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EFFECTS OF PROCESSING ON THE MICROSTRUCTURE OF OAT (AVENA SATIVA) BRAN CONCENTRATE AND THE PHYSICOCHEMICAL PROPERTIES OF ISOLATED β-GLUCANS

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Abstract

Fluorescence microscopy was used to study the microstructure of oat cell walls during concentration of oat bran and isolation of β-glucans. The bran concentrate separated from c.v. Nasta contained mainly the aleurone and subaleurone endosperm layers, whereas that separated from a commercial bran mixture contained more endosperm. In contrast to Nasta, the commercial bran mixture contained β-glucan degrading enzymes, which survived the β-glucan isolation procedure. In the presence of enzymes, the solubility and yield of β-glucans improved but the viscosity decreased when it was measured at the same β-glucan concentration. For inactivation of the enzymes, 80% ethanol at 78 °C was more effective than 80% or 94% ethanol at 60 °C or 94% ethanol at 78 °C. The yield of β-glucan extracted was higher from Nasta bran concentrate than from commercial bran concentrate, and after alkaline extraction the Nasta solid residue exhibited intense red fluorescence in the aleurone and subaleurone endosperm cell walls. The solid residue from commercial bran had areas of starchy endosperm cell walls provided the β-glucan degrading enzymes were inactivated.

Key Words: oat, bran, cell walls, β-glucan, β-glucanase, viscosity, fluorescence microscopy.

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Introduction

Oat is an excellent source of soluble dietary fiber, the main component being water-soluble (1-3)(1-4)-β-D-glucan (Wood, 1986). Fluorescence microscopy studies, using fluorochromes such as Congo Red and Calcofluor White as markers, have revealed that oat β-glucan tends to be concentrated in the subaleurone endosperm cell walls of certain oat cultivars (Fulcher, 1982; Wood *et al.*, 1983; Yiu, 1986). β-glucan can thus be enriched by separation of the coarser bran particles from the fine fraction. According to Wood *et al.* (1991a), the enrichment of β-glucan is dependent on the degree to which the cell wall thickness varies throughout the endosperm. Relevant to this, variation in the enrichment during milling has been observed for different cultivars.

Recent reports on the effectiveness of oat and barley fiber in lowering blood cholesterol suggest that the effect is related to the increased viscosity of the gastrointestinal contents which contained the soluble fiber (Bengtsson *et al.*, 1990; Mälkki, Autio, *et al.*, submitted).

Mean molecular weight, chemical structure, amount of β-glucan, and endogenous β-glucanase activity are the main factors influencing the rheological properties of bran water slurries and water solutions of isolated β-glucans. β-glucans isolated from c.v. Hinoat oat flours formed more viscous solutions than β-glucans extracted from c.v. Rodney flour (Wood *et al.*, 1978). Wood *et al.* (1991b) reported that there was no essential variation in the chemical structure of oat β-glucan between cultivars, nor between whole groat and bran. This finding suggests that other effects besides chemical structure are responsible for the observed rheological differences. Little has been published on the β-glucanase activity in oats, but it is known that it can persist through the gum extraction procedure (Wood *et al.*, 1978).

In the work reported here, the microstructure of two sources of oats, Nasta, a Finnish oat cultivar and a commercial oat bran, were studied at different stages of the β-glucan isolation procedure to determine the key factors in the creation of viscous conditions. The enzyme inactivation procedure was varied to determine optimal conditions for inactivation.

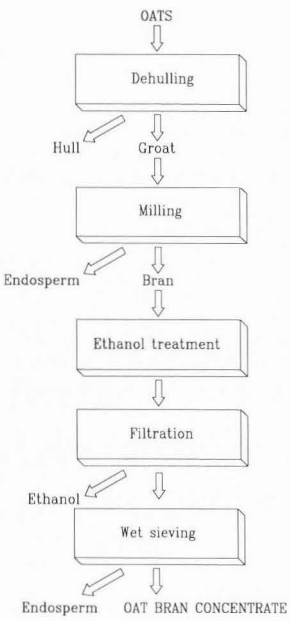


Fig. 1. Flow chart summarizing concentration of oat bran.

