Extraction of Oat Gum from Oat Bran: Effects of Process on Yield, Molecular Weight Distribution, Viscosity and (1→3)(1→4)-β-D-Glucan Content of the Gum

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ABSTRACT

Different processing technologies were examined for their efficiency in production of large amounts of good quality oat gum rich in (1→3)(1→4)-β-D-glucan. Untreated and enzyme-deactivated oat bran concentrate were extracted with aqueous sodium carbonate at pH 10 and 40°C. Oat gums were subsequently isolated either by dialysis, ultrafiltration, or alcoholic precipitation on small, medium, and pilot plant scales. It was possible to produce oat gums with a β-glucan content of ≈60–65% with all three methodologies. The viscosity of solutions of the gums and the molecular weights differed, depending on the production process. Using dialysis, the production of highly viscous gums was possible, whereas ultrafiltration and alcoholic precipitation yielded gums with lower solution viscosity. Enzyme-deactivated oat bran concentrate gave an oat gum with an increased β-glucan content and solution viscosity, but in lower yield. To produce large amounts of oat gum, alcoholic precipitation would be the process of choice, but ultrafiltration and dialysis are useful alternatives.

Research on oat and oat products has been intensified by the findings that oat bran possesses serum-cholesterol-lowering properties (Storch et al 1984, Anderson and Chen 1986, Gold and Davidson 1988, Anderson et al 1991). Mixed-linkage (1→3)(1→4)-β-D-glucans (β-glucans) are the predominant cell-wall components of oat endosperm and are believed to play an important role in lowering serum cholesterol. The subaleurone layers of dehulled oat are particularly rich in β-glucan. β-Glucan content of commercial oat bran ranges from 5 to 10%, depending on the process used. Therefore, a minimum β-glucan content of 5.5% for commercial oat bran was recommended by the American Association of Cereal Chemists in a guideline definition (AACC 1989).

To further study the physicochemical and especially the physiological properties of oat β-glucan, large quantities of isolated β-glucan, also called oat gum, are needed. Wood et al (1989) produced sufficient amounts of oat gum in the pilot plant for extensive chemical and physiological studies. In this study several technologies were examined for their influence on β-glucan content, viscosity, and molecular weight of oat gum and their effectiveness for production of large amounts of good quality oat gum.

MATERIALS AND METHODS

Oat bran concentrates (*Avena sativa* cv. Sang) with β-glucan contents of 15.3% (B0188) and 16.0% (B1413) were obtained from Swedish Protein AB (Väröbacka, Sweden). Oat flour (2.4% β-glucan) was purchased from Klingenthal Mühlen (Kaiseraugst, Switzerland). Barley β-glucan samples were obtained from Megazyme Australia Pty. Ltd. (Warriewood, Australia) and Sigma Chemicals (St. Louis, MO).

Enzyme Deactivation

In small scale experiments, typically 27.5 g of oat bran was blended with 250 ml of aqueous ethanol solutions. The treatments used were 4 hr at: 1) room temperature (RT) with 75% ethanol (v/v); 2) RT with 96% ethanol (v/v); 3) RT with absolute (100%) ethanol; and 4) 80°C with 75% ethanol (v/v). The supernatant was decanted, and the wet bran was allowed to desolventize for two days at 40°C in a forced-air desolventizer.

In the pilot plant, typically 3 kg of oat bran was blended with 27.3 L of aqueous ethanol (75%, v/v) in a 100-L reactor vessel prepared for reflux circulation at ≈80°C and stirred for 4 hr. The mixture was cooled to ≈30°C, and the supernatant was carefully decanted. The wet bran was reslurried in 10 L of absolute (100%) ethanol and after 15 min it was pumped to a filter press. The ethanol was removed and the wet bran was allowed to desolventize for 30 hr at 35°C in a forced-air desolventizer.

