancreatine (Sigma P-7545, St Louis, USA) added. The mixture was kept at 40°C for 3 h in a water-bath with shaker and after dilution to 400 ml with water, 600 ml absolute ethanol was slowly added with magnetic stirring and the bottle was then stored overnight at 4°C. The crude precipitate of mixed-linked β-glucans was isolated by centrifugation (2300 ***g***, 20 min), slurried with a glass rod in a 100-ml beaker using about 2 ml 60% aqueous ethanol, followed by gentle addition of water under stirring until a homogeneous gum was formed. The gum was heated at 70–80°C under magnetic stirring with 250 ml water in an open Duran bottle until a clear solution was obtained and most of the alcohol had evaporated (3–4 h). After cooling, the lukewarm solution was adjusted to 300 ml water, 60·0 g $(NH_4)_2SO_4$ slowly added with vigorous stirring and the suspension was kept for 72 h at 4°C. The mixture was then centrifuged (2300 ***g***, 15 min), the supernatant decanted and later used for isolation of arabinoxylans (see below). The pellet was washed by centrifugation with 50 ml 20% (w/v) aqueous $(NH_4)_2SO_4$, slurried in 50 ml water, dissolved by heating at 80°C for 1–2 h in a dialysis tube (Spectrapor, molecular cut-off 12 000 Da) containing a few drops of chloroform and then dialysed against deionized water for at least 3 × 24 h. The suspension was then centrifuged (2300 ***g***, 15 min), the supernatant weighed, and stored at 4°C after addition of 0·02% (w/w) NaN_3. This liquid extract, containing highly purified mixed-linked β-glucans, was used for various analytical purposes, either directly

or after freeze-drying. The pellet from centrifugation was discarded as sugar analysis showed that the mixed-linked β-glucans were less pure than those in the liquid extract.

Isolation of arabinoxylans

The supernatant obtained on precipitation of mixed-linked β-glucans with 20% $(NH_4)_2SO_4$ (see above) was concentrated by rotary evaporation until salt began to precipitate, and thereafter kept overnight at 4°C. The mixture was centrifuged (2300 ***g***, 20 min), the supernatant discarded (since it contained only trace amounts of polysaccharides) and the pellet redissolved in about 80 ml water by occasional shaking for about 1 h (till all lumps had dissolved). After dialysis for at least 3 × 24 h, again with a few drops of chloroform added, and freeze-drying, an extract enriched in arabinoxylans was obtained.

Analytical methods

All chemicals were of analytical grade. Analyses were performed in at least duplicate unless otherwise stated. Quantitative results are reported on a dry weight basis. Liquid extracts of mixed-linked β-glucans (5·0–10·0 mg polysaccharide) were hydrolysed in 0·36 M H_2SO_4 for 1 h at 125°C, whereas arabinoxylan extracts (5·0–10·0 mg) were hydrolysed in 1 M trifluoroacetic acid (containing 1·0 mg *myo*-inositol as internal standard) for 1·5 h at 121°C (Bengtsson *et al.*, 1992). Neutral non-starch polysaccharide residues were then quantified as alditol acetates by capillary GLC on a CpSil 88 column with *myo*-inositol as internal standard (Theander & Westerlund, 1986).

Viscosity was monitored with a Bohlin Visco 88 instrument (Bohlin Reologi AB, Sweden) equipped with concentric cylinder geometry (C 30, DIN 53019). Oat fractions (3·00 g) were suspended in 20·0 ml water, the suspension was transferred to the viscometer and viscosity monitored for 60 min at 38°C at a shear rate of 1200 s^{-1}.

Nuclear magnetic resonance of polysaccharide extracts (single samples) was conducted on a Varian VXR 400 instrument. ^{13}C (101 MHz) spectra of mixed-linked β-glucan extracts (30 mg freeze-dried material) were recorded in 3 ml dimethylsulphoxide-d_6 at 90°C with solvent reference (39·5 ppm). About 70 000 pulses were given, pulse repetition time was 4·5 s, and radio frequency pulse angle 67°. ^1H (400 MHz) spectra of isolated mixed-linked β-glucans (5·0–10·0 mg) and of arabinoxylan extracts (5 mg) were obtained in D_2O at 85°C with Na-3-trimethylsilylpropionate-d_4 as reference, using about 800 pulses with repetition time 3·75 s and r.f. pulse angle 45°. Processing of NMR spectra was performed with computer software from New Methods Research Incorporated (East Syracuse, New York, USA).

