Large-Scale Preparation and Properties of Oat Fractions Enriched in (1→3)(1→4)-β-D-Glucan[1]

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ABSTRACT

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Large amounts (kilograms) of oat gum, the major component of which is (1→3)(1→4)-β-D-glucan (β-glucan) and oat bran were prepared for nutritional studies using pilot plant equipment. Bran fractions, containing from 10.9 to 16.6% β-glucan and which were used to increase the yield of gum, were prepared by air-classification of hexane-defatted flour or of full-fat flour, and by simple sieving techniques. Defatted, air-classified bran after refluxing in ethanol to deactivate enzymes, was extracted with sodium carbonate at ph 10 to give oat gum containing 78% β-glucan. This material was found to exhibit significantly lower viscosity than material prepared at the bench. Monitoring the pilot plant process showed that the major viscosity losses occurred during centrifuging. Oat gums were pseudoplastic in aqueous solution, but under conditions of high shear there was a permanent loss in viscosity. This was reflected in reduced molecular size as shown by high-performance gel chromatography, which also revealed bench-prepared oat gum to be of higher molecular weight than pilot plant gum. No difference in the major structural characteristics was detected between pilot plant material and bench material or between β-glucan derived from the inner and outer parts of the grain.

The current interest in dietary fiber has been stimulated by the epidemiologically derived hypothesis that many diseases of Western developed societies might be attributed to a lack of fiber in the diet (Trowell and Burkitt 1975). Animal and clinical studies have clearly established specific beneficial effects of dietary fiber, although studies have been confounded by problems with definition and lack of recognition that dietary fiber cannot be treated as a single entity. Soluble and insoluble dietary fiber can have quite different effects, and even within these categories there may be some variation (Jenkins et al 1978, Anderson and Chen 1979, Eastwood et al 1986). The potential value of oat bran as a source of dietary fiber, particularly soluble dietary fiber, was first recognized by Anderson and colleagues (Anderson and Chen 1979, Kirby et al 1981). In addition to being a valuable component of a high-carbohydrate, high-fiber diet useful to diabetics for control of glucose metabolism, oat bran brought about a selective decrease in serum low-density lipoprotein cholesterol levels (Kirby et al 1981). In studies of the effects of fiber supplements, soluble fibers such as guar gum have been shown to lower postprandial glucose and insulin levels and to lower serum cholesterol (Jenkins et al 1975, Jenkins et al 1978).

To further study the value of oat bran as a source of dietary fiber and to develop palatable products containing higher levels of soluble fiber than are currently available without recourse to supplements, large quantities of oat brans are required. To more clearly delineate the role of the soluble fiber (oat gum) in oat bran, large quantities of this product, mainly a (1→3)(1→4)-β-D-glucan, are also needed. The method of preparation and properties of these materials, which are currently in use in human and animal nutritional studies, are described here.

MATERIALS AND METHODS

Oats: Source and Preparation

Oats (*Avena sativa,* cv. Hinoat) were grown in the Ottawa area. Dehulling was carried out with either the series 14 Entoleter impact mill (CEA Simon-Day, Winnipeg, MB) or a pneumatic dehuller, laboratory model (Hydromechanique et Frottement, Andrezieux Boutheon, France). The impact mill was operated at 120–125 psi and a throughput of 475–1,000 kg/hr. Hulls were removed on a multipass aspirator (Kice Metal Products, Wichita, KS) with an airflow of 450 ft^3/min and a throughput of 750 kg/hr. Groats were separated from remaining whole seed on a gravity table (Kipp-Kelly Ltd., Winnipeg, MB) and intact grains recycled. Dehulled seed and subsequently prepared flours were stored at −17°C.

Analysis and Characterization of Samples

β-Glucan was analyzed by the method of McCleary and Glennie-Holmes (1985) using the Biocon kit (Biocon U.S.A.,

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Lexington, KY). For structural characterization, the oligosaccharides obtained by the action of the (1→3)(1→4)-β-D-glucan-4-glucanohydrolase were not treated by β-glucosidase as described in the kit, but were fractionated on a Bio-Rad HPX-42A or HPX-65A column maintained at 85° C and eluted with deionized distilled water at 0.4 ml/min. Sugars were detected with the orcinol-sulfuric acid reaction using a Technicon Autoanalyzer (Kennedy et al 1980, Wood 1985).

Starch was analyzed by the method of Batey (1982) using amylase preparations that did not release glucose from β-glucan. Glucose was then determined by an automated glucose oxidase assay using the Technicon Autoanalyzer. Recovery of pure cornstarch in the assay was 98–99%.

Standard deviations are reported for analyses replicated three or more times. Duplicate values were within 5% of each other.

Viscosity. Kinematic viscosities were measured at 25° C in Cannon-Manning calibrated semi-micro Ubbelhode type capillary viscometers as previously described (Wood et al 1978). Apparent viscosities and shear stress-shear rate studies were done in a Carri-Med (Dorking, Surrey, UK) controlled stress rheometer using a 4-cm, 1° cone and plate or, when extended periods of shear were examined, a 4-cm, 2° cone and plate with solvent trap.