Extraction of Oat Gum (Small Laboratory Scale)

Extraction. Typically 6 g of deactivated oat bran concentrate (OBC) was blended with 90 ml of water at 40°C. The mixture was adjusted to pH 10 with 20% sodium carbonate and stirred for 30 min. The mixture was centrifuged for 10 min at 2,000 × *g* (MSE Mistral 1000 centrifuge, Zivy & Cie SA, Oberwil, Switzerland) and the supernatant was decanted. The residue was again blended with 90 ml of aqueous sodium carbonate at pH 10 and reextracted at 40°C for 30 min. The liquid extracts (E1 and E2) were cooled to 20°C and adjusted to pH 4.5 by addition of 20% (v/v) hydrochloric acid with vigorous stirring. The resulting mixture was centrifuged at 2,000 × *g* for 10 min, and the supernatant gum extract was retained.

Ethanol precipitation. The gum extract was cooled to 10°C and an equal volume of absolute ethanol was added with vigorous stirring. The mixture was centrifuged at 2,000 × *g* for 10 min and the supernatant was carefully decanted. The gum solids were collected and desolventized for 3 hr at RT, then dried at 60°C for 6 hr. The dried oat gum was ground (Retsch ZM1 mill, Schieritz & Hauenstein AG, Arlesheim, Switzerland) to pass a 250-μm screen.

Dialysis. The gum extract was transferred into a dialysis tubing #44146 (MW cut-off ≈10 kDa, Bender & Hobein, Zürich, Switzerland) and dialyzed at 20°C against distilled water for 48 hr. The retentate was freeze-dried and the dried gum was ground to pass a 250-μm screen.

Extraction of Oat Gum (Large Laboratory Scale)

In large laboratory-scale experiments, 2.6 kg of deactivated OBC was blended at 40°C with 39 L of water. The mixture was

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adjusted to pH 10 with 20% sodium carbonate and stirred in a 100-L reactor vessel for 30 min, then centrifuged (SAOOH 205, Westfalia Separator AG, Oelde, Germany) at 4,000 rpm. The liquid extract was removed (extract 1), and the residue was reextracted with 30 L of aqueous sodium carbonate at 40°C (extract 2). Extracts 1 and 2 were combined, cooled to 20°C, and adjusted to pH 4.5 by addition of 20% (v/v) hydrochloric acid with vigorous stirring. The resulting mixture was centrifuged at 4,000 rpm, and the supernatant was pumped to a Carbosep 2F V7 ultrafiltration system (Société de Fabrication d'Elements catalytiques, Bollène, France) with a cut-off of 20 kDa and a membrane area of 0.316 m^2. The volume of the supernatant (110 L) was reduced to 25 L in 9 hr. A 10-L aliquot of the retentate was freeze-dried and ground to pass a 250-µm screen (A). The remaining 15 L was blended with 20 L of water, and the volume reduced to 15 L in 3 hr by ultrafiltration. This retentate also was freeze-dried and ground to pass a 250-µm screen (B).

Extraction of Oat Gum (Pilot Plant Scale)

Deactivated OBC (90 kg) was blended with 1,350 L of water at 40°C. The mixture was adjusted to pH 10 by addition of sodium carbonate (20%w/v) and stirred at 40°C for 30 min before cooling to 20°C and adjusting to pH 4.5 by addition of 20% (v/v) hydrochloric acid with vigorous stirring. The resulting mixture was centrifuged in a decanting centrifuge (CA 150-01-33, Westfalia Separator AG) at 4,000 rpm. The residue was blended with 1,350 L of water, adjusted to pH 10, and reextracted at 40°C for 30 min and treated as for the first extraction. The liquid fraction was cooled, pumped to a mixing tank, and an equal volume of propan-2-ol was added with vigorous stirring. The precipitated gum was collected by centrifugation in the decanting centrifuge at 4,000 rpm and desolventized for 24 hr at 30°C in a forced-air desolventizer and ground to pass a 500-µm screen.