RESULTS AND DISCUSSION

Chemical characterization of oat fractions

Dehulled oats were milled, without or after steam-flaking, and separate fractions of bran, outer starchy endosperm and inner starchy endosperm collected. In these fractions, the contents of crude protein, fat, ash and dietary fibre were, as a rule, highest in bran, whereas the content of starch was highest in the starchy endosperm fractions (Table 1). Dietary fibre comprises non-starch polysaccharides and Klason lignin, and in bran the most abundant of these components were arabinoxylans and mixed-linked β-glucans. The content of insoluble arabinoxylans was generally higher than that of soluble for all fractions and this is consistent with previous results on oats (Petterson *et al.*, 1987; Frölich & Nyman, 1988). The proportion of soluble to insoluble mixed-linked β-glucans was significantly lower in steam-flaked fractions than in non-steam-flaked fractions. This may have been due to decreased activity of endogenous β-glucanase after steam-flaking, resulting in lower analytical values for soluble mixed-linked β-glucans. The fractions also contained significant amounts of Klason lignin (0·6–3·3%). The contents of mixed-linked β-glucans and arabinoxylans were similar in outer and inner starchy endosperm of steam-flaked fractions. This finding is not consistent with that for corresponding non-steam-flaked fractions, suggesting that steam-flaking changed the milling properties and caused less efficient separation of outer and inner endosperm fractions. Otherwise, no major differences were observed between steam-flaked and non-steam-flaked samples. Viscosity was studied for each oat fraction under conditions allowing endogenous enzyme activity (Fig. 1). A high shear rate was necessary to maintain the samples in suspension so that measurements were reproducible. Bran developed considerably higher viscosity than endosperm samples due to a higher mixed-linked β-glucan content. This was particularly pronounced for the sample of steam-flaked bran, possibly due to partial gelatinization of starch and decreased activity of endogenous β-glucanase as a result of the heat treatment. Growth reduction and serum cholesterol-lowering properties have been demonstrated on chickens fed bran from these steam-flaked oat fractions (Petterson & Åman, 1991).

Isolation of arabinoxylans and mixed-linked β-glucans

Isolation of oat mixed-linked β-glucans in high yield and purity is difficult because of the presence of starch,

Table 1. Chemical composition of dehulled oat fractions, without or after steam-flaking (% of dry matter)

Components	Non-steam-flaked oat			Steam-flaked oat		
	Bran	Outer endosperm	Inner endosperm	Bran	Outer endosperm	Inner endosperm
Crude protein	16·1	10·6	9·2	15·6	10·0	9·2
Crude fat	11·5	7·4	7·0	12·2	7·3	6·9
Ash	3·8	1·3	1·0	3·9	1·1	1·0
Starch	46·7	73·0	76·3	45·8	73·4	75·8
Dietary fibre	19·7	5·7	4·0	20·4	5·2	4·1
Arabinoxylans[a]						
Soluble	0·7	0·2	0·2	0·7	0·2	0·3
Insoluble	4·5	1·0	0·8	4·1	0·8	0·7
Mixed-linked β-glucans						
Soluble	4·2	1·3	1·1	4·1	0·9	0·9
Insoluble	3·7	1·0	0·9	4·3	1·3	1·4
Klason lignin	3·3	0·8	0·6	2·7	0·7	0·6

[a]Calculated as the sum of arabinose and xylose residues from the analysis of non-starch polysaccharides.

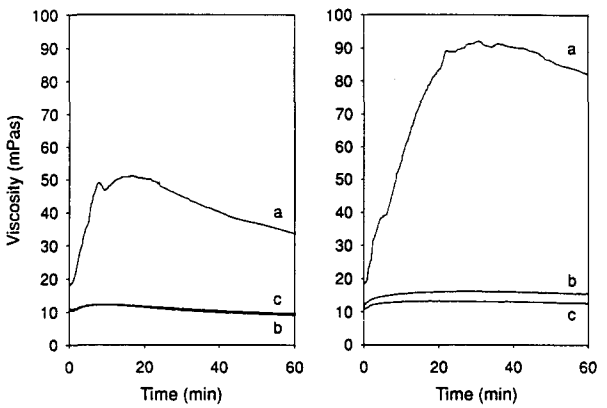

Fig. 1. Changes in viscosity with time of aqueous suspensions of oat fractions at 38°C at a shear rate of 1200 s^{-1}. Non-steam-flaked fractions shown to the left and steam-flaked fractions to the right. a, Bran; b, outer starchy endosperm; c, inner starchy endosperm.

other polysaccharides and protein. The properties of isolated mixed-linked β-glucans, such as molecular weight, may also be significantly changed if endogenous β-glucanase is allowed to operate during the isolation procedure. The procedure developed by Wood and co-workers (Wood *et al.*, 1978, 1986, 1991*a*, *b*) involves mild alkaline extraction, removal of co-extracted protein by precipitation at pH 4·5, followed by isolation and purification of soluble mixed-linked β-glucans by repeated precipitations in 50% 2-propanol. The mixed-linked β-glucans thus isolated contained about 5% protein but by further precipitation, employing 20% (w/v) aqueous $(NH_4)_2SO_4$ as developed by Preece and Hobkirk (1953), about 98% purity could be achieved (Wood, 1986).

