Effect of Hydrothermal and Enzymic Treatments on the Viscous Behavior of Dry- and Wet-Milled Oat Brans

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ABSTRACT

The effect of hydrothermal, Termamyl α-amylase, and Finase S40 phytase treatments on viscous behavior of dry- and wet-milled oat brans was studied. Regular oat bran from a dry-milling procedure was higher in starch and lower in β-glucan and other dietary fibers than the fiber-concentrated oat bran from a wet-milling procedure. The high content of dietary fibers, especially β-glucan, in the wet-milled oat bran had a marked effect on the viscosity of heat- and α-amylase-treated bran slurries. Heating increased the amount of soluble β-glucan, on average, from 29 to 84%. The molecular weight of β-glucan was 8.4×10^5 in regular oat bran and 6.0×10^5 in fiber-concentrated oat bran, and it remained unchanged during the hydrothermal and α-amylase treatments. The phytase preparation used for hydrolysis of phytic acid also caused a reduction in viscosity of oat bran slurries. When the phytase preparation was added before heating, the viscosity of bran slurries remained at low levels during the whole temperature cycle, owing mainly to degradation of β-glucan. Rapid reduction in viscosity in the postheating treatments with phytase preparation was caused by the degradation of gelatinized starch together with β-glucan. During both treatments with phytase preparation, the amount of soluble β-glucan increased to 90%. Phytic acid content in the bran slurries decreased more (54%) in the postheating treatment than in the preheating treatment with Finase S40 phytase (36%). Enzymic studies showed that, at high levels of nonstarch polysaccharides, the viscosity of oat bran slurries changes only a little when starch is completely degraded to water-soluble oligosaccharides; but the viscosity clearly decreases when β-glucan is only partially hydrolyzed to high molecular weight products (M_w 4.0×10^4).

A high intake of oats and oat products has been shown to reduce blood total cholesterol (Ripsin and Keenan 1992) and post-prandial glucose resorption (Lund et al 1989). These effects are probably due to the presence of mixed-linked β-glucans (β-glucans), the main soluble dietary fiber component of oats. β-Glucans are cell-wall polysaccharides that can form highly viscous solutions. In physiological conditions, a high viscosity probably modifies intestinal absorption, which may partly explain the beneficial effects on cholesterol and glucose metabolism. High viscosity is favored by the high molecular weight of the β-glucan polymer and the high degree of solubilization (Autio et al 1987). The resistant starch component has also been found to be important in lowering the glycemic response (Björck 1993).

In oat varieties with low β-glucan content, the β-glucans are concentrated in the subaleurone layer of endosperm that passes into the bran fractions during milling (Fulcher and Miller 1991). Industrial-scale milling procedures generally provide a bran fraction containing 5.8–8.9% β-glucan and 41–52% starch (Wood et al 1991a). Even higher β-glucan contents have been obtained in more advanced dry- or wet-milling processes (Knuckles et al 1992, Mälkki et al 1992). However, the yield is usually low for bran with the highest β-glucan concentration (Wood et al 1989). Newer wet-milling methods, such as that described by Lehtomäki et al (1993), may yield both a high-bran and high-fiber content. Commercially available products of this type are reported to contain 15–30% β-glucan.

Oat bran is generally used in foods such as baked goods and breakfast cereals. One novel application for oat bran, a snack-type food resembling yogurt, was introduced by Salovaara and Kurka (1991). This slightly sour-tasting product is a modification of a traditional product, oat flummery, formerly used in countries such as Finland and Scotland. In the traditional procedure, oat meal is fermented before heating, whereas in the newer procedure, fermentation is performed after heating, thus providing a product with viable lactic acid bacteria or bifidobacteria that have potential probiotic benefits.

In the production of oat flummery (Fig. 1), oat bran is exposed to hydrothermal treatment, either with or without enzyme additions. During heating, the viscosity of the bran-water slurry increases as a result of a number of simultaneously occurring events: the bran particles bind water and swell, some bran components are leached into the surrounding medium, starch is gelatinized, and some components undergo changes in molecular structure. Oat polysaccharides, starch, and β-glucan play an important role in the viscosity of oatmeal suspensions (Wikström Jansson and Lindahl 1991). Viscosity is a limiting factor when a high solids content is required. One way to achieve an acceptable consistency is to degrade starch with amylolytic enzymes. The resulting malto-oligosaccharides may later contribute growth energy for the fermenting bacteria.

The bioavailability of nutritionally valuable minerals can be improved by degrading the phytate-mineral complexes. In oats, the endogenous phytase activity is low or absent (Lasztity and Lasztity 1990) and, moreover, it is likely to be inactivated during the kilning procedure (Frølich et al 1988). Treatment of preheated oat bran flummery with exogenous phytase (30 min, 55°C) degraded over 90% of the phytic acid (Aalto-Kaarlehto et al 1992). The study also showed, however, that the phytase preparation caused variable changes in the viscosity of oat bran flummery, whether added before or after heating. Contaminating hydrolase activities that were proposed for the unexpected behavior were further examined in this study.