The gum similarly obtained from extraction 2 was reconstituted with water (1:10, w/w) and incubated with 103.2 g of pancreatin (Fluka AG, Buchs, Switzerland) at 40°C for 2 hr with stirring. An equal volume of propan-2-ol was added with vigorous stirring. The precipitated gum was collected with a silk bolting cloth (PES-200/45, Schweizerische Seidengazefabrik AG, Zürich, Switzerland) and the remaining propan-2-ol was removed in a filter press. The gum was desolventized for 24 hr at 30°C in a forced-air desolventizer and ground to pass a 500-µm screen.

TABLE I
β-Glucanase-Activity of Untreated and Processed Oat Flour and Oat Bran Concentrate (OBC)

Product	Deactivation Process: Temperature	Deactivation Process: Ethanol Concentration	β-Glucanase Activity[a] (U/kg)
Oat flour[b]	Untreated		80.0 ± 7.1[c]
	80°C	75%	70.5 ± 1.3
	RT[d]	75%	76.1 ± 1.2
	RT	96%	80.2 ± 5.9
	RT	100%	78.3 ± 8.1
OBC B0188[e]	Untreated		77.7 ± 4.1
	80°C	75%	70.5 ± 1.3
OBC B1413[e]	Untreated		76.8 ± 5.8
	80°C	75%	67.9 ± 3.0

[a] $n = 3$.
[b] Laboratory scale.
[c] Mean ± standard deviation.
[d] Room temperature.
[e] Pilot plant scale.

Analytical Methods

β-Glucan content was analyzed by the method of McCleary and Glennie-Holmes (1985) using a β-glucan assay kit (Megazyme Australia Pty. Ltd., Warriewood, Australia). β-Glucanase activity (U/kg) was analyzed by the method of McCleary and Shameer (1987) using a β-glucanase assay kit (Megazyme). Starch, protein, and ash were analyzed as described in the Swiss Manual for Food Analysis (1991). Neutral sugars were determined by gas chromatography after hydrolysis and derivatization as aldonitrilacetates as described by Niederer (1993). Kinematic viscosity (0.2% w/v, in dimethyl sulfoxide) was measured at 25°C in a calibrated Ubbelohde capillary viscometer (Type Ia and 0a, Schott Geräte GmbH, Hofheim, Germany) by the method of Wood et al (1978).

Solutions for molecular weight determination were prepared by dissolving 25 mg of oat gum in 50 ml of water at 60°C and stirring vigorously for 1 hr. The solutions were stirred for another 14 hr at RT before chromatography.

High-performance size-exclusion chromatography (HPSEC). A precolumn filter (A318, Upchurch Scientific Inc., Oak Harbor, USA), a PL-GFC 1,000 Å and a PL-GFC 300 Å column were connected in series (Polymer Laboratories Ltd., Shropshire, England) to a pump (2200, Bischoff GmbH, Leonberg, Germany) for HPSEC. The columns were maintained at 30°C in a column oven (Waters Associates, Milford, CN). Samples (200 µl) were injected via a sample loop, eluted with water at a flow rate of 1 ml/min, and detected using a refractive index (RI) detector (Erna Inc., Tokyo, Japan). Quantitation was done using a D-2520 GPC integrator (Hitachi Inc., Tokyo, Japan).

TABLE II
Yield, β-Glucan Content, and Viscosity of Oat Gum from Untreated and Enzyme-Deactivated Oat Bran Concentrate (OBC) Produced by Laboratory-Scale Dialysis and Alcoholic Precipitation

Process	Starting Material	Extraction[a]	Yield (% of Starting OBC)	β-Glucan (%)[b]	Kinematic Viscosity (cS)[c,d]
Dialysis	Untreated	1	7.63 ± 0.31[e]	33.13 ± 1.23	3.49 ± 0.03
		2	4.60 ± 0.23	43.48 ± 0.77	6.94 ± 0.04
		3	3.60 ± 0.17	51.50 ± 0.87	10.64 ± 0.03
		Total	15.83		
	Enzyme-deactivated	1	2.89 ± 0.12	48.50 ± 2.67	9.50 ± 0.03
		2	2.94 ± 0.16	64.35 ± 0.77	16.82 ± 0.02
		3	2.74 ± 0.13	65.99 ± 1.59	13.66 ± 0.04
		Total	8.57		
Alcoholic precipitation	Enzyme-deactivated	1	6.24 ± 0.33	57.00 ± 0.50	9.35 ± 0.02
		2	3.49 ± 0.30	62.50 ± 2.00	11.30 ± 0.00
		Total	9.73		

[a] Consecutive extracts.
[b] $n = 4$.
[c] $n = 3$.
[d] 0.2% (w/v) in dimethyl sulfoxide.
[e] Mean ± standard deviation.