In the present study a method for isolation of water-soluble mixed-linked β-glucans was developed that also produced an arabinoxylan extract (Fig. 2). First, extraction of bran, outer endosperm and inner endosperm was performed with a mixture of hot isopropanol and petroleum ether (Hara & Radin, 1978) in order to inactivate endogenous β-glucanase and remove lipophilic substances. Extractions with 90%

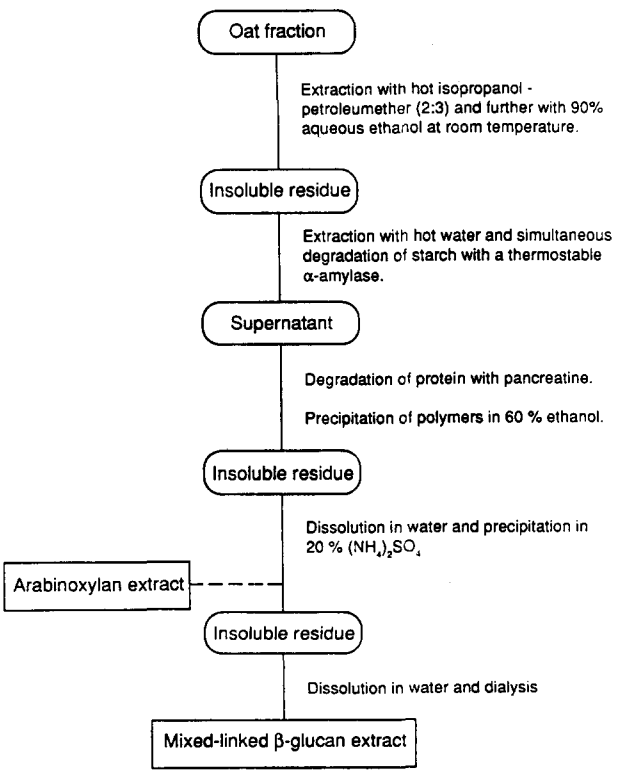

Fig. 2. Scheme for the isolation of arabinoxylan and mixed-linked β-glucan extracts from different oat fractions.

aqueous ethanol then removed more polar substances as low molecular weight sugars. Starch was degraded by treatment of the insoluble residue with a bacterial thermostable α-amylase which has proved to be very effective for this purpose (Theander & Westerlund, 1986). Extraction of lipophilic substances was necessary to optimize the yield and purity of the mixed-linked β-glucans since otherwise there is insufficient phase separation on centrifugation following the enzymatic removal of starch.

The supernatant was treated with pancreatine to decrease protein content in the pellet containing mixed-linked β-glucans insoluble in 60% aqueous ethanol. This alcohol concentration precipitated most of the water-soluble polysaccharides, whereas oligomers from starch degradation remained in solution as evidenced by sugar and starch analyses. During the development of this isolation procedure, direct precipitation by 20% (w/v) aqueous $(NH_4)_2SO_4$ was tried instead of 60% ethanol. However, incomplete precipitation of mixed-linked β-glucans occurred when using 20% $(NH_4)_2SO_4$ directly, probably due to the high concentration of starch oligomers and other components present in the mixture. In the present procedure the mixed-linked β-glucans in the insoluble residue were further purified by dissolution in water and then precipitation with 20% $(NH_4)_2SO_4$ (Fig. 2). A liquid extract containing mixed-linked β-glucans in 96–99% purity was then obtained by dissolution of the precipitate, dialysis and centrifugation.

An arabinoxylan extract was also obtained by further precipitation of the polysaccharides soluble in 20% $(NH_4)_2SO_4$, followed by centrifugation and dialysis of the insoluble residue (Fig. 2). Arabinoxylans in oats have previously been isolated from water extracts digested with α-amylases and characterized by methylation analysis (Aspinall & Carpenter, 1984) or by sugar analysis after further purification on DEAE-cellulose and graded ammonium sulphate precipitation (MacArthur & D'Appolonia, 1980).