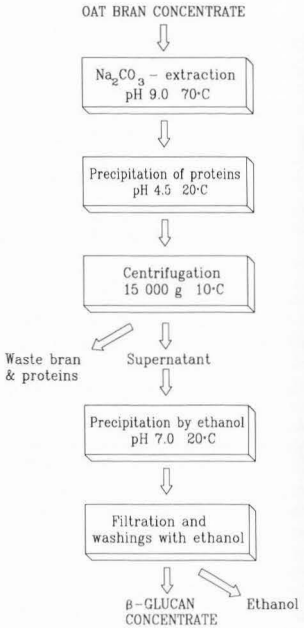


Fig. 2. Flow chart summarizing isolation of β-glucans.

Table 1. Experimental conditions for the enzyme inactivation and sample codes.

Experimental conditions:				
Temperature	78 °C		60 °C	
Ethanol conc. (%, w/w)	80	94	80	94
Sample codes:				
Nasta	Ia	Ib	Ic	Id
Commercial mixture	IIa	IIb	IIc	IId

Materials and Methods

Oat samples

Commercial bran mixture was obtained from Raisio Group (Raisio, Finland), and the Nasta cultivar from the Institute of Plant Breeding (Jokioinen, Finland).

Oat bran concentration

The bran concentration diagram is presented in Figure 1. Whole grain oats were dehulled using a dehuller and milled in a Brabender roller mill. Duplicate samples of two hundred grams of bran was heated with 600 ml of ethanol-water solutions for two hours while mixing. The experimental conditions for the enzyme inactivation are shown in Table 1. In this process, the components concentrated are soluble and insoluble fiber and protein. To distinguish this concentrate from the commercial oat fiber, prepared from oat hulls and con-

taining solely insoluble fiber, this end product is called oat bran concentrate.

Oat β-glucan isolation and preparation of β-glucan solutions

Oat β-glucans were isolated according to the diagram shown in Figure 2, by stirring duplicate samples of 35 grams of bran concentrate with 1500 ml of Na_2CO_3 (pH 9.0; 70 °C) for 2 hours. β-glucan solutions of 0.37% (w/v) were prepared by solubilizing β-glucan precipitates in buffer (citric acid-phosphate buffer, pH 5.0 or pH 8.0) at room temperature.

The determination of particle size distribution

Ten grams of bran concentrates were sieved in a laboratory sieve (Bühel MIAG DLKP-2040, Germany) fitted with different screens and separated into four fractions: > 720 μm, 720-530 μm, 530-355 μm, and < 355 μm. The fractions were weighed.

Analytical methods

The β-glucan content was determined by the enzymatic method of McCleary and Glennie-Holmes (1985) with the use of a Biocon kit. The total carbohydrate content was determined by the phenol-sulfuric acid method of Dubois *et al.* (1956) and the protein by the Kjeldahl nitrogen method.

Rheological methods

Viscosity measurements were made with a Bohlin VOR instrument (Bohlin Rheology AB, Lund, Sweden). A concentric cylinder system (DIN 53019) was used. The viscosity was measured both in the crude extract (after removal of solids) and in solutions of β-glucan precipitate. Solutions of β-glucan were prepared from wet-ethanol precipitates. The viscosity was measured in the shear rate range of 18.6 - 463 s^{-1} at 25 °C. Up- and down-curves were measured.