Solutions of β-glucan were prepared by vigorous stirring in water containing 5 m*M* sodium azide at 55° C for at least 3 hr. Care was taken to avoid lumps of gel-like material forming on initial mixing and "skin" formation associated with evaporation. Losses of solvent were prevented by covering vessels with Parafilm. Solutions of gum were then centrifuged at 33,000× *g* for 0.5 hr, and supernatants were used for viscosity measurement. Final solids content was determined by evaporation of aliquots in vacuo at 60° C with corrections for azide content. (Note: The high viscosity of 1% bench oat gum made this process inaccurate; however, the bench gum did not produce significant residue on centrifuging, and thus concentration as estimated from the weighed amount approximates true concentration.) Except where otherwise noted, bench gum was from ethanol-treated Hinoat flour as described by Wood et al (1978).

An Oster 10-speed blender ("grate" setting for 45 sec) and Brinkman Instruments Polytron (PT-35 probe; setting 6 for 5 min) were used to subject samples to high shear.

High-performance gel chromatography. Samples of polysaccharide were dissolved (1 mg/ml) in 0.05*M* sodium 2-(*N*-morpholino) ethane sulfonate buffer, ph 6.5, containing 5 m*M* sodium azide and filtered (0.45 μm) prior to analysis on a Bio-Rad TSK 60 column maintained at 40° C and eluted with the buffer at 0.4 ml/min. The polysaccharides were detected with the orcinol-sulfuric acid reaction (Kennedy et al 1980, Wood 1985). The column was calibrated using pullulan standards (Shodex P-82 series, Showa-Denka K. K., Tokyo).

Effect of high shear (Oster blender) on viscosity and molecular size of oat gum isolated at the bench from oat bran. Full-fat, sieved, air-classified, ethanol-deactivated bran was extracted (20:1 liquid to solids) in the usual fashion (Wood et al 1978), but following adjustment to ph 4.5 and centrifugation, the supernatant was divided into two parts, one part of which was isolated by alcohol precipitation as usual. The remaining portion was blended in the Oster blender, allowed to stand for 20 hr at 2° C, and then precipitated as usual. Viscosities of the supernatant were determined before, immediately after, and after standing for 20 hr. Yields and viscosities of isolated gums were determined.

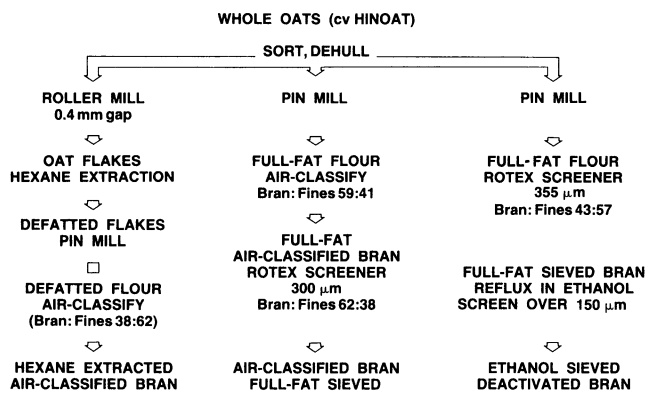

Fig. 1. Flow chart summarizing pilot plant production of oat brans.

Preparation of β-Glucan-Rich Samples

Flow diagrams (Figs. 1 and 2) summarize the process used.

Hexane-extracted, air-classified bran. In the laboratory, groats were adjusted to 10% moisture and flaked in a laboratory roller mill at gap settings of 0.25, 0.33, 0.40, and 0.48 mm. The flaked groats (10 g) were extracted with 6 × 600 ml warm *n*-hexane to simulate pilot plant solvent extraction. Oil was determined by the Butte tube method (AOCS Ba-3-38).

In the pilot plant, the groat was flaked at ≈100 kg/hr on a roller mill (Simon-Day, 45 × 30 cm) with a roll gap setting of 0.40 mm. Flaked groats (≈500kg) were fed at 65.6 kg/hr to a counter-current extracter (Crown Iron Works, Minneapolis, MN) with a retention time through the hexane wash cycle of ≈80 min. Fresh solvent was added at the rate of 3.0–3.3 L/min. Extracted oil was recovered in a rising film evaporator, and residual meal was desolventized in a two-tray toaster (52–60° C) followed by air desolventizing for two to four days.

The defatted flaked groats were pin-milled at 11,800 rpm housing and 3,200 rpm door side in a Contraplex A250WC pin mill (Alpine, Augsberg, Germany) at 136 kg/hr to obtain a flour of 90% through a 600 μm sieve. The flour was fed at 142 kg/hr to a Bauer Centri-Sonic 751 air classifier (Bauer Bros., Brantford, ON), with the classifier rotor speed at 2,200 rpm and collector pressure at −73 cm (H_2O), to give hexane-extracted, air-classified coarse (bran) and fines fractions.