Chemical characterization of arabinoxylans

Sugar analysis of the arabinoxylan extracts showed that glucose, arabinose and xylose residues predominated, whereas the contents of galactose and mannose residues were considerably lower (Table 2). The total yields were significantly higher for bran than for endosperm extracts. This observation is consistent with the fact that the contents of soluble arabinoxylans and mixed-linked β-glucans were also much higher in the original bran than in starchy endosperm fractions (Table 1). The comparatively high concentration of glucose residues in all arabinoxylan extracts (0·04–0·12% of the original oat fraction) indicated that small amounts of the soluble mixed-linked β-glucans were not precipitated by 20% $(NH_4)_2SO_4$. However, compared to the amount of soluble mixed-linked β-glucans isolated (Table 3), less than 5% of the mixed-linked β-glucans were lost in this step.

The fine structure of soluble arabinoxylans in oats has not been extensively studied. Methylation analysis has shown (Aspinall & Carpenter, 1984) that terminal arabinofuranosyl residues are linked mainly to O-3 but also both to O-2 and O-3 of the xylose residues. Structural features of soluble pentosans can also be studied by analysis of the anomeric region of ^{1}H-NMR spectra of the polysaccharides.

Generally, for cereal polysaccharides, signals in the region 5·2–5·4 ppm arise from anomeric protons of differently linked arabinofuranosyl residues, whereas signals at 4·4–4·8 ppm arise from xylo- and galactopyranosyl residues (Westerlund *et al.*, 1990; Bengtsson & Åman, 1990). Since there were no major differences between the spectra of non-steam-flaked and steam-flaked fractions, only the latter will be discussed. ^{1}H-NMR of these arabinoxylan extracts showed resonances at the same chemical shifts in the anomeric region (Fig. 3). The signal at 5·44 ppm arises from terminal arabinose residues linked to position O-3 in xylose residues. The two signals of similar intensity at 5·22 and 5·29 ppm are due to terminal arabinose residues substituted at O-2 and O-3, respectively, of the same xylose residue (Westerlund *et al.*, 1990; Bock *et al.*, 1991). This is consistent with the previous findings (Aspinall & Carpenter, 1984) that two structural features are characteristic for soluble arabinoxylans in oat, i.e. terminal arabinofuranosyl residues linked to xylose residues at O-3 (monosubstitution) or at both O-2 and O-3 (disubstitution). Additional structural information

Table 2. Yield of carbohydrate residues in arabinoxylan extracts (% of dry oat fraction)

Carbohydrate residues	Non-steam-flaked oat			Steam-flaked oat		
	Bran	Outer endosperm	Inner endosperm	Bran	Outer endosperm	Inner endosperm
Glucose	0·12	0·06	0·04	0·10	0·04	0·05
Arabinose	0·07	0·02	0·02	0·05	0·02	0·02
Xylose	0·08	0·02	0·03	0·06	0·02	0·03
Galactose	0·01	0·01	0·01	0·01	<0·01	<0·01
Mannose	0·01	<0·01	<0·01	0·01	<0·01	<0·01
Total	0·29	0·10	0·10	0·23	0·08	0·10

Table 3. Yield of mixed-linked β-glucans (% of dry oat fraction) and the relative composition of mixed-linked β-glucan extracts

Components	Non-steam-flaked oat			Steam-flaked oat		
	Bran	Outer endosperm	Inner endosperm	Bran	Outer endosperm	Inner endosperm
Mixed-linked β-glucans	3·25	0·99	0·83	2·47	0·76	0·73
Relative composition:						
Glucose residues	93·6	96·0	96·6	91·9	99·6	99·5
Arabinose residues	1·7	1·3	0·9	1·9	trace	trace
Xylose residues	1·7	1·4	0·8	2·6	trace	trace
Galactose residues	0·6	0·7	0·8	0·4	trace	trace
Mannose residues	1·4	0·3	0·5	2·1	trace	trace
Amino acid residues	1·0	0·4	0·5	1·1	0·4	0·5

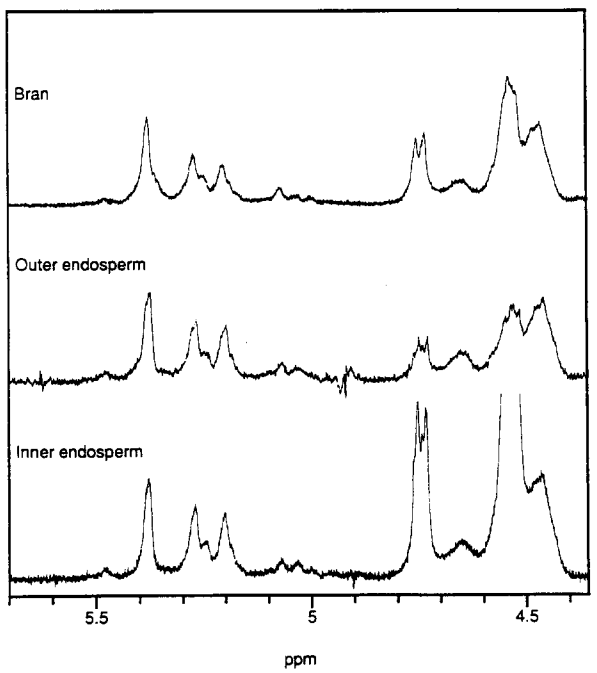

Fig. 3. ^{1}H-NMR spectra at 400 MHz of the anomeric region of polysaccharides in arabinoxylan extracts isolated from steam-flaked oat.