Incubation of β-glucan solutions with endo-β-glucanase

Since the impurities present in β-glucan might affect the viscosity, experiments were performed in which the β-glucans were degraded with a pure endo-β-glucanase. Endo-β-glucanase produced by *Trichoderma reesei*, was isolated and purified by the Biotechnical Laboratory of this Research Centre. The preparation showed activity only towards β-glucan. No amylase or xylanase activity were detected. Enzyme activity was determined by incubating the enzyme solution in 0.1 M acetate-buffer, pH 5.0 at 50 °C for 10 minutes with 0.1% barley β-glucan (Biocon, Wadhurst, Victoria, Australia). The reducing sugars formed were assayed by adding 3 ml of DNS-reagent (Sumner and Somers, 1949), boiling for 5 minutes, cooling and measuring the absorbance at 540 nm.

Solutions of β-glucan were incubated with this enzyme (final activity 6.2 nkat/ml) at pH 5.0 and 50 °C for one hour.

Embedded sections

Bran concentrate samples were embedded in agar gels. Small pieces of kernel or agar gel were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) according to the method described by Yiu (1986). Fixed samples were dehydrated through a series of changes of ethanol and embedded in historesin (Reichert-Jung AG, Heidelberg, Germany). Sections (5 μm) were cut with a rotary microtome (Reichert-Jung AG) and stained with 0.01 % aqueous Congo Red (Wood *et al.*, 1983).

Fluorescence microscopy

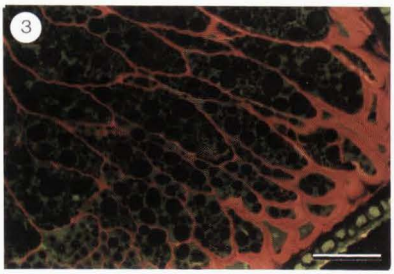
Samples were examined with an Olympus BH-2 microscope. The sections stained with Congo Red were examined with an exciter/ barrier filter set BG12 with maximum transmission at 400 nm / > 500 nm. Photomicrographs were obtained using Kodak Ektachrome 400 Daylight film.

Results and Discussion

Investigations using the Microscope

For ease of comparison the results on two samples are presented in sequence of treatments. Figures 3-6 are for Nasta at different stages of β-glucan isolation process: Figure 3 (the groat), Figure 4 (the bran), Figure 5 (the concentrated bran), Figure 6 (solid residue after β-glucan isolation). Figures 7-10 are for the commercial bran: Figure 7 (commercial bran), Figure 8 (concentrated bran), Figure 9 (solid residue of sample IIa after isolation) and Figure 10 (solid residue of sample IIc after isolation of β-glucan).