Full-fat, sieved, air-classified bran. Pin-milled, full-fat groats were fed 102 kg/hr to the air classifier but at a collector pressure of

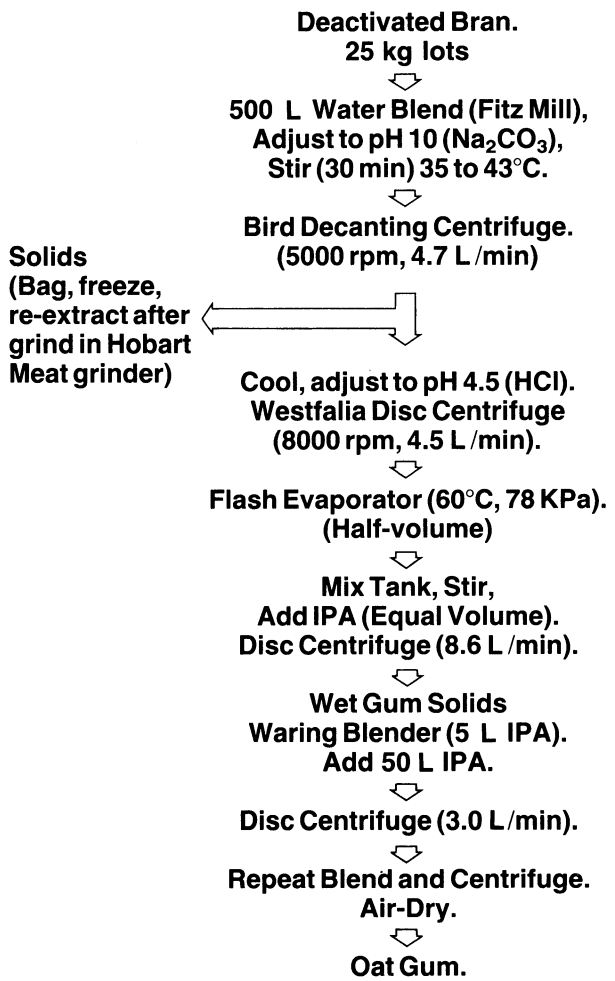

Fig. 2. Flow chart summarizing pilot plant production of oat gum.

−77 cm (H_2O). The coarse-to-fines ratio was high (59:41), and in order to upgrade the "bran" it was further fractionated by sieving over a Duralloy bolting cloth (300 μm) in a model 111A Rotex screener (Sullivan, Strong, Scott, Winnipeg, MB) at 105 kg/hr to give full-fat, sieved, air-classified bran.

Full-fat, sieved bran. Bran was also prepared by sieving. Pin-milled, full-fat groats were screened over 355 μm Duralloy bolting cloth in the Rotex screener at 100 kg/hr to give bran that was further processed in refluxing 75% ethanol, cooled, and pumped over 150 μm bolting cloth in the Rotex screener. The retained wet bran was reslurried in 100% ethanol, screened, and air-dried to give ethanol sieved, deactivated bran.

Enzyme deactivation in refluxing ethanol. Typically, 55 kg of bran was mixed with 500 L of aqueous ethanol (75%, v/v, ethanol) in a 560-L reactor vessel prepared for reflux circulation at ≈80° C and stirred for 4 hr. The cooled (50° C) contents were pumped to a plate and frame filter press and purged of free ethanol with N_2. Then the wet bran was removed, reslurried in 100% ethanol, and after 15 min stirring returned to a fresh filter press and purged with N_2. The wet bran was allowed to desolventize for three days in a stainless steel, forced-ambient-air desolventizer.

Extraction of oat gum. Deactivated bran (25 kg) was blended with water (500 L) at 33–35° C using a model DAS06 comminuting mill (Fitzpatrick Co., Elmhurst, IL) equipped with a 1.6-mm screen. The pH was adjusted to 10 by adding 20% sodium carbonate, and the mixture was stirred in a holding tank for 30 min. Liquid extract was separated from residue using a 15× 30 cm decanting centrifuge (Bird Machine, S. Walpole, MA) operating at 5,000 rpm and a flow of 4.7 L/min. Solids were bagged and held at −20° C prior to further extraction. Frozen bran residue was ground in a meat grinder fitted with a 4.5-mm die (Hobart Mfg., Troy, OH) prior to reextraction with 500 L of pH 10 sodium carbonate at ≈45° C.

The liquid extract was cooled by pumping through a CV-5 shell and tube chiller at 18° C (Chester Jensen, Chester, PA) to a holding tank where the pH was adjusted to 4.5 with 20% (v/v) hydrochloric acid. The resulting mixture was centrifuged in an SA-7-01-576 Westfalia disk centrifuge (Centrico, Northvale, NJ) at 8,400 rpm and 4.5 L/min with the desludge cycle set at 10-min intervals. The supernatant was pumped to a model R56 flash evaporator (APV-Crepaco, Tonawanda, NY) and reduced to 40–50% of the original volume at ≈4.2 L/min with a heat exchange temperature of 60° C and a system pressure of 23 kPa. The liquid was cooled and pumped to a mixing tank, and 100% propan-2-ol or ethanol was added in equal volume with vigorous stirring. The precipitated gum was collected under nitrogen in the disk centrifuge at a feed rate of 8.6 L/min with desludge intervals of 20 min. The gum solids, after blending in 100% propan-2-ol or ethanol in a 5-L Waring Blendor, were made up to 50 L with the alcohol and separated at 3 L/min in an explosion-proof decanting centrifuge (Bird, 15× 30 cm) at a speed of 5,000 rpm. The gum cake was again blended, resuspended in alcohol, centrifuged, air desolventized for two to three days, then weighed and ground in a model 66B Jacobsen hammer mill (Kipp-Kelly) equipped with a 250-μm screen.