Objectives of this study were to determine effects of hydrothermal, α-amylase, and phytase treatments on viscosity of dry- and wet-milled oat brans as related to molecular weight distribution of β-glucan and starch.

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MATERIALS AND METHODS

Oat Brans, Oat Polysaccharides, and Enzymes

Two commercial oat brans were studied: a regular oat bran (ROB) (Melia Ltd., Raisio, Finland) manufactured by a dry-

milling process; and a fiber-concentrated oat bran (FCB) (NatuReal Essence, Alko Ltd., Rajamäki, Finland) manufactured by a wet-milling process (Lehtomäki et al 1993). To make the brans more comparable, ROB was ground to a smaller particle size using a custom-made laboratory mill (University of Helsinki, Finland). Each bran originated from a single batch of an industrial process. Commercially available oat polysaccharides, starch (80.9%; NatuReal Textural), and β-glucan (82%; Megazyme Pty. Ltd., Sydney, Australia) were used as reference materials.

The enzyme preparations were Termamyl α-amylase, (Novo-Nordisk A/S, Bagsvaerd, Denmark) and Finase S40 phytase (Alko). α-Amylase activity was 1,721 U/L, as determined by the Biocon α-amylase kit (Megazyme). Phytase activity was 40,000 PU/ml, assayed as follows: 1 ml of an appropriate enzyme dilution was mixed with 25 ml of sodium inositol hexaphosphate (Sigma) in 0.2*M* sodium phosphate buffer (10 mg/ml, pH 5.0). After incubation at 37°C for 15 min, the reaction was stopped by adding 2.0 ml of 15% tricarboxylic acid solution. The released orthophosphate was measured spectrophotometrically according to Chen et al (1956).

Hydrothermal and Enzymic Treatments

Hydrothermal treatments were performed in a Brabender viscoamylograph (Brabender OHG, Duisburg, Germany). The temperature was raised (1.5°C/min) from 25 to 95°C, held at 95°C for 20 min, and cooled (1.5°C/min) to 25°C, with constant stirring (75 rpm). Application of enzymes was studied in three separate experiments: 1) in Termamyl α-amylase treatments, the enzyme (2.8 mU/g of starch) was added immediately before heating; 2) in Finase S40 phytase treatments, the enzyme (14,300 U/g of phytate) was added immediately before heating; or 3) the enzyme was added during cooling, when the temperature had reached 55°C (Fig. 1). After processing, oat bran slurries were frozen until used for further analyses. The treatments were performed at a 7.0% solids content, and also at a 13.3% concentration of FCB, to reach the same starch content (3.3%) as the ROB slurries at 7.0% solids. Total sample size was 420 g. Each experiment was repeated four times.

Oat starch and β-glucan used as a reference materials were dispersed in water at 3.3% (w/v) and 1.1% (w/v) concentrations, respectively. They were treated in the viscoamylograph using the same temperature profile and enzyme applications as described above. The enzyme-carbohydrate ratio was the same as in experiments with oat brans: Finase S40 phytase at 3,500 U/g of β-glucan and 500 U/g of starch; Termamyl α-amylase at 1.0 U/g of β-glucan. For starch solution, total sample size was 420 g. Total sample size for β-glucan solution was 50 g (in a small bowl).

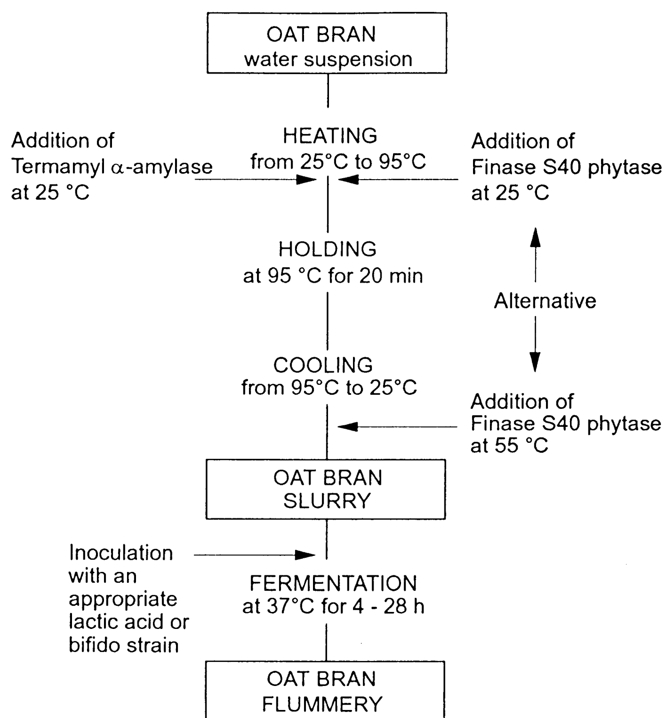

Fig. 1. Flow diagram of the production of oat bran flummery.