Congo Red staining of the oat kernel revealed the presence of (1-3)(1-4)-β-D-glucan as a red fluorescence (Figure 3). Nasta cultivar has thick cell walls in the subaleurone endosperm layer and thin cell walls in the inner endosperm (Figure 3). The micrographs of the original brans are shown in Figures 4 and 7. The commercial bran is thicker and contains more endosperm than the Nasta bran. Commercial oat brans in Finland typically consist of 40% Veli, 20% Puhti, 9% Virma, 8% Ryhti, 5% Karhu and less than 5% other varieties. The photographs shown in this study were chosen as representatives of a number of samples. The main appearance was that the cell wall thickness varied less in the commercial bran than in Nasta kernel which could account for the poorer β-glucan enrichment in the bran fraction of this sample. It has been suggested that endosperm cell wall thickness is the basis for β-glucan enrichment in oat bran (Fulcher, 1986), since the cells with the thickest cell walls are usually at the periphery of the kernel, thus are in the bran portion after milling.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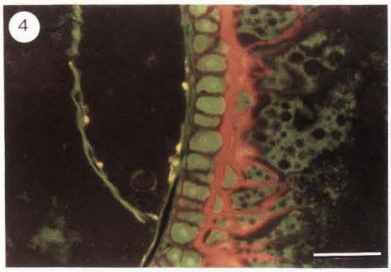
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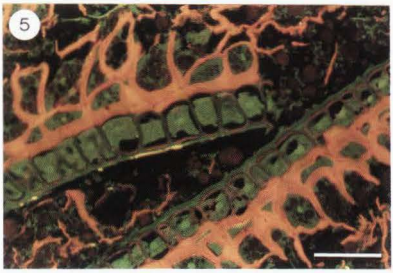
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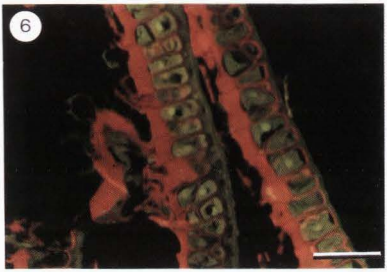
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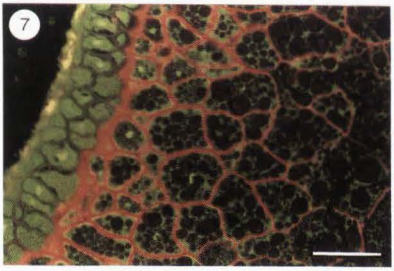
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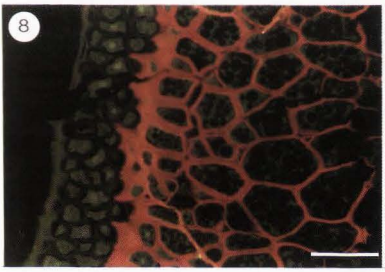
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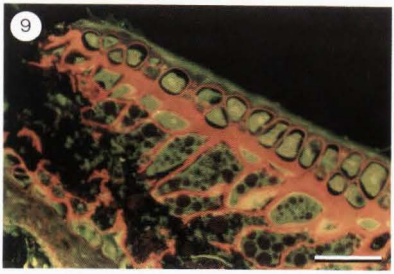
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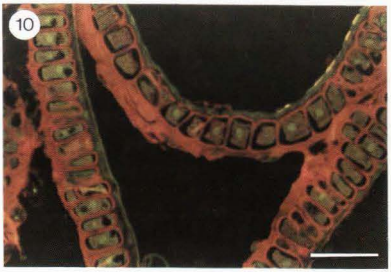
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Legends for color illustrations (Figs. 3-10).
Bar = 100 μm (in each of the Figures).

Fig. 3. Embedded section of Nasta kernel. β-glucans appear red.

Fig. 4. Embedded section of original Nasta bran.

Fig. 5. Embedded section of Nasta bran concentrate.

Fig. 6. Embedded section of Ia residue after isolation of β-glucans. Ia is Nasta bran concentrate heated with 80% ethanol at 78 °C.

Fig. 7. Embedded section of original commercial bran.

Fig. 8. Embedded section of concentrate obtained from commercial bran.

Fig. 9. Embedded section of IIa residue after isolation of β-glucans. IIa is concentrate from commercial bran heated with 80% ethanol at 78 °C.

Fig. 10. Embedded section of IIc residue after isolation of β-glucans. IIc is concentrate from commercial bran heated with 80% ethanol at 60 °C.

The high lipid content of oats causes rancidity on storage of milled fractions, and limits efficient fractionation. The ethanol treatment was included in concentration of oat bran since it decreases fat content, and deactivates enzymes (Wood *et al.*, 1989). The microstructure of the bran concentrate prepared from Nasta (Samples I) is illustrated in Figure 5. The particles contain the aleurone and subaleurone layers of oat kernel. Although much of the starchy endosperm portion of the bran had been dispersed, the structural integrity of the subaleurone cells and aleurone cells did not appear to be affected. There is some loss of aleurone cell contents which is due to ethanol extraction rather than fixation or dehydration, since no shrinkage of aleurone cells is observed in the original brans. Generally, aleurone cells contain a high percentage of fat, which is extracted during the ethanol treatment. Concentrate from commercial bran (Samples II) contained, in addition to intact aleurone and subaleurone layers, large areas of apparently undamaged inner starchy endosperm (Figure 8). The different inactivation procedures (Table 1) had no visible effect on the microstructure of the samples.