Gel-filtration chromatography (GFC). For GFC, samples were applied to a 135 × 1.7 cm Sephacryl S-400 high-resolution gel column (Pharmacia LKB, Uppsala, Sweden). The column was maintained at RT and eluted with water at ≈23 ml/hr using a peristaltic pump (Perpex, Werner Meyer, Luzern, Switzerland). Sample aliquots of 1.5 ml (β-glucans, pullulan and dextran standards) were applied to the column. Fractions of 3.8 ml were collected and assayed for neutral sugars (Bailey 1958) by automated segmented flow analysis (Skalar Analytica B.V., Breda, Netherlands) using a method similar to that of Thibault (1979).

TABLE III
Yield, β-Glucan Content, and Viscosity of Oat Gum from Enzyme-Deactivated Oat Bran Concentrate (OBC) Produced by Large Laboratory-Scale Ultrafiltration

Extract	Yield (% of Starting OBC)	β-Glucan (%)[a]	Kinematic Viscosity (cS)[b,c]
A	2.70	57.75 ± 3.21[d]	3.08 ± 0.00
B	6.60	66.15 ± 4.61	3.54 ± 0.02
Total	9.30		

[a] $n = 6$.
[b] $n = 3$.
[c] 0.2% (w/v) in dimethyl sulfoxide.
[d] Mean ± standard deviation.

RESULTS

Enzyme Deactivation

Deactivation of the β-glucanase at RT with ethanol at concentrations between 75 and 99.9% was not very effective, whereas treatment at 80°C in 75% ethanol (v/v) reduced the β-glucanase activity by 12% (Table I). The β-glucanase activity of native oat flour was reduced from 80.0 U/kg to 70.5 U/kg by this incubation. A similar reduction was seen in trials on the pilot plant scale. The activity of the β-glucanase of OBC was reduced in the pilot plant from 77.7 to 70.5 U/kg and from 76.8 to 67.9 U/kg (Table I).

Preparation of Oat Gum

Different technologies for the production of oat gum were evaluated on small laboratory, large laboratory, and pilot plant scales. The influence of reduction of β-glucanase activity by incubation with ethanol (75%, v/v) at 80°C for 4 hr on the β-glucan content and kinematic viscosity of oat gum was determined in laboratory experiments. Oat gum from enzyme-deactivated and untreated OBC was prepared by dialysis (Table II). The β-glucan content was lowest in oat gum from the first extract, and highest in oat gum of the third extract in both preparations. Kinematic viscosity was highest in the second and third extract. The yield of

TABLE IV
Yield, Viscosity, and Composition of Oat Gum from Enzyme-Deactivated Oat Bran Concentrate (OBC) Produced by Precipitation with Alcohol in the Pilot Plant

Extract	Yield (% of Starting OBC)	Kinematic Viscosity (cS)[a,b]	β-Glucan (%)[c]	Starch (%)[a]	Protein (%)[c,d]	Pentosan (%)[a,e]	Ash (%)[a]
1	22.6	3.96 ± 0.02[f]	21.82 ± 0.34	35.91 ± 1.76	17.55 ± 0.24	1.91 ± 0.01	6.45 ± 0.08
2	5.8	4.62 ± 0.02	39.65 ± 0.34	21.89 ± 0.59	9.42 ± 0.08	1.32 ± 0.05	8.92 ± 0.11
2PA[g]	4.0	5.30 ± 0.00	62.23 ± 1.47	6.17 ± 0.74	4.02 ± 0.24	1.84 ± 0.07	11.94 ± 0.49

[a] $n = 3$.
[b] 0.2% (w/v) in dimethyl sulfoxide.
[c] $n = 6$.
[d] Nitrogen × 6.25.
[e] (Arabinose + xylose) × 0.88.
[f] Mean ± standard deviation.
[g] 2PA = Oat gum extraction 2, incubated with pancreatin.