Composition of Oat Brans

For chemical analyses, both ROB and FCB were ground in a Cyclotec sample mill (Tecator AB, Sweden) to pass a 0.5-mm screen. Moisture was determined as the weight loss on heating at 130°C for 1 hr according to method 44-15A (AACC 1983). Total, soluble and insoluble fibers were assayed by the enzymic-gravimetric method of Asp et al (1983). β-Glucan was determined according to the method of McCleary and Codd (1991), using an assay kit from Megazyme. Pentosans were assayed colorimetrically (Douglas 1981). Starch (method 76-20), protein (method 46-12) (N × 6.25), fat (method 30-10), and ash (method 08-01) were analyzed by standard methods (AACC 1983). Phytic acid was measured as elemental phosphorus by inductively coupled plasma atomic emission spectrometry on samples extracted with 2.4% HCl, according to the procedure of Plaami and Kumpulainen (1991). The particle-size distribution of the brans was determined by sieve analysis, and the results were expressed on a fresh weight basis. The data reported are means of at least duplicate determinations unless stated otherwise.

Viscosity Measurements

The viscosity of oat bran slurries was measured continuously during the heating, holding, and cooling periods by a Brabender viscoamylograph equipped with a 700 cm·gf sensitivity cartridge. For the starch and β-glucan solutions, the end viscosity was monitored (229 sec^{-1}, 25°C) with a Visco 88 viscometer (Bohlin Rheology AB, Lund, Sweden) equipped with a concentric cylinder system C30 (DIN 53019).

Molecular Weight Determination of β-Glucan, Starch, and Their Hydrolysis Products

The molecular weight distribution of β-glucan was characterized by gel-permeation chromatography (GPC) as described by Suortti (1993), with the exception that μHydrogel columns 2,000, 500 and 250 (Millipore/Waters, Millford, MA) were used. Standards were two β-glucan preparations extracted from oat according to Autio et al (1992): one had a high molecular weight (8.8×10^5) and the other one, treated with cellobiohydrolase II from *Trichoderma reesei* as described by Henriksson et al (1995), had a reduced molecular weight (2.5×10^5). After characterization by GPC/dual-angle laser light scattering detection (DALLS, PD 2,000 W, Pecision Detectors, Amherst, MA), these preparations were used as standards for determining the weight average molecular weight (M_w) and number average molecular weight (M_n) of β-glucan in oat bran using GPC-fluorimetric detection (M-470, Millipore/Waters) with postcolumn Calcofluor reaction. For calculations, the Millenium/GPC option program was used. In GPC-light-scattering detection, β-glucan concentration per injection was 1,000–2,000 mg/L of 0.1*N* NaOH; in GPC/Calcofluor reaction, it was 20–100 mg/L.

The molecular weight distribution of starch was assayed using the same column combination as described above (Suortti and Pessa 1991). A 0.2-g sample was suspended in 5 ml of distilled water and dissolved by adding 1*N* NaOH solution (35 ml) and stirring with a magnetic stirrer for 6 hr at room temperature. All the samples were diluted with 1*N* NaOH to give a starch concentration of 750–1,500 mg/L. The detection was spectrophotometric at 560 nm (M-991, Millipore/Waters) after postcolumn

reaction with iodine. Pullulans (M_w 5.80 × 10^3 – 1.66 × 10^5) (Shodex standard P-82, Showa Denko K.K., Japan) were used as standards.

Low molecular weight saccharides were determined by high-performance liquid chromatography (HPLC) (M-6000A, Millipore/Waters) with a μHydrogel DP column and two fast fruit juice columns (Millipore/Waters) in series. The samples were diluted with 0.1% H_3PO_4 to an oligosaccharide content of 25–5,000 mg/L. Chromatography was performed at 70°C with a flow rate of 0.5 ml/min using 0.5% H_3PO_4 as eluent. A refractive index detector was used. Malto-oligosaccharides (DP 1–7, Boehringer Mannheim GmbH, Germany) and glucose (BDH AnalaR, Poole, England) were used as standards.

Determination of Soluble β-Glucan

Soluble β-glucan was determined using a modified method of Åman and Graham (1987). Oat brans and oat bran slurries (two samples per experiment) (2% solids, 30 ml of water) were incubated in four replicates in a shaking water bath (37°C, 2 hr). The insoluble material was separated from the supernatant by centrifugation (8,965 × *g*, 10 min) and filtration (Whatman No. 4) and then frozen until used for analysis. The insoluble material and the original bran or bran slurry were analyzed for β-glucan content by the Calcofluor-fluorimetric GPC method of Suortti (1993). The amount of soluble β-glucan was expressed as the difference between total and insoluble β-glucan.

Statistical Evaluation

Analysis of variance was used to assess the statistical significance of each treatment on oat bran data. Student's *t* test was used to compare paired-sample means.