RESULTS

Preparation of β-Glucan Enriched Fractions

Hexane extraction of rolled oats. Laboratory studies showed that hexane extraction of flaked groats would reduce oil content to levels suitable for air-classification of flour (<2%) but this could not be achieved with whole groats.

Counter-current extraction in the pilot plant reduced the oil content from 5.5% to an average of 1.5% (range 0.7–2.0%). A thick flake (0.4 mm) adjusted to 10% moisture was used, but release of starch fines (2–4 mm) into the hexane occurred, leading to pump and cyclone blockages and accumulation of solids in the extractor bed. Continuous operation beyond 3–4 hr was thus not possible. About 500 kg of defatted flakes was prepared before cleanup of the extractor became necessary. A sludgy oil fraction was obtained in 5.9% yield, and fines losses totalled 3.3%.

Classification of oats to produce coarse, or bran, fractions. Defatted, flaked groats (5.5% β-glucan) were milled and air-classified to yield, in the ratio of 38:62, a bran (coarse) fraction (11.2% β-glucan) and a fines fraction (1.3% β-glucan).

Although trial air-classification of milled full-fat groats (5.6% β-glucan) gave bran containing as much as 13.8% β-glucan, large-scale air-classification of full-fat pin-milled groats (468 kg) was less successful. A final overall "bran"/flour split of 59:41 was obtained, and examination of the air classifier revealed that the vane openings were half-filled and rotor honeycombs were partially blocked by flour.

The air-classified full-fat bran was further fractionated by sieving to give a 62:38 bran/flour split and a final bran product of 12.8% β-glucan in 34% overall yield

Sieving alone was also used to prepare large quantities of oat bran enriched in β-glucan. Sieving full-fat pin-milled groats on a 355-μm screen gave a 43% yield of bran with a β-glucan content of 10.9%. This preparation of bran was further fractionated by sieving in ethanol after treatment in refluxing ethanol, to give a final bran product (overall yield 21%) containing 16.6% β-glucan. The β-glucan and dietary fiber contents of the different brans obtained are summarized in Table I.

Extraction of oat gum. Milled groats used in the preparation of hexane-extracted brans were extracted at the bench level as described by Wood et al (1978). Yields and viscosities of gums (Table II) were similar to those previously reported and showed the same effect of refluxing in ethanol to deactivate enzymes. (Note: extract numbers 1, 2, and 3 refer to consecutive extractions as previously reported.) Because of the development of unmanageable viscosity when bran was extracted at 10:1 (v/w) ratio, a 20:1 ratio was used in pilot plant extractions.

A total of 2,000 kg of bran was extracted (Fig. 2) yielding 18.6 kg of gum. Examination of the first and second extract from each 25 kg lot of bran processed did not reveal a difference in kinematic viscosities between extracts 1 and 2 (8.1 ± 0.9 centistokes [cS] and 9.4 ± 2.1 cS, respectively) as is noted with bench extractions. On two occasions a further third consecutive extraction was done, but

TABLE I
β-Glucan and Dietary Fiber Content (percent, dwb) of Oat Fractions Obtained by Air-Classification and Sieving[a]

Sample	Yield % of Starting Flour	β-Glucan	Dietary Fiber[b] Soluble	Dietary Fiber[b] Total
Dehulled Hinoat	···	4.7–5.7	5.4–5.8	12.7–14.2
Hexane-extracted, air-classified bran	38	11.2 ± 0.3	nd[c]	nd[c]
Full-fat sieved, air-classified bran	34	12.8 ± 0.1	14.2	38.6
Full-fat sieved bran	43	10.9 ± 0.2	12.1	27.9
Ethanol-sieved deactivated bran	21	16.6 ± 0.3	18.8	40.1

[a] As shown in Fig. 1.
[b] Mongeau and Brassard 1986 (average of duplicates).
[c] Not done.

TABLE II
Yields and Viscosities of Gums Extracted from Untreated and Enzyme-Deactivated[a] Hinoat Flour Used in the Pilot Plant

Flour	Extract No.[b]	Gum Yield (% dwb)[c]	Kinematic Viscosity (cS)[d]
Untreated	1	2.6 ± 0.2	5.0 ± 0.3
	2	1.5 ± 0.1	14.3 ± 0.6
	3	0.7 ± 0	31.9 ± 2.8
Enzyme-deactivated	1	1.3 ± 0.1	34.0 ± 2.9
	2	1.5 ± 0.1	61.8 ± 6.9
	3	1.1 ± 0.1	60.6 ± 2.7

[a] By a 2 × 2 hr reflux in 70% (v/v) ethanol.
[b] Consecutive extracts.
[c] Dry weight basis.
[d] 0.2%, w/v, in dimethylsulfoxide; cS = centistokes.

for the total from extracts 1 and 2 alone the average yield was 8.8 ± 2.2% (not greatly different from the overall 9.3% yield). (A subsequent pilot plant production of gum using essentially the same process but extracting 50 kg lots of bran [13% β-glucan] in 1,000 L, gave a similar gum preparation, containing 76.4 ± 0.6% β-glucan and with a kinematic viscosity (0.2% in dimethylsulfoxide) of 8.4 ± 0.5 cS, in 8.9% yield.)