TABLE V
Retention Volumes (RV) of Oat Bran β-Glucans, Pullulans, and Commercial Barley β-Glucans[a]

Sample	RV (ml)		K_{av}[b]	
	HPSEC	GFC	HPSEC	GFC
Pullulan[c]				
P-5 (5.8×10^3)[d]	16.53 ± 0.05[d]	191.6	0.85	0.87
P-10 (12.2×10^3)	16.03 ± 0.05	184.7	0.80	0.81
P-20 (23.7×10^3)	15.47 ± 0.04	175.7	0.74	0.73
P-50 (48.0×10^3)	14.59 ± 0.04	160.9	0.64	0.60
P-100 (100.0×10^3)	13.58 ± 0.03	145.8	0.53	0.47
P-200 (186.0×10^3)	12.54 ± 0.03	137.5	0.41	0.40
P-400 (380.0×10^3)	11.51 ± 0.03	119.0	0.30	0.23
P-800 (853.0×10^3)	10.60 ± 0.02	109.9	0.20	0.15
β-Glucan[e]				
Pilot plant oat gum, extraction 2, pancreatin incubated	10.30 ± 0.10	101.8	0.16	0.11
Pilot plant oat gum, extraction 1	10.32 ± 0.16	...	0.16	...
Pilot plant oat gum, extraction 2	10.31 ± 0.10	103.7	0.16	0.10
Small laboratory-scale oat gum, extraction 1	10.46 ± 0.03	...	0.18	...
Small laboratory-scale oat gum, extraction 2	11.75 ± 0.31	139.5	0.32	0.41
Large laboratory-scale oat gum, retentate A	14.40 ± 0.42	151.1	0.62	0.51
Large laboratory-scale oat gum, retentate B	10.88 ± 0.04	121.5	0.23	0.26
Biocon β-glucan	10.83 ± 0.02	115.6	0.22	0.20
Sigma β-glucan	11.16 ± 0.35	...	0.26	...

[a] Determined by high-performance size-exclusion chromatography (HPSEC) and gel-filtration chromatography (GFC).
[b] $K_{av} = V_e - V_0/V_t - V_0$.
[c] n = 3. Number in parentheses is molecular weight as quoted by supplier.
[d] Mean ± standard deviation.
[e] $n = 4$.

each consecutive extract (1–3) and the total yield from untreated OBC was higher than from enzyme-deactivated OBC, but the β-glucan content and viscosity were lower. The total yield of extracted β-glucan from the untreated OBC was also higher: ≈23% greater than the enzyme-deactivated OBC and ≈11% greater than the alcohol-precipitated gum (6.37 g/100 g vs. 5.16 g/100 g vs. 5.74 g/100 g).

Oat gum preparations obtained from enzyme-deactivated OBC by alcoholic precipitation showed similar compositions to oat gums produced by dialysis, although the yield and β-glucan content of the first extract (6.24 and 57.0%, respectively) were higher than the values obtained by dialysis (2.89 and 48.50%). Gums precipitated from the second extract showed similar β-glucan content and yield for the two methods, but the kinematic viscosity of gum obtained by alcoholic precipitation was lower.

In large laboratory-scale ultrafiltration experiments, β-glucan content of the oat gum was 57.75% for retentate A and 66.15% for the washed retentate B (Table III), and it was similar to the gums produced by dialysis and alcoholic precipitation in laboratory-scale experiments (Table II). The overall yield of 9.3% for the two extractions was similar to the yield obtained by laboratory-scale alcoholic precipitation (9.7%). The kinematic viscosity of oat gums produced by ultrafiltration was low: retentates A and B had viscosities of 3.08 cS and 3.54 cS, respectively.