RESULTS AND DISCUSSION

Characterization of Oat Brans

The chemical compositions of two commercial oat brans, originating from different milling procedures, are shown in Table I. ROB produced by a dry-milling process was typical of a commercial oat bran, containing 9.1% β-glucan and 47.4% starch. FCB produced by the wet-milling method of Lehtomäki et al (1993) contained significantly more β-glucan (17.4%), pentosan, and dietary fiber, but less starch (24.9%) than the ROB. The FCB was also higher in protein, fat, ash, and phytic acid than the ROB. Both samples met the AACC definition for oat bran (AACC 1989).

The compositional data suggest that ROB was quite high in starchy endosperm and low in outer fiber-rich layers, whereas the FCB was concentrated with the subaleurone layers of the grain. The two oat brans also differed significantly in particle-size distribution (Table I). ROB particles were larger than the FCB

TABLE I
Oat Bran Components (%) and Particle-Size Distribution (μm)[a]

	Regular Oat Bran	Fiber-Concentrated Bran
Component		
Moisture	6.96 ± 0.06 a	6.05 ± 0.06 a
Total dietary fiber	15.38	32.00
Soluble dietary fiber	7.40 ± 0.06 a	14.52 ± 0.45 b
Insoluble dietary fiber	7.98 ± 0.54 a	17.48 ± 0.71 b
β-glucan	9.13 ± 0.01 a	17.43 ± 0.29 b
Pentosan	3.95 ± 0.50 a	7.42 ± 1.06 b
Starch	47.38 ± 0.16 a	24.93 ± 0.04 b
Protein	18.63 ± 0.04 a	21.12 ± 0.16 b
Fat	9.21 ± 0.01 a	11.31 ± 0.14 b
Ash	2.44 ± 0.26 a	4.59 ± 0.05 b
Phytic acid	1.70 ± 0.04 a	3.20 ± 0.06 b
Particle-size distribution		
1,600	0.4 a	0 a
600	50.1 a	44.8 b
400	27.5 a	36.7 b
200	12.8 a	18.0 b
150	7.1 a	0.4 b
<150	2.1 a	0.1 a

[a] Moisture basis. Mean ± standard deviation; *n* = 2, component; *n* = 4, particle-size distribution. Values followed by same letter between pairs are not significantly different ($P < 0.05$).

TABLE II
Amount of Soluble β-Glucan in Oat Brans and Slurries After Hydrothermal and Enzymic Treatments[a]

	Soluble β-Glucan (%)[b]	
Treatment	ROB[c]	FCB[d]
None	33.7 ± 4.9 a	23.6 ± 1.0 b
Hydrothermal (no enzyme)	85.3 ± 9.8 c	82.4 ± 1.0 c
Termamyl α-amylase	83.3 ± 4.5 c	82.1 ± 5.8 c
Finase S40 phytase (pre)[e]	93.7 ± 5.0 d	90.0 ± 4.3 d
Finase S40 phytase (post)[f]	95.3 ± 3.0 d	90.8 ± 4.0 d

[a] Mean ± standard deviation, *n* = 4. Values followed by same letter are not significantly different ($P < 0.05$).
[b] Expressed as difference between total and insoluble β-glucan.
[c] Regular oat bran.
[d] Fiber-concentrated oat bran.
[e] Enzyme added to oat bran slurry immediately before heating at 25°C.
[f] Enzyme added to oat bran slurry during cooling at 55°C.

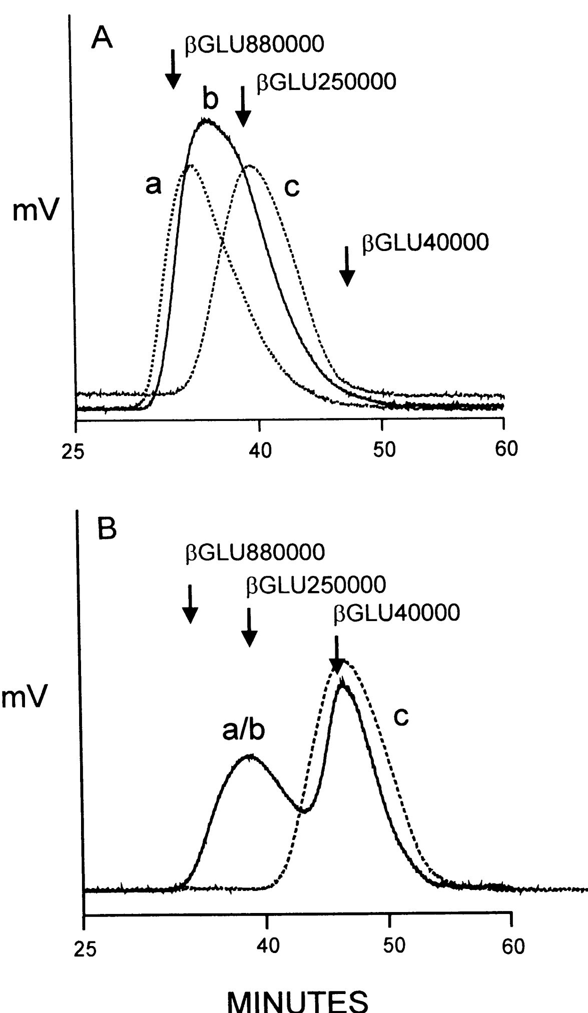

Fig. 2. Gel-permeation chromatogram of β-glucan in regular oat bran (a); fiber-concentrated oat bran (b); reference oat β-glucan (Megazyme) (c). Fluorescence detection after postcolumn reaction with Calcofluor. **A,** No treatment. **B,** Treatment with Finase S40 phytase (before heating).