Microbiological examination of the combined 18.6 kg of gum revealed levels of microorganisms ($>10^5$) that were not acceptable for food use. To correct this, the gum was treated with refluxing 75% ethanol, washed with 100% ethanol, and dried, to give a final product (16.2 kg) showing <100 microorganisms per gram and no evidence of aerobic or anaerobic spore formers. Other components of the pilot plant gum were as follows (dwb, average of duplicates): β-glucan, 78 ± 0.7%; pentosan, 2.9% (determined by acid hydrolysis using 1*M* trifluoroacetic acid for 1 hr and high-performance liquid chromatography [HPLC] with a Bio-Rad HPX-85 column); starch, 7.8 ± 0.3%; protein, 7.2% (N × 6.25); ash, 1.6%; and phytic acid, 0.8% (analyzed by a ^{31}P nuclear magnetic resonance technique based on that of O'Neill et al 1980).

Viscosity loss in pilot plant. Viscosity loss in the pilot plant was monitored by sampling the extract at different stages through the extraction scheme. These samples were then processed to completion in the laboratory (Wood et al 1978), and viscosity of the final gums was determined (Table III). Viscosity declined during the extraction process, and this occurred most rapidly during the centrifugation step.

Characterization of Products

Effect of shear stress on oat gum viscosity and molecular size. The effects of shear rate on viscosity of aqueous solutions of bench-prepared (0.5 and 1%) and pilot plant (1%) oat gums are shown in Figure 3. Solutions showed pseudoplastic behavior typical of high molecular weight polysaccharides. Analysis of the shear rate-shear stress curves (Table IV) indicated a good fit ($r > 0.99$) to the power law relationship $S = cR^n$, where S = shear stress, R = shear rate, and c and n are constants. (For pseudoplastic solutions $0 < n < 1$; the smaller n, the greater the shear sensitivity.) Duplicate values were within 5% of each other except for the plastic viscosity coefficient of 1% solutions of bench gum where difficulties in getting exact concentrations cause curve displacement. Thus the calculated viscosity at 30 sec^{-1} was about 14.5 poise, but the measured value in the sample shown was 16% greater. Solutions were subject to shear rates up to 1,500/sec for 5–15 min (the longer periods require a solvent trap to prevent evaporation). No permanent viscosity loss was observed under these conditions. At high shear rates the linearized data have a different slope than at low shear rates. The data in Table IV were obtained with shear rates <100 sec^{-1}. Bench gum was more shear sensitive ($n = 0.61$) than the pilot plant gum ($n = 0.76$) even at about half the concentration.

The effect of shear stress on isolation of gum was monitored at the bench level. Bran extracts were prepared as usual (Wood et al 1978), and the effect of high shear (Oster blender) on the crude extracts and isolated gums examined. The results (Table V) show a loss of viscosity in the supernatant after blending that does not greatly change in standing. Yields of the final product were unaffected but viscosities were significantly reduced. Blended samples showed a small increase in retention time on high-performance gel chromatography (Fig. 4). The kinematic viscosities of aqueous solutions (0.5%, w/v) of pilot plant gum were further reduced from 36 to 13 cS by very high shear in a Polytron blender. Bench-prepared gum (≈650 cS) was reduced to similar viscosities in the Polytron. Retention time of the pilot plant gum on high-performance gel chromatography showed a corresponding increase from 22.5 to 24.1 min (Fig. 4). (Note: Dextran from Sigma Chemical Co., quoted mol wt 5–40 × 10^6, had a peak retention time of 18.8 min.)

HPLC analysis of enzyme-treated oat flours and gums. The low molecular weight products from the action of (1→3)(1→4)-β-D-glucan-4-glucanohydrolase on oat flours, brans, and gums were analyzed by HPLC. Profiles from purified oat β-glucan (bench preparation) and pilot plant oat gum are compared in Figure 5. Essentially no differences were observed between oligosaccharide patterns from fines, bran, or gums regardless of whether pilot plant

TABLE III
Viscosity of Gums Isolated from Extracts Sampled at Different Stages of the Pilot Plant Extraction Process

Process Stage	Viscosity[a] (cS) Run 1	Run 2
30 min @ pH 10, 43°C	29.7	21.4
Decanter feed	26.7	···
Decanter effluent	16.5	16.3
After pump and heat exchanger	15.2	···
Adjusted to pH 4.5	14.6	13.7
After 2 hr @ pH 4.5, disk centrifuge feed	18.9	14.3
Disk centrifuge effluent	10.9	8.1
Final product	8.6	8.2

[a] 0.2% in dimethylsulfoxide; cS = centistokes.

TABLE IV
Summary of Analysis of Shear Rate (R) Against Shear Stress (S) Curves for Bench[a] and Pilot Plant Prepared Gum[b]

Sample	Concentration[c] (%, w/v)	c[d,e]	n[d]
Bench gum	0.5	7.9	0.61
Bench gum	1.0	150	0.30
Pilot plant gum	1.0	10.1	0.76

[a] Wood et al 1978, Hinoat extract 2.
[b] A pseudoplastic power-law relationship of the form $S = cR^n$ was assumed.
[c] Nominal concentration, true solids content of pilot plant gum was 0.93%.
[d] Average of duplicates.
[e] Values given are in dynes sec^n cm^{-2}.