Oat gum produced in the pilot plant by alcoholic precipitation had a low β-glucan content and high starch and protein contamination (Table IV). This was a result of poor separation of solids during the centrifugation after the extractions. The yields were therefore very high (22.6 and 5.8%, respectively). The kinematic viscosity of both gums was low: 3.96 cS for gum 1 and 4.62 cS for gum 2. The pancreatin-incubated gum 2 (2PA) had a β-glucan content of 62.23% and a higher kinematic viscosity (5.3 cS). Protein and starch contents were reduced by the pancreatin incubation. Pentosan contamination was low in all three gums, and ash content was between 6 and 12%.

Molecular Weight Distribution

The molecular weight distribution was examined using both HPSEC and GFC. Retention volumes and distribution coefficients (K_{av}) of β-glucans and pullulan standards are shown in Table V. The retention volumes determined with HPSEC were highly reproducible. Similar results were obtained using GFC. HPSEC and GFC chromatograms of β-glucans are shown in Figures 1 and 2.

DISCUSSION

Oat β-glucan is believed to be the physiologically active substance in oat and oat products responsible for lowering blood cholesterol and reducing postprandial blood glucose levels, although reported effects are variable (Davidson et al 1991, Törrönen et al 1992, Braaten et al 1994, Wood et al 1994). Oat β-glucan is located in the endosperm cell walls and is frequently concentrated in the subaleurone region of the outer endosperm (Fulcher and Miller 1993). The extraction of β-glucans was investigated in several studies (Wood et al 1978, Autio et al 1992, Dawkins and Nnanna 1993, Westerlund et al 1993), but in these trials only small quantities β-glucans were isolated. Wood et al (1989) were the first to produce large amounts of good quality oat gum, making physiological trials possible (Wood et al 1990; Braaten et al 1991, 1994; Wood et al 1994). In the present study, new technologies for good quality oat gum production were evaluated and the practicality for large-scale production was tested.

The use of enzyme-deactivated OBC led to an oat gum with increased β-glucan content and kinematic viscosity but low yield, which confirmed the results of Wood et al (1989). The native OBC showed relatively low β-glucanase activity. The commercial OBC has been heat-treated by the producer (steaming); untreated oat bran would have higher β-glucanase activity. Nevertheless, there is a residual small activity that can be further reduced by hot-alcohol treatment. This further reduction seems to be important for production of good quality oat gum and confirms that the longer the processing time, the lower the viscosity and molecular weight of the product (Wood et al 1989).

It was possible to produce oat gums with a β-glucan content of 60–65% using three different methodologies in small laboratory, large laboratory, or pilot plant scales. The kinematic viscosity and the molecular weight distribution of the gums differed according to the production process. Using dialysis, the production of good quality, high-viscosity gums was possible, although the process was time-consuming and therefore not suited for large scale production. The large laboratory-scale ultrafiltration process was less