particles, although ROB had been ground in a laboratory mill to achieve a particle-size distribution comparable to that of the FCB.

The amount of soluble β-glucan was greater in ROB (34%) than in FCB (24%) when determined after incubation of the oat brans in water at 37°C for 2 hr (Table II). As oat β-glucan binds more tightly to the thicker cell walls in the outer bran layers than to the inner endosperm (Wood and Fulcher 1978), the lower solubilization of the β-glucan was quite reasonable. Similar results for β-glucan solubility in low- and high-fiber oat brans were published earlier (Knuckles et al 1992; Mälkki et al 1992). Our results were, however, somewhat lower than those reported by Knuckles et al (1992) for low-fiber oat brans (59%) and for high-fiber oat brans (55%), and clearly lower than those reported by Åman and Graham (1987) for oat grains (80%). Extraction of β-glucan was lower than in earlier studies even though extraction conditions were similar. This may be explained, at least partly, by the large particle size of the brans studied.

The M_w and the M_n of β-glucan were slightly higher in the ROB (8.4×10^5 and 4.2×10^5, respectively) than in the FCB (6.0×10^5 and 2.6×10^5, respectively). The M_w of reference β-glucan (2.2×10^5) was about three times lower than the M_w values of β-glucan in oat brans (Fig. 2A). This result is consistent with earlier reports (Vårum and Smidsrod 1988; Wood et al 1989, 1991b), which showed that the molecular weight of β-glucan decreases during the isolation and purification procedure due to enzyme action or shear forces.

Effect of Hydrothermal Treatment

FCB developed much higher viscosity than ROB during the hydrothermal treatment (Fig. 3A). The end viscosity (BU) of the FCB slurry was over two times greater than that of the ROB slurry at the same solids level (7%), and over five times greater when the solids content of the FCB was increased (13.3%) to give the same starch content (3.3%) as that of the ROB. When reference oat starch was studied in water dispersion at 3.3.% concentration, its viscosity was so low that it was hardly detected with the Brabender viscoamylograph (Fig. 3A). This curve, however, does not adequately explain the effect of starch on viscosity of oat bran slurries, because starch behaves quite differently when divided within the bran particles and when evenly dispersed in water (K. Autio, *personal communication*).

In any case, the dissimilar polysaccharide composition of the ROB and FCB is the most likely explanation for their differences in viscous behavior. FCB slurries (13.3%) differed primarily from ROB slurries (7%) in having four times greater amount of total dietary fiber (TDF), such as β-glucan and pentosan, at equal starch content. This indicates that the TDF had very much greater influence on the viscosity of oat bran slurries than did starch, at least when a high mixing rate (75 rpm) was used. At lower mixing rates, the difference of the effects of TDF and starch on viscosity is probably not as great because starch is known to be more shear-thinning than β-glucan (Paton 1986).

Another factor contributing to the viscosity of the bran slurries is the microstructure of the bran particles. Decrease in particle size contributes to an increased viscosity (K. Autio, *personal communication*). Slightly lower particle size of the FCB may explain some of its high-viscosity-forming ability in comparison to that of ROB.

The M_w of β-glucan in ROB (8.4×10^5) and FCB (6.0×10^5) remained unchanged during hydrothermal treatment. ROB and FCB also were similar in the amount of solubilized β-glucan; ≈84% of the β-glucan in ROB and FCB was solubilized during heating (Table II). High values for the standard deviation indicate the difficulty of determining soluble β-glucan from heat-treated bran slurries. The separation of solids from the supernatant was difficult, especially in ROB slurries that contained high amounts of gelatinized starch.