can, however, be obtained by integration of anomeric protons of the individual arabinofuranosyl residues. In this way, the ratio of mono- to double-substituted xylose residues was found to be lower for inner endosperm (1·3:1·0) and outer endosperm (1·5:1·0) than for bran (1·9:1·0) arabinoxylans.

^{1}H-NMR studies of water-soluble arabinoxylans in rye has also revealed the presence of the same structural units. It was found for rye that these may occur in different regions of the same polymer (Bengtsson & Åman, 1990; Åman & Bengtsson, 1991; Bengtsson *et al.*, 1992) or originate from two different polymers.

The small resonance at 5·26 ppm, previously observed in spectra of wheat pentosans (Westerlund *et al.*, 1990), indicates the presence of terminal arabinose residues, probably in an arabinogalactan-protein (Fincher *et al.*, 1974). The major multiplets at 4·75 and 4·55 ppm originate from the anomeric protons of the 3- and 4-linked glucopyranosyl residues in mixed-linked β-glucans, respectively (Bock *et al.*, 1991). In conclusion, ^{1}H-NMR and sugar analysis of the arabinoxylan extracts showed that all oat fractions contained arabinoxylans, mixed-linked β-glucans and probably also traces of arabinogalactan-proteins.

Chemical characterization of mixed-linked β-glucans

Sugar analysis showed that, as expected, the mean yields of mixed-linked β-glucans (glucose residues) were significantly higher for bran than for starchy endosperm extracts (Table 3). Also, the differences between the duplicates were found to be higher for untreated and steam-flaked bran (3·14–3·38% and 2·51–2·95%) compared to outer endosperm (0·95–1·04% and 0·76–0·77%) and inner endosperm (0·82–0·83% and 0·68–0·78%). This shows that the reproducibility of the method was best for the endosperm samples. The relative composition of the extracts showed that, besides glucose residues (93·9–99·5%), small amounts of other sugar and amino acid residues also were present, suggesting that some pentosans or proteins co-precipitated with the mixed-linked β-glucans or were an integral part of the polymers. There are previous indications that some protein may be covalently bonded to mixed-linked β-glucans in oats (Vårum & Smidsrød, 1988), although the existence of such bonds has not yet been established. Mixed-linked β-glucans isolated from endosperm of steam-flaked samples were found to have the highest purity. The levels of amino acid residues remaining were lower for endosperm (0·4–0·5%) than for bran (1·0–1·1%), showing efficient removal of this component from all samples.

Analysis of the isolated mixed-linked β-glucans by ^{13}C-NMR spectroscopy showed that all samples were very similar (only spectra from steam-flaked oat are therefore shown, Fig. 4), suggesting a common chemical structure for these highly pure mixed-linked β-glucans.

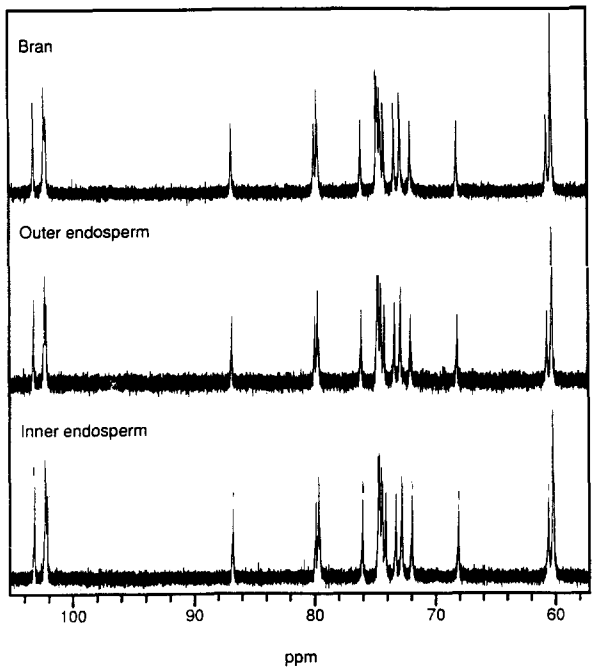

Fig. 4. ^{13}C-NMR spectra at 101 MHz of mixed-linked β-glucan extracts isolated from steam-flaked oat.