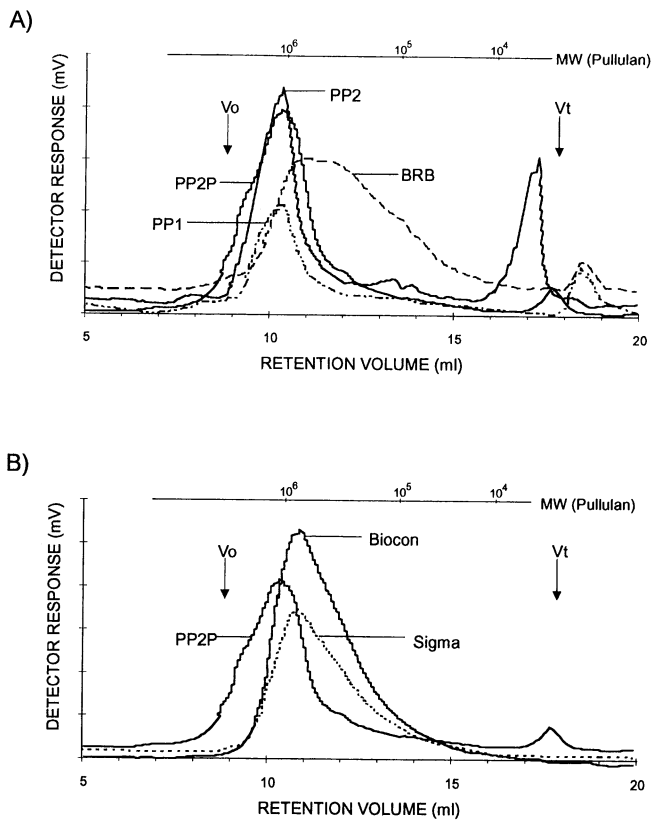

Fig. 1. High-performance size-exclusion chromatography of oat β-glucans. **A,** Pilot plant (PP) oat gum, extractions 1, 2, and 2P (pancreatin incubated); large laboratory-scale oat gum, retentate B (BRB). **B,** PP2P; commercial barley β-glucans (Biocon and Sigma). $V_{0(amylopectin)}$ = 8.8 ml. $V_{t(glucose)}$ = 17.8 ml.

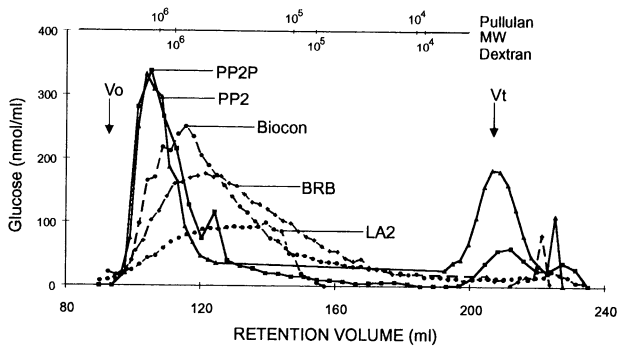

Fig. 2. Gel-filtration chromatography of oat β-glucans. Pilot plant (PP) oat gum, extractions 2, and 2P (pancreatin incubated); large laboratory-scale oat gum, retentate B (BRB); small laboratory-scale oat gum, alcoholic precipitation, extraction 2 (LA2); and commercial barley β-glucan (Biocon). $V_{0(amylopectin)}$ = 92.3 ml; $V_{t(glucose)}$ = 206.7 ml.

time-consuming. Because of the high shear forces produced during ultrafiltration, kinematic viscosity and peak molecular weight of the gum was low. With the use of special membranes (cross-flow and spiral wound systems) and larger membrane areas, the forces during the ultrafiltration and the time of filtration could be reduced. Ultrafiltration does not require the large volumes of alcohol used for alcoholic precipitation, making this process potentially less hazardous and expensive.

Oat gums produced by precipitation with alcohol in the pilot plant had high β-glucan contents after an additional pancreatin treatment. The pancreatin treatment did not affect the molecular weight distribution of the gum (Table V); the β-glucanase activity of the used pancreatin seems to be very low or absent. To reduce starch and protein contamination of oat gum for physiological trials, incubation with pancreatin is an effective process.

Wood et al (1989) reported a viscosity of 8.4 cS for oat gums produced by precipitation with alcohol in the pilot plant. The lower viscosity of our gums (5.3 cS) may be partly due to the high shear forces during decanting centrifugation, by the time required for this process, and by differences in β-glucan content. High centrifugation capacity is needed to produce large amounts of oat gum in one batch. As shown by Wood et al (1989), viscosity, peak molecular weight, and gum yield can be increased by making the centrifugation step as fast as possible. The commercial cereal β-glucans had lower peak molecular weights than did the gums described in this article, but they were purer β-glucan sources.

The gums produced by dialysis and ultrafiltration were easily and rapidly dissolved in water, which may be important for physiological activity in the gastrointestinal tract of humans. The oat gum isolated by precipitation with alcohol was difficult to dissolve in water.

For production of large amounts of oat gum, alcoholic precipitation would seem to remain the process of choice, but ultrafiltration and dialysis are useful alternatives.

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