Sections of the waste bran plus proteins left behind after the isolation of β-glucan were also examined under the microscope. The residue of samples I exhibited intense red fluorescence in the outer subaleurone cell walls, indicating that a substantial portion of β-glucan in these parts of the bran was insoluble under alkaline

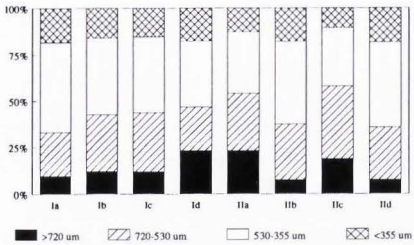

Fig. 11. Particle size distribution of fiber concentrates.

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conditions of extraction (Figure 6). Wood and Fulcher (1978) reported that the residual β-glucan was largely removed by a second alkaline extraction. An interesting observation was that the gum obtained from the second or third alkaline extraction showed higher viscosity than did that from the first extraction.

Although the inactivation procedure did not affect response of the microstructure to alkali in samples I (from c.v. Nasta), it did affect that in samples II (from commercial bran): Thus, when the inactivation temperature was 78 °C and the ethanol concentration 80%, much of the endosperm cell walls did not dissolve in the hot alkaline solution (Figure 9). However, when the bran was treated at 78 °C with 94% ethanol or at 60 °C with 80% or 94% ethanol the endosperm cell walls including the subaleurone endosperm layers disappeared in alkali (Figure 10).

Yields of brans and mixed-linked β-glucan extracted from fiber concentrates

Yields of bran concentrate range from 42 to 50% of the original bran, with the higher β-glucan contents being associated generally with the lower concentrate yields (Table 2). From β-glucan in the bran, 71 to 76% was transferred into the concentrate.

Yields of mixed-linked β-glucan extracted from bran concentrate differed for the two raw materials. Samples I contained more β-glucan (Table 2), and the yield was also higher, varying from 51 to 58% and from 42 to 52% for samples I and II, respectively. The particle size of samples Ia,b,c and d varied, with the smaller particle size being associated with the higher yield of β-glucan (Figure 11). The yield of β-glucan was substantially lower for sample IIa than for samples IIb,c and d. Histochemical examination of the extraction residue of sample IIa indicated that it contained more residual β-glucan than samples IIb, c and d (Figures 9 and 10). The larger particle size of sample IIa can not be the only reason for the lower yield of extracted mixed-linked β-glucan.

Table 2. β-glucan content of brans and bran concentrates, concentrate yield and yields of mixed-linked β-glucan in the concentration and isolation steps. Duplicate experiments are from different concentrates and extractions.

Sample	Bran concentrate yield (%)	β-Glucan content (%)	β-Glucan yield (%)	
			from bran to concentrate	from concentrate to isolate
Bran I		9.7		
Bran II		7.8		
Ia	50.0±2.0	14.8±0.3	76.3	57.6±2.5
Ib	48.1±2.1	15.1±0.5	74.9	52.4±0.3
Ic	47.1±0.3	15.2±0.1	73.8	52.8±4.8
Id	43.8±0.1	16.9±0.1	76.3	51.3±1.2
IIa	50.0±1.0	11.9±0.2	76.3	41.6±0.5
IIb	42.7±0.7	13.0±0.2	71.2	48.8±1.5
IIc	44.3±0.2	13.2±0	75.0	49.6±1.3
IId	47.1±2.5	11.9±0.5	71.9	52.2±2.8

Table 3. Apparent viscosity of crude β-glucan extracts (pH 5.0) and 0.37% β-glucan solutions (pH 8.0), and viscosity loss of the β-glucan solution during incubation at pH 5.0 and 50 °C for one hour expressed as a percentage of the original viscosity value before incubation. Duplicate experiments are from different concentrates and extractions.