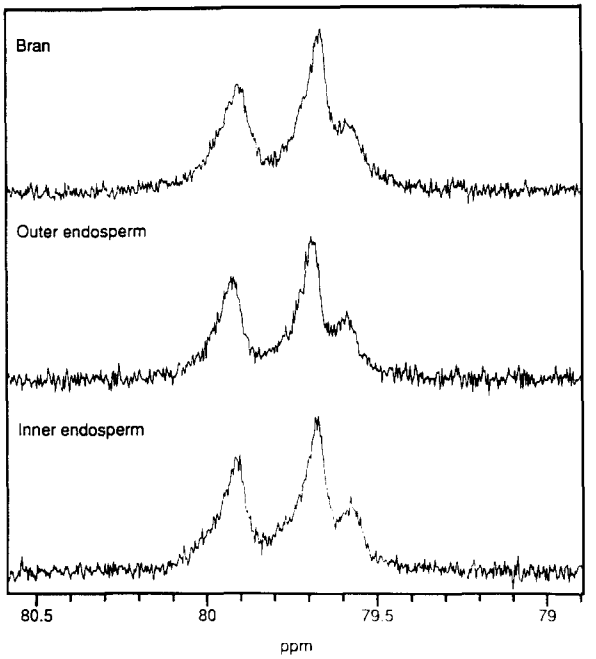

Fig. 5. ^{13}C-NMR spectra at 101 MHz of C-4 signals of 4-linked glucopyranosyl residues in mixed-linked β-glucan extract isolated from steam-flaked oat.

The signals that appeared as singlets (103·2, 86·8, 76·1, 72·0, 68·1, and 60·6 ppm) were attributed to glucopyranosyl residues linked in 3-position (G3), occurring isolated in the polymer, according to previous evidence (Dais & Perlin, 1982; Wood, 1986). Some carbons in the 4-linked glucopyranosyl residues (G4), in particular C-1 (102·2–102·3 ppm) and C-4 (79·5–80·0 ppm) exhibited more than one signal each, showing that the chemical shift of these carbons was differently affected by neighbouring sugar residues in the polysaccharide backbone (Dais & Perlin, 1982; Wood *et al.*, 1991*b*).

Enlargements of the resonances from C-4 in G4 residues revealed the presence of three signals (Fig. 5). This is because the G4 residues in a mixed-linked β-glucan are flanked by glucopyranosyl residues in three ways, viz. G3–G4–G4, G4–G4–G3 or G4–G4–G4. The minor upfield signal has been assigned to C-4 in the G4–G4–G4 unit (Dais & Perlin, 1982). Assuming that the polysaccharide is mainly composed of cellotriosyl and cellotetraosyl units, the proportion of the three signals may be used for calculating the ratio of these cellosyl units in the polymer. Wood *et al.* (1991*b*), however, have observed that the signal-to-noise ratio for crude and purified oat β-glucans prevented highly accurate integration of the three signals. As a better alternative in order to determine the ratio of cellotriosyl to cellotetraosyl units, oligosaccharides formed on enzymatic hydrolysis of oat samples by lichenase was quantified. A molar ratio of 2·1 was found with a total anhydroglucose recovery of 83·7% on average and with no significant difference between samples from oat brans and the whole groat. The resultant polysaccharide structure was composed mainly of cellotriosyl and cellotetraosyl units, separated by (1–3)-β-linkages, with a small (5–6%) portion of 4–8 consecutive 4-linked residues.

In view of the abovementioned and other drawbacks when using ^{13}C-NMR like differences in relaxation times, ^{1}H-NMR analysis was used in the present study in order to achieve more accurate quantification of signals. In this way the proportion of G3 residues in the polymer was obtained, since in D_2O at 85°C integration of the signals arising from anomeric protons in the G3 and G4 residues were well-separated at 4·52–4·59 ppm and 4·74–4·79 ppm, respectively (cf. Bock *et al.*, 1991). The proportion of (1–3)-β-linkages was found to be 28·3% (SD 0·8, n = 6). The findings of Wood *et al.* (1991*b*) for a purified oat β-glucan that cellotriosyl and tetraosyl units are present in a ratio of 2·0:1 together with about 5% of cellopentaosyl and higher units, corresponds to 28·5% of (1–3)-β-linkages in the polymer. Within the limitations of the methods used, it is interesting that this value is in good agreement with that found in the present study, especially when quite different extraction methods were used for isolating the polysaccharides.