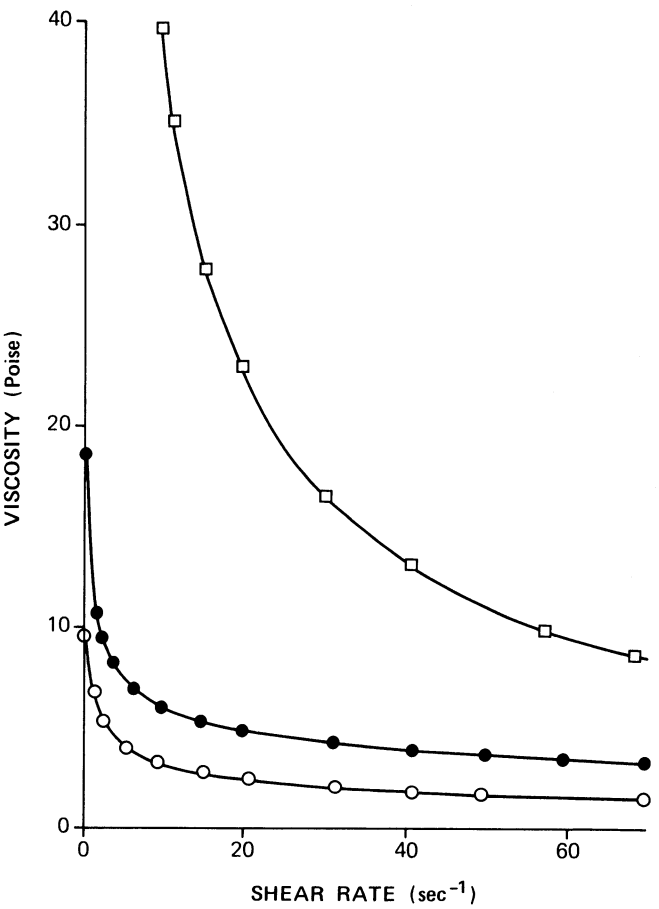

Fig. 3. Effect of shear on apparent viscosity of bench-prepared oat gum (Wood et al 1978, Hinoat extract 2) (o and □ = 0.5 and 1%, w/v, in water, respectively) and pilot plant prepared oat gum (● = 1%, w/v, in water).

or bench prepared. High-speed blending did not result in observable structural changes. In all cases the ratio of trisaccharide to tetrasaccharide peak height was 1.40:0.05, and similar amounts of penta- and hexasaccharide were observed. However, the higher oligosaccharides from flour preparations were obscured somewhat by polymeric peaks, such as pentosan.

DISCUSSION

The (1→3)(1→4)-β-D-glucan of oats is located mainly in the endosperm cell walls, which, in the cultivar Hinoat, are thicker in the subaleurone region than in the inner endosperm (Wood et al 1983). As a consequence, milling tends to produce coarser particles from the aleurone and subaleurone region than from the inner endosperm, and fractionation of coarse (or "bran") particles from fines yields a β-glucan enriched bran (Acker et al 1955, Hohner and Hyldon 1977, Wood and Fulcher 1978). Histochemical evidence suggests that this enrichment is dependant upon the presence of the thick subaleurone endosperm cell walls, since the aleurone cell walls stain markedly less with Congo red than the endosperm walls (Wood et al 1983). It is known that most oat cultivars including commercial oats, have increased β-glucan concentrations in the outer endospermic cell walls (Fulcher and Wood 1983; Yiu et al 1987; S. H. Yiu, *personal communication*). Thus, similar processes should apply to cultivars more easily available than Hinoat.

In these studies, Hinoat, although not a commercial cultivar, was initially used because of its high protein content and known morphology. In other studies (*unpublished*), we found that the major structural features and molecular size of extracts from different cultivars were similar. The methods used do not represent an optimized process, because resources and equipment were limited. For example, hexane extraction of lipids would probably be more efficient with steel-cut rather than flaked groats.

The high lipid content of oats, distributed throughout the kernel, in addition to causing rancidity on storage of milled fractions, limits efficient fractionation during milling. For this reason we used a hexane extraction step before air-classification, essentially as suggested by Hohner and Hyldon (1977). Air-classification without defatting initially produced bran containing 13.8% β-glucan, which declined to less than 10% as air-classification continued. Air-classification was less useful than sieving for fractionation of full-fat oat flours.

Oughton (1980) reported that problems associated with lipid in oats could be avoided by effecting separations in organic solvents using centrifuges and liquid cyclones. Our analysis of bran from Oughton's process (which used Hinoat) showed a β-glucan content of ≈15%, which is similar to the β-glucan content we have achieved by sieving in ethanol. The maximum β-glucan content we have measured was 19.1% in bran (cultivar Marion) from an aqueous process, patented by Burrows et al (1984). Commercial bran is generally analyzed at 8-10% β-glucan. Yields of bran range from 20 to 40% of the dehulled oat, with the higher β-glucan contents being associated generally with the lower bran yields (Table I). The major product of the fractionation is therefore a fine flour fraction. Because our interest was to produce bran for physiological studies, we did not further examine the fine fractions in detail. Commercially, however, these may have as yet unrecognized functionality, and may be of value because of the low β-glucan content (Gordon et al 1986). The lowest value we observed was from ethanol sieved flour from which a fines fraction of 0.7% β-glucan and 1.5% total dietary fiber was obtained.