Sample	Apparent viscosity (at 18.6 s^{-1}) (mPa s)		Decline in viscosity[1] (%)
	Crude extract	0.37% solution*	
Ia	106.5±7.8	339.5±47.4	14.1
Ib	131.0±1.4	512.0±119	4.6
Ic	193.0±9.9	482.0±2.8	12.0
Id	240.5±13.4	463.5±0.7	0
IIa	38.9±3.5	500.0±46.7	0
IIb	47.9±4.9	341.0±84.9	81.5
IIc	51.9±8.6	303.0±12.7	83.2
IId	52.9±8.3	340.0±2.8	82.7

* β-glucan concentration; [1] during incubation.

Table 4. Chemical composition of isolated β-glucan preparates. Duplicate experiments are from different concentrations and extractions.

Sample	β-Glucan content (%)	Protein content (%)	Total carbohydrates (%)
Ia	58.2±1.4	2.9±0.1	83.3±1.1
Ib	59.2±2.5	3.8±0.5	80.2±0.1
Ic	55.3±2.5	4.9±0.9	83.0±0.6
Id	57.3±0.3	5.2±1.1	80.4±1.9
IIa	57.9±0.5	1.8±0.1	80.3±0.6
IIb	61.6±0.5	1.3±0.2	91.3±2.0
IIc	65.0±0.5	1.5±0	92.6±3.3
IId	59.9±4.2	1.5±0.2	79.8±6.4

Characterization of the preparations

The solutions of β-glucans were pseudoplastic, but did not exhibit time dependent behavior at pH 5.0 (crude extract) or at pH 5.0/8.0 (isolates). A low shear rate value of apparent viscosity of the samples is presented in Table 3. The apparent viscosity of the crude β-glucan concentrate of samples I, after alkaline extraction and removal of solids, was substantially higher than that of samples II, indicating that Nasta oat contained more soluble fiber than the commercial bran (sample II, Table

2). Although samples Ia and Id contained almost equal concentrations of β-glucan in the crude extract (0.2%), the apparent viscosity of sample Id was significantly higher (Table 3).

Nasta is a protein-rich variety, so samples I contained substantially more protein than samples II (Table 4). Conditions of ethanol treatment had an effect on the protein content of the β-glucan preparations: Samples Ic and Id had higher protein contents than samples Ia and Ib.

Inclusion of a purified endo-β-glucanase in solutions of β-glucan eliminated viscosity, indicating that the viscosity was solely a property of β-glucan. Control solutions with no added enzyme were included in the enzyme experiment. Regardless of the inactivation procedure the viscosities of the controls of samples I, were the same before and after incubation suggesting that the preparations isolated from Nasta cultivar did not or only slightly contained β-glucan degrading enzymes. Wood *et al.* (1978) measured enzyme activity viscometrically and found that viscosities of β-glucan were rapidly reduced by endoenzyme action. The viscosity loss was over 80% in the controls of samples II (b,c,d) after incubation at pH 5.0 and 50 °C suggesting that the preparation isolated from the bran concentrate contained β-glucanase enzymes, which had survived through the isolation procedure (Table 3). The only procedure effective for the inactivation of the enzymes was 78 °C with 80% ethanol and the sample (IIa) treated in this way, even though its yield of isolated β-glucan was lowest, had the highest viscosity when values were compared at the same β-glucan concentration (Table 3). Enzyme-degraded gum is more rapidly solubilized, leading to an increased yield (Wood, 1986). In a study by Wood *et al.* (1989), it was reported that bran deactivated by refluxing in 75% ethanol showed no decline in viscosity of crude extracts at pH 4.5 in 1 hour. It was suggested that these enzymes are of bacterial or fungal origin, since they are located in the outer layer of the kernel and are very heat resistant.