The viscosity properties of the mixed-linked β-glucans isolated are being further investigated. Thus, it has been found (Wikström Jansson *et al.*, unpublished results) that the mixed-linked β-glucans isolated from bran on average had a much higher viscosity (7·0 ± 1·2 mPa s) than those from endosperm fractions (1·5 ± 0·4 mPa s), when measured at a shear rate of 37·1 s^{-1}.

CONCLUSIONS

A mild procedure was developed for isolation of water-soluble mixed-linked β-glucans from oat fractions in good yield and with low contamination of other sugar residues and protein. The structure of mixed-linked β-glucans was found to contain 28·3% of 3-linked glucopyranosyl residues occurring isolated within the polymer, and to be very similar for bran and starchy endosperm fractions. Water-soluble oat arabinoxylans were composed of a main chain of (1–4)-β-D-linked xylopyranosyl residues, some of which were substituted by terminal L-arabinofuranosyl residues, mainly at O-3 but also at both O-2 and O-3. The proportion of xylose residues substituted solely at O-3 was higher for arabinoxylans from bran than from endosperm. Variations in the content and structure of oat polysaccharides can be expected to affect the physiological and technological properties of oats.

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REFERENCES

Åman, P. & Bengtsson, S. (1991). Periodate oxidation and degradation studies on the major water-soluble arabinoxylan in rye grain. *Carbohydr. Polym.*, **15**, 405–14.

Åman, P. & Graham, H. (1987). Analysis of total and insoluble mixed-linked (1-3), (1-4)-β-glucans in barley and oats. *J. Agric. Food Chem.*, **35**, 704–9.

Åman, P. & Hesselman, K. (1984). Analysis of starch and other main constituents of cereal grains. *Swedish J. Agric. Res.*, **14**, 135–9.

Andersson, J.W. & Chen, W-J.L. (1986). Cholesterol-lowering properties of oat products. In *Oats, Chemistry and Technology*, ed. F.H. Weber. American Association of Cereal Chemists, St Paul, MN, pp. 309–33.

Anonymous (1974). Fedt. Bestemmelse i kød og kødvarer efter SBR (Schmid-Bonzynski-Ratzloff). *Nordisk metodikkommitte för livsmedel*, 88.

AOAC (1980). *Official Methods of Analysis* (14th edn). Association of Official Analytical Chemists, Washington, DC, p. 1141.

Asp, N-G., Johansson, C-G., Hallmer, H. & Siljeström, M. (1983). Rapid enzymatic assay of insoluble and soluble dietary fiber. *J. Agric. Food Chem.*, **31**, 476–82.

Aspinall, G.O. & Carpenter, R.C. (1984). Structural investigations of the non-starchy polysaccharides of oat bran. *Carbohydr. Polym.*, **4**, 271–82.

Bengtsson, S. & Åman, P. (1990). Isolation and chemical characterization of water-soluble arabinoxylans in rye grain. *Carbohydr. Polym.*, **12**, 267–77.

Bengtsson, S., Andersson, R., Westerlund, E. & Åman, P. (1992). Content, structure and viscosity of soluble arabinoxylans in rye grain from several countries. *J. Sci. Food Agric.*, **58**, 331–7.

Bock, K., Duus, J.O., Norman, B. & Pedersen, S. (1991). Assignments of structures to oligosaccharides produced by enzymic degradation of a β-D-glucan from barley by ^{1}H- and ^{13}C-n.m.r. spectroscopy. *Carbohydr. Res.*, **211**, 219–33.

Clarke, A.E. & Stone, B.A. (1963). Chemistry and biochemistry of β-glucans. *Rev. Pure Appl. Chem.*, **13**, 134–56.

Dais, P. & Perlin, A.S. (1982). High-field, ^{13}C-N.M.R. spectroscopy of β-glucans, amylopectin, and glycogen. *Carbohydr. Res.*, **100**, 103–16.

Ernst, P.A., Thye, F.W., Palmer, J.K. & Saltmarch, M. (1985). The effects of unprocessed and processed oat bran on cholesterol levels of healthy males. *Fedn Proc.*, **44**, 758.

Fincher, G.B., Sawyer, W.H. & Stone, B.A. (1974). Chemical and physical properties of an arabinogalactan-peptide from wheat endosperm. *Biochem. J.*, **139**, 535–45.

Forrest, I.S. & Wainwright, T. (1977). The mode of binding of β-glucans and pentosans in barley endosperm cell walls. *J. Inst. Brew.*, **83**, 279–86.

Frölich, W. & Nyman, M. (1988). Minerals, phytate and dietary fibre in different fractions of oat-grain. *J. Cereal Sci.*, **7**, 73–82.