Effect of Termamyl α-Amylase Treatment

Treatment of the bran slurries (7% solids) with Termamyl α-amylase (0.0028U/g of starch) resulted in a greater decrease in viscosity for ROB than for FCB, due in part to higher starch content in the ROB (Fig. 3B). When the solids content of the FCB slurry was increased to 13.3%, the decline in the viscosity after α-amylase treatment was strikingly small. Although the viscoamylograph is not sufficiently sensitive to detect changes in BU values in this range (>2,000 BU), the data clearly showed that α-amylase treatment has a very small effect on the viscosity of oat

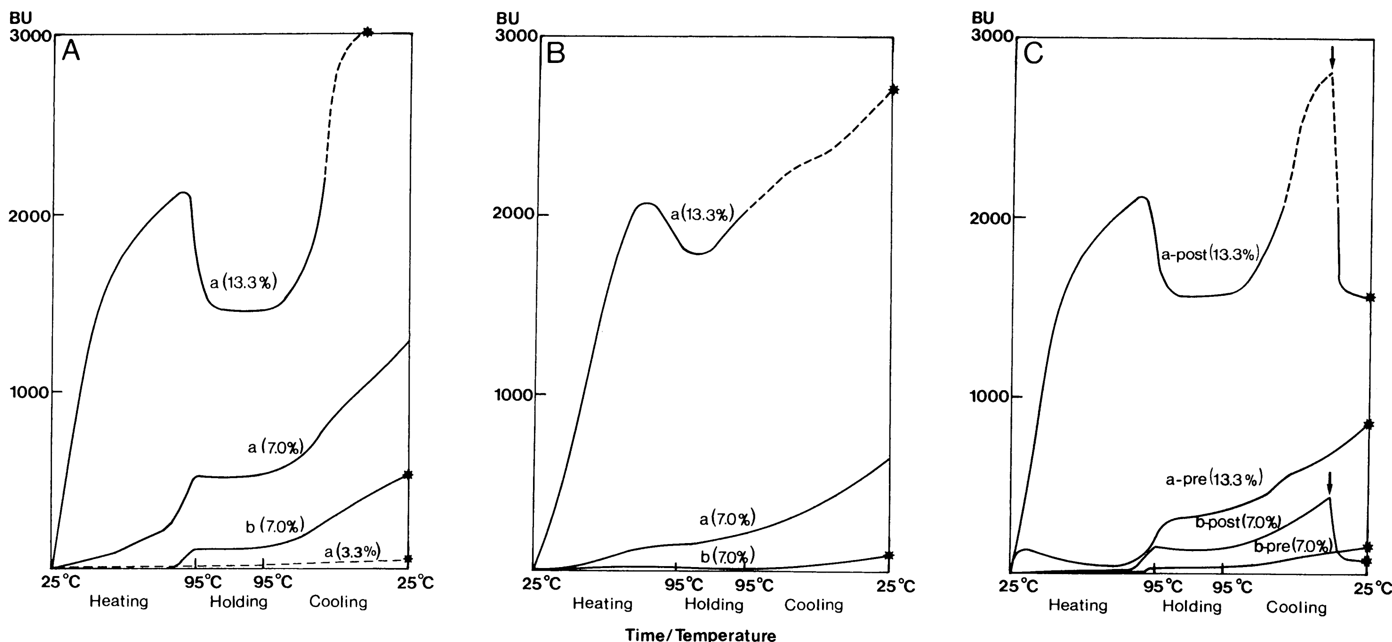

Fig. 3. Viscoamylograms of fiber-concentrated oat bran (a) and regular oat bran (b) slurries at 7 or 13% (w/v) solids content and of a reference (c) oat starch dispersion at 3.3% (w/v) concentration. ✱ = Identical starch content (3.3%, w/v). **A,** Hydrothermal treatment. **B,** Treatment with Termamyl α-amylase. **C,** Treatment with Finase S40 phytase. Arrow indicates stage of enzyme addition (postheating). – – – = range of BU values over detection limit of Brabender viscoamylograph.

bran slurries when TDF content is high (4.2%).

Because the viscosity of α-amylase-treated FCB slurries was so high, degradation of starch was analyzed by GPC with postcolumn iodine detection, which showed that original starch polymers were degraded and that a product peak appeared at 52 min (Fig. 4A and B). These end products of the α-amylase-treated bran and reference starch were further characterized by HPLC, which surprisingly indicated a complete degradation of starch to water-soluble dextrins of varying chain lengths (Table III).

When α-amylase was added to reference oat β-glucan solution before heating, the molecular weight (2.2×10^5) or viscosity-forming ability of the β-glucan remained unchanged (Table IV). This suggests that the α-amylase preparation is appropriate for use when β-glucan is to be kept undegraded. Furthermore, α-amylase did not change the solubility of β-glucan as did the hydrothermal treatment (Table II).

The viscosity and molecular weight studies showed that, even with extensive hydrolysis of starch, the viscosity of oat bran slurry does not change when the TDF level is high. It was also concluded that the action of α-amylases was independent of the high content of solids or viscous β-glucan, which conflicts with earlier suggestions (Björck 1993). This might be caused by the large enzyme dosage used in this study.

Effect of Finase S40 Phytase Treatments

Finase S40 phytase (14,300U/g of phytate) caused a vital and immediate decrease in the viscosity of oat bran slurries when added either before heating or during cooling (55°C). In the preheating treatment, the viscosity remained low during the whole temperature cycle. In the postheating treatment, a sudden reduction in the viscosity occurred (Fig. 3C). The viscosity reduction was greater in FCB slurries than in ROB slurries at equal starch content (3.3%, w/v). In FCB slurries, the end viscosity (25°C) was lower when phytase preparation was added before heating, in ROB slurries viscosity was lower when phytase preparation was added after heating. The phytic acid content of the bran slurry decreased more in the postheating treatment (54%) than in the preheating treatment (36%) with the phytase preparation used. These results are analogous to those obtained by Aalto-Kaarlehto et al (1992).