The method used for extraction of oat gum was similar to that of Hohner and Hyldon (1977) but included an enzyme deactivation step. In our experience, using the viscosity reducing assay with oat β-glucan as substrate, bran showed higher endogenous β-glucanase levels than whole flour. Moist heat treatment, such as used commercially (15% H_2O, 104–116°C, 1–4 hr) reduced, but did not eliminate, β-glucanase activity. Viscosity measurements of the supernatant from full-fat, sieved, air-classified bran extracted with 0.2*M* sodium acetate buffer (pH 4.5) showed a viscosity loss of 24% in 1 hr, whereas bran deactivated by refluxing in 75% ethanol showed no loss in that period. Similarly, reductions in viscosity at pH 10 were somewhat greater in untreated bran (25%) compared to deactivated bran (14%). The initial values of viscosity as determined in extracts immediately after centrifugation were considerably lower (7.3 and 2.5 cS at pH 10 and pH 4.5, respectively) for the untreated bran than for the deactivated bran (29 and 21 cS, respectively.)

The origins of these declines in viscosity of crude extracts, despite prior enzyme deactivation steps, are uncertain. The final effect was greater in the pilot plant (Table IV) than at the bench because of the longer time taken to complete processing. It is possible that at pH 4.5, some residual enzyme activities might be responsible, particularly if these were of bacterial or fungal origin, since such enzymes may have considerable heat resistance (Wood and Weisz 1987). The potential for such contamination is greater in the outer layers of the oat groat. At pH 10, the effect is probably nonenzymic (Madacsi et al 1983) with the higher viscosity in

TABLE V
Effect of High Shear in Oster Blender on Gum Extracts Prepared as Described in the Text (averages of duplicates)

Sample	Extract	Viscosity of Supernatant (cP)[a] Initial	After Blend	After Hold	Yield (%)	Viscosity of Gum[b] (cS)	Retention Time (min)[c]
Normal	1	18.9	na[d]	na	2.9	40.2	21.3
	2	41.7	na	na	3.6	52.4	21.3
	3	17.2	na	na	2.5	48.5	21.5
Blended	1	18.9	7.3	6.9	2.8	11.6	22.1
	2	41.7	18.9	17.8	3.4	14.1	21.9
	3	17.2	7.5	7.1	2.5	14.8	22.1

[a] Measured in a Brookfield viscometer with a CP-51 spindle at 60 rpm; cP = centipoise.
[b] 0.2% in dimethylsulfoxide; cS = centistokes.
[c] TSK-60 column.
[d] Not applicable.

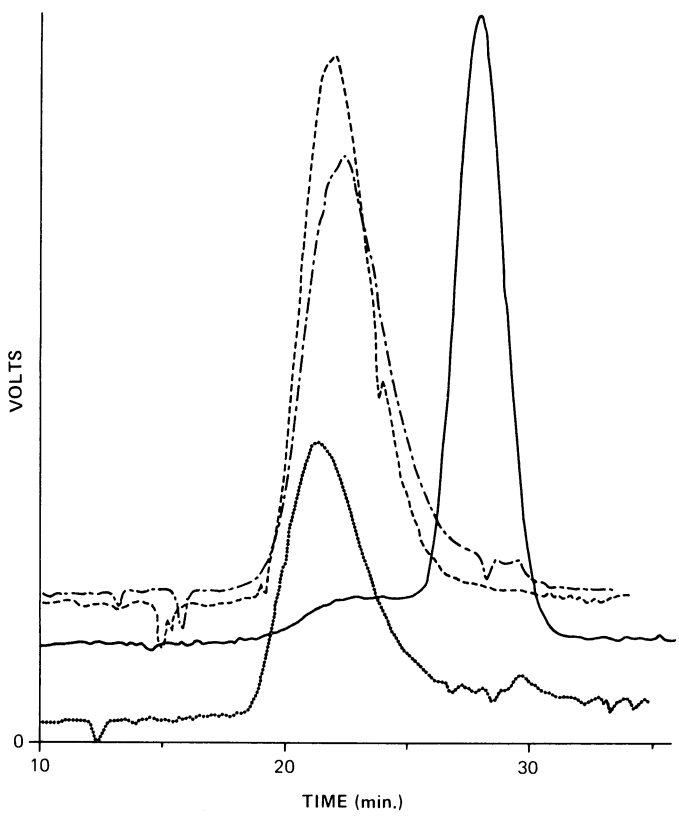

Fig. 4. Elution of oat gums from TSK-60 columns with water at 0.4 ml/min and detection by the orcinol-sulfuric acid reaction (baselines offset for clarity): pilot plant gum,-·-·-; bench gum,·····; Oster blended bench gum,----; Polytron bench gum,——.

deactivated bran possibly related to extraction of phenolics into 75% ethanol. It has been reported that anthraquinone induces chain scission of polysaccharides in alkaline solution (Arbin et al 1980).

Losses in viscosity in the pilot plant accelerated during decanter and disk centrifugation. A possible explanation for this is that high shear forces are imposed by these centrifuges.