Sample Ia had lower viscosity than the samples Ib, Ic and Id. Recent studies in this laboratory have shown that there exist great differences in viscosity between β-glucan solutions isolated from different Finnish oat varieties (Autio *et al.*, 1992). The more significant differences in the viscosities could be explained in terms of differences in mean molecular weight. It has been suggested that a protein or peptide residue that remains attached to β-glucan improves the rheological properties (Vårum and Smidsrod, 1988). β-glucan solution of Nasta was substantially more shear-thinning than the others. The shear-thinning behavior is attributed to the disaggregation of aggregated β-glucan molecules or very small fibre particles as a result of shear. Using total intensity light scattering Vårum and Smidsrod (1991) suggested that a portion of β-glucan monomers are able to associate into large micelle like aggregates which might involve the protein. Further studies on the supermolecular structure of β-glucan and its relationship to the physical properties of β-glucans are needed.

Conclusions

Factors important for the creation of viscous conditions in oat bran concentrate and in isolated β-glucan solutions were studied with the following results:

1. Oat bran concentrate prepared from c.v. Nasta was found to have higher β-glucan content than the bran concentrate from the commercial bran mixture, and its crude extract was more viscous. Fluorescence microscopic examination showed it to contain intact aleurone and subaleurone layers, whereas concentrate from commercial bran also contained large portions of endosperm cell walls.

2. The commercial bran mixture contained β-glucan degrading enzymes which survived the entire isolation procedure. Heating at 78 °C with 80% ethanol proved most effective for the inactivation of β-glucan degrading enzymes. The solubility and recovery of β-glucan improved in the presence of enzymes in the extraction step while the viscosity decreased, when values were measured at the same β-glucan concentration.

3. Sample Ia was less viscous than samples Ib, Ic, and Id. This difference can not be explained in terms of β-glucan content or the presence of β-glucanase. The only significant difference between the samples was the lower protein content of sample Ia.

Acknowledgement

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Discussion with Reviewers

S.H. Yiu: What direct evidence did the authors have in claiming that differences in the yield were related to the presence of β-glucan degrading enzymes?
Authors: The viscosity loss of over 80% after incubation at pH 5.0 and 50 °C indirectly suggests that the preparates IIb, IIc and IId contained β-glucanase enzymes. Wood (1986) reports that residual Calcofluor positive material after alkaline extraction is largely removed by the action of a specific endo-β-glucanase. Enzyme-degraded gum is more rapidly solubilized, leading to an increased yield.

S.H. Yiu: Do the authors have any direct evidence to support the suggestion that the molecular weight of β-glucan influences its viscosity?
Authors: It is well known from the polymer science that at a molecular weight exceeding a critical value the low shear rate viscosity of undiluted polymers increases with molecular weight. In our laboratory, we studied the controlled hydrolysis of β-glucan with purified endo-β-glucanase, and observed a dramatic decrease in viscosity as a function of decreased molecular weight.

R. Chinnaswamy: Tables 2 and 4 of your report show that oat bran concentrate and β-glucan concentrate contain proteins and other carbohydrates. In the text, you mention, however, that the viscosity of β-glucan treated with pure endo-β-glucanase was not higher than that of water. Could you comment on this?
Authors: The viscosity measurements were performed in 0.37% β-glucan concentration. In this solution, the concentration of impurities, such as starch and protein, is less than 0.15%, and at this concentration level, the viscosity of the starch or protein water solution is not higher than that of water.

P.I. Wood: IIb, c, d, are evidently unstable at pH 5.0, so what happens during dissolution?
Authors: During dissolution there was no apparent loss of viscosity. Enormous loss of viscosity occurred during incubation at 50 °C.

P.I. Wood: The aleurone of oat is single celled in thickness. In Figs. 7 and 8 the bran is shown to have a multiple-layered aleurone. Could the angle of cut have made the layer appear 2-3 cells thick?
Authors: If the section is cut from the round end of the seed, the aleurone layer will appear two or more cells thick, independent of the angle of section.