Hara, A. & Radin, N.S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.*, **90**, 420–6.

Heims, H. & Steinhart, H. (1991). Determination of structural features of the water-insoluble dietary fiber from oats by the reductive-cleavage method. *Carbohydr. Polym.*, **15**, 207–14.

Henry, R.J. (1987). Pentosan and (1-3), (1-4)-β-glucan concentrations in endosperm and wholegrain of wheat, barley, oats and rye. *J. Cereal Sci.*, **6**, 253–8.

Hurt, H.D., Mathews, R. & Ink, S.L. (1988). Biomedical considerations of oat dietary fibre and β-glucans. In *Proceedings of the 3rd International Oat Conference*, eds B. Mattson & R. Lyhagen. Lund, Sweden, pp. 206–22.

Kirby, R.W., Andersson, J.W., Sieling, B., Rees, E.D., Chen, W.J.L., Miller, R.E. & Kay, R.M. (1981). Oat-bran intake selectively lowers serum low-density lipoprotein cholesterol concentrations in hypercholesterolemic men. *Am. J. Clin. Nutr.*, **34**, 824–9.

MacArthur, L.A. & D'Appolonia, B.L. (1980). Comparison of nonstarchy polysaccharides in oats and wheat. *Cereal Chem.*, **57**, 39–45.

Petterson, D. & Åman, P. (1991). Production results, serum cholesterol concentration and carcass composition of broiler chickens fed diets based on bran or inner endosperm from oats with and without enzyme supplementation. *J. Sci. Food Agric.*, **57**, 273–86.

Petterson, D., Graham, H. & Åman, P. (1987). The productive value of whole and dehulled oats in broiler chicken diets, and influence of β-glucanase supplementation. *Nutr. Report. Int.*, **36**, 745–50.

Preece, I.A. & Hobkirk, R. (1953). Non-starchy polysaccharides of cereal grains. III. Higher molecular gums of common cereals. *J. Inst. Brew. London*, **59**, 385–92.

Staudte, R.G., Woodward, J.R., Fincher, G.B. & Stone, B.A. (1983). Water-soluble (1-3), (1-4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. III. Distribution of cellotriosyl and cellotetraosyl residues. *Carbohydr. Polym.*, **3**, 299–312.

Theander, O. & Westerlund, E. (1986). Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. *J. Agric. Food Chem.*, **34**, 330–6.

Vårum, K.M. & Smidsrød, O. (1988). Partial chemical and physical characterisation of (1–3), (1–4)-β-glucans from oat (*Avena sativa* L.) aleurone. *Carbohydr. Polym.*, **9**, 103–17.

Vollendorf, N.W. & Marlett, J.A. (1991). Dietary fiber methodology and composition of oat groats, bran, and hulls. *Cereal Foods World*, **36**, 565–70.

Westerlund, E., Andersson, R., Åman, P. & Theander, O. (1990). Effects of baking on water-soluble non-starch polysaccharides in white bread fractions. *J. Cereal Sci.*, **12**, 33–42.

Wood, P. (1986). Oat β-glucan: structure, location and properties. In *Oats, Chemistry and Technology*, ed. F.H. Weber. American Association of Cereal Chemists, St Paul, MN, pp. 121–52.

Wood, P.J., Siddiqui, I.R. & Paton, D. (1978). Extraction of high viscosity gums from oats. *Cereal Chem.*, **55**, 1038–49.

Wood, P.J., Weisz, J., Fedec, P. & Burrows, V.D. (1989). Large-scale preparation of oat fractions enriched in (1–3) (1–4)-β-D-glucan. *Cereal Chem.*, **66**, 97–103.

Wood, P.J., Weisz, J. & Fedec, P. (1991*a*). Potential for β-glucan enrichment in brans derived from oat (*Avena sativa* L.) cultivars of different (1–3), (1–4)-β-D-glucan concentrations. *Cereal Chem.*, **68**, 48–51.

Wood, P.J., Weisz, J. & Blackwell, B.A. (1991*b*). Molecular characterization of cereal β-D-glucans. Structural analysis of oat β-D-glucan and rapid structural evaluation of β-D-glucans from different sources by high-performance liquid chromatography of oligosaccharides released by lichenase. *Cereal Chem.*, **68**, 31–9.

Wood, P.J., Weisz, J. & Mahn, W. (1991*c*). Molecular characterization of cereal β-glucans. II. Size-exclusion chromatography for comparison of molecular weight. *Cereal Chem.*, **68**, 530–6.

Woodward, J.R., Fincher, G.B. & Stone, B.A. (1983). Water soluble (1–3) (1–4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure. *Carbohydr. Polym.*, **3**, 207–25.