Both bench-prepared and pilot plant gum suffered considerable viscosity loss when subject to high shear stress in a Polytron homogenizer. High molecular weight polymers are known to be degraded by shear above a certain critical value of stress (Harrington and Zimm 1965). The reduced viscosity of ultrasonicated polysaccharides (e.g., Szu et al 1986) is presumably mainly due to high shear stress. We also observed considerable viscosity loss in ultrasonicated oat gums. Although we are aware of no previous reports of viscosity loss in shear-stressed cereal β-glucans, the observation of Letters (1977) of a decrease in solubility of wort and beer β-glucans following disk centrifuging or high-speed homogenization may be relevant.

There is clearly a need for better knowledge of the effect of shear stress on oat β-glucan, in particular to determine a critical value (i.e., the value at which bond breakage commences). Shear stress achieved in the viscometer had not, apparently, reached this level. It is worth noting that theory suggests (Harrington and Zimm 1965) that bond breakage due to shear stress will generally occur near the center of high molecular weight polymers, with the consequence that bond scission by this mechanism is very effective in reducing viscosity.

At low shear rates oat gums show typical pseudoplastic behavior, i.e., shear-thinning is not permanent. We were unable to detect permanent shear thinning at shear rates attained by viscometers.

Both bench and pilot plant gums eluted from the TSK-60 gel column before the highest molecular weight pullulan standard (23.3 min, 8×10^5 daltons [Da]), preventing reliable estimation of molecular weight. However, an extrapolated calibration curve indicated that both gums have molecular weights in excess of 10^6 Da, and bench-prepared (Wood et al 1978) gum (21.5 min) was at least two to three times the molecular size of pilot plant gum (22.4 min). Severely shear-stressed samples (Polytron and ultrasonication) eluted after pullulan (P 800) suggesting a molecular weight less than 10^6 Da (Fig. 4).

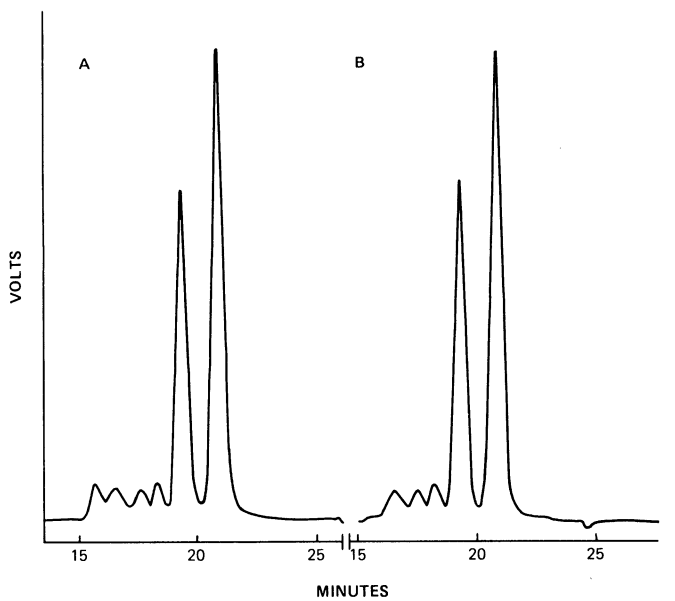

Fig. 5. Oligosaccharides produced by action of (1→3)(1→4)-β-D-glucan-4-glucanohydrolase on pilot plant (**A**) and bench-prepared gum (**B**), as separated by high-performance liquid chromatography with a Bio-Rad HPX-42A column with elution by water at 0.4 ml/min and detection by the orcinol-sulfuric acid reaction.

HPLC analysis of the products of action of (1→3)(1→4)-β-D-glucan-4-glucanohydrolase revealed no major structural differences between bench and pilot plant prepared oat β-glucan or between β-glucan derived from inner and outer parts of the seed. The proportions of the major products of the enzyme (3-*O*-β-D-cellobiosyl-D-glucose and 3-*O*-β-D-cellotriosyl-D-glucose) reflect the major structural variations in mixed linkage β-glucans, more so than does the ratio of (1→3) to (1→4) linkages (Wood et al 1987). The elution profile, however, does not include the enzyme degradation products from areas of contiguous (1→4)-linked units that form insoluble products (Woodward et al 1983). Such regions (or other structures such as areas of contiguous (1→3)-linked units), although present as minor features, may significantly influence properties (Buliga et al 1986). Both pilot plant and bench β-glucan yielded a small amount of precipitate after enzyme treatment. In general it seems that the structure as assessed in flour, bran, pilot plant gum or bench-prepared gum has the same overall characteristic features. What is markedly different from bench and presumably also the native cell wall β-glucan is the molecular weight and viscosity of the pilot plant β-glucan. This factor will require consideration in physiological trials since Jenkins et al (1978) have established a relationship between viscosity and effectiveness of gums in regulating post-prandial glucose levels.

In conclusion, the production of fine and coarse fractions of oats (cultivar Hinoat) containing from less than 1% to 16% or more β-glucan is possible. The oat bran and gum fractions described here are presently under investigation in clinical and animal feeding trials in an attempt to establish relationships between these components and lipid and glucose metabolism. Oat bran fractions containing in excess of 15% β-glucan are excellent natural (i.e., unsupplemented) sources of viscous soluble dietary fiber.

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