GPC studies of bran starch showed that the molecular weight distribution of starch changed little during the treatments with phytase preparation (Fig. 4), and there was no systematic difference in GPC chromatograms of pre- and postheated bran starches. However, the GPC method used was not capable of detecting all changes in molecular weight of starch. This was probably due to inadequate solubility of high molecular weight amylopectin. Degradation of starch was also studied by assaying the hydrolysis products with HPLC and measuring the viscosity of the reference oat starch after treatments with phytase preparation.

The HPLC results indicated that, in the postheating treatment with phytase preparation, the amount of glucose in ROB and FCB slurries increased to 41% of the original starch content; but in the preheating treatment with phytase, the sugar composition remained unchanged (Table III). When the reference starch dispersion was treated with phytase preparation after heating, 54% of the original starch was degraded to glucose; when treated before heating, the glucose content did not increase (Table IV). This suggests that the glucose in phytase-treated brans originates from starch, and not from other bran polysaccharides. The HPLC

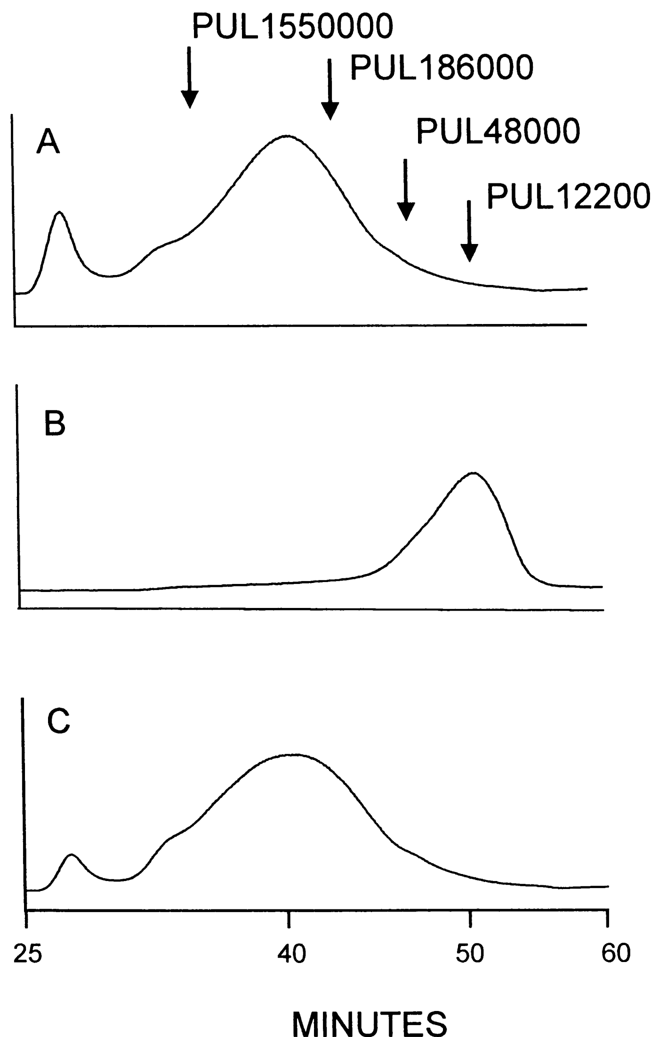

Fig. 4. Gel-permeation chromatogram of starch in fiber-concentrated oat bran with postcolumn iodine detection at 560 nm. **A,** No treatment. **B,** Treatment with Termamyl α-amylase. **C,** Treatment with Finase S40 phytase (before heating).

TABLE III
Carbohydrate Content (%) in Oat Bran Slurries After Hydrothermal and Enzymic Treatments[a]

Treatment	Glucose		Maltobiose		Maltotriose		HMW Oligosaccharides[b]	
	ROB[c]	OS[d]	ROB	OS	ROB	OS	ROB	OS
Hydrothermal (no enzyme)	1.0 ± 0.4 a	0 a	0 a	0 a	0 a	0 a	7.4 ± 2.4 a	0.5 ±0.1 a
Termamyl α-amylase	1.6 ± 0.3 a	0 a	3.1 ± 0.6 b	1.9 ± 0.6 a	5.1 ± 1.2 b	3.9 ± 0.4 b	79.6 ± 5.5 b	88.6 ± 2.7 b[e]
Finase S40 phytase (pre)[f]	1.3 ± 0.2 a	2.0 ± 0.2 a	0 a	0 a	0 a	0 a	10.7 ± 0.4 a	0 a
Finase S40 phytase (post)[g]	40.9 ± 1.5 b	53.8 ± 5.0 b	0 a	0 a	0 a	0 a	10.3 ± 1.8 a	0 a

[a] Initial starch content (%) of slurry. Mean ± standard deviation; $n = 4$, bran; $n = 3$, starch. Values followed by same letter within a column are not significantly different ($P > 0.05$).
[b] Calculated using maltotriose as standard. Soluble in 0.1% H_3PO_4 solution. HMW = high molecular weight.
[c] Regular oat bran.
[d] Reference oat starch.
[e] $n = 2$.
[f] Enzyme added to oat bran slurry immediately before heating at 25°C.
[g] Enzyme added to oat bran slurry during cooling at 55°C.

TABLE IV
Viscosity of Reference β-Glucan Solution (1.1%, w/v) and Starch Dispersion (3.3%, w/v) After Hydrothermal and Enzymic Treatments[a]

Treatment	Oat β-Glucan Solution[b]	Oat Starch Dispersion[c]
Hydrothermal (no enzyme)[d]	61 b	47 b
Termamyl α-amylase	61 b	nd [e]
Finase S40 phytase (pre)[f]	<10 a	47 b
Finase S40 phytase (post)[g]	<10 a	35 a

[a] Mean ± standard deviation, $n = 3$. Values followed by same letter within a column are not significantly different ($P < 0.05$).
[b] Viscosity (mPas) measured with Bohlin viscometer (25°C, 229 sec^{-1}) immediately after each treatment.
[c] β-Glucan wetted with a few drops of 90% ethanol and dissolved in water (25°C) using a magnetic stirrer. Solution incubated in boiling water bath for 2 min and cooled to 25°C.
[d] Starch dispersed in water (25°C) and treated.
[e] Not determined.
[f] Enzyme added to oat bran slurry immediately before heating at 25°C.
[g] Enzyme added to oat bran slurry during cooling at 55°C.

results were further confirmed with viscosity measurements of the reference starch dispersion, which indicated that the starch dispersion lost its viscosity only when phytase preparation was added after heating, when starch was first gelatinized (Table IV).

Molecular weight studies of β-glucan in ROB and FCB showed that this polymer, in turn, degraded similarly in phytase preparation treatments. The chromatogram exhibited a bimodal β-glucan distribution: the first peak eluted at the same time as undegraded β-glucan at ≈39 min (M_w 2.2×10^5); the second peak eluted at ≈46 min (M_w 4.0×10^4) (Fig. 2B). In the reference solution of β-glucan, hydrolysis had presumably progressed further: only one peak at ≈46 min was detected in the pre- and postheated solutions (Fig. 2B). The viscosity of reference β-glucan decreased in a similar manner when phytase was added before or after heating (Table IV). Furthermore, no small molecular fragments were detected in the phytase-treated β-glucan solutions.

It seems that the Finase S40 phytase preparation contains unusual β-glucanase activity, specific for susceptible sequences, of which there are only a few in the β-glucan chain. Such an enzyme could be a β(1→4)-endo-glucanase that cleaves the β-glucan at sites containing more than three consecutive β-(→4)-linkages. Yin and MacGregor (1989) reported a β-(1→4)-endo-glucanase was associated with the solubilization of barley β-glucan during malting. Recently, Johansen et al (1993) suggested that a similar enzyme is responsible for the hydrolysis of oat β-glucan in the pig jejunal. In both studies, high molecular weight degradation products were reported: $2.0 \times 10^4 - 2.5 \times 10^4$ (Yin and MacGregor 1989) and 1.0×10^5 (Johanssen et al 1993).

Solubilization of the β-glucan bound in the cell walls was almost complete in phytase-treated bran slurries (Table II). This gives further evidence that the Finase S40 phytase preparation contains endo-β-(1→4)-glucanase, which increases the solubilization of β-glucan (Yin and MacGregor 1989).

The molecular weight and viscosity studies of phytase-treated oat brans and reference polysaccharides showed that, in the preheating treatment, the viscosity reduction was mainly due to the degradation of β-glucan, whereas in the postheating treatment, degradation of both β-glucan and gelatinized starch caused the rapid decrease in viscosity. This explains why the viscosity reduction after both treatments with phytase preparation was much greater in FCB slurries with high β-glucan content than it was in ROB slurries.

CONCLUSIONS

At high levels of TDF (4.2%), the effect of starch on oat bran slurry viscosity is minimal when a high mixing rate (75 rpm) is used together with heating. In other words, the viscosity of an oat bran product with high dietary fiber content does not necessarily change, even though the starch is completely degraded to water-soluble oligosaccharides. A partial degradation of the bran β-glucan (M_w 6.0×10^5) to a product of M_w 4.0×10^4 greatly reduces the viscosity of the oat bran slurry with high TDF. This was indicated in experiments with a phytase preparation containing an unusual β-glucanase, probably specific for sites in the β-glucan chain that contain more than three consecutive β-(1→4)-linkages, and for a glucoamylase that was active only on gelatinized starch.

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