Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 96 (2006) 56–65
Food Chemistry
www.elsevier.com/locate/foodchem

The influence of α-amylase-hydrolysed barley starch fractions on the viscosity of low and high purity barley β-glucan concentrates

A. Faraj [a], T. Vasanthan [a,*], R. Hoover [b]

[a] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada T6G 2P5
[b] Department of Biochemistry, Memorial University of Newfoundland, St. John's, Canada A1B 3X9

Received 30 August 2004; received in revised form 27 January 2005; accepted 27 January 2005

Abstract

Gelatinized barley starch was hydrolysed using porcine pancreatic α-amylase for various time intervals and the hydrolysate fractionated according to molecular weight distribution (low, medium and high) by gel permeation chromatography. The effects of hydrolysed starch fractions (2.5%, w/w) on the solution viscosity of low- (~50%, w/w) and high- (~88%, w/w) purity barley β-glucan (0.75%, w/w,) at different temperatures (20 and 37 °C) were determined and compared to that of a control. The results indicated that none of the hydrolysed starch fractions significantly influenced the solution viscosity of high purity β-glucan. However, addition of the medium molecular weight fraction to low purity β-glucan significantly increased its viscosity when determined at low shear rates (1.29–12.9 s^{-1}). Marginal changes in viscosity were observed at shear rates exceeding 12.9 s^{-1}. This study suggested that a non-β-glucan component in the low purity β-glucan concentrate probably influences the solution viscosity of "β-glucan–hydrolysed starch" blends.

Keywords: Barley β-glucan; Starch; Porcine pancreatic α-amylase; Viscosity; Fractionation

1. Introduction

Barley β-glucan is a water-soluble dietary fibre that can form highly viscous aqueous solutions at concentrations as low as 0.5% (w/v) (Autio, Myllymäki, & Mälkki, 1987; Bohm & Kulicke, 1999; Burkus & Temelli, 1999; Cui & Wood, 2000; Doublier & Wood, 1995; Lazaridou, Biliaderis, & Izydorczyk, 2003; Skendi, Biliaderis, Lazaridou, & Izydorczyk, 2003; Vaikousi, Biliaderis, & Izydorczyk, 2004). Research on barley β-glucan has demonstrated its multiple human health benefits (Anderson et al., 1984; Bourdon et al., 1999; Braaten et al., 1994; Hallfrisch & Behall, 2000; Keagy, Knuckles, Yokoyama, Kahlon, & Hudson, 2001; Mälkki & Virtanen, 2001; Wood et al., 1994; Yokoyama et al., 1997). Thus, the industrial demand for this natural cereal based compound is fast growing. Functional food products containing β-glucan are now being commercially introduced to the market (Burkus & Temelli, 2000; Inglett, 1990; Morin, 2001; Pszczola, 1996; Temelli, 2001; Wood, 1986). Since starch is one of the major components of foods, understanding the mechanism of interaction of β-glucan with native starch and its hydrolytic products and its implication for rheological properties is highly important in order to achieve a product with a high sensory appeal.

Studies focussing on the rheological response arising from the interactions between β-glucan and other biopolymers, especially the interaction of β-glucan with starch amylolytic products, are limited. The effect of starch on the viscosity of barley β-glucan gum was investigated by Burkus and Temelli (1998). The authors reported that the viscosity of β-glucan (0.5% w/w) in the

* Corresponding author. Tel.: +1 780 492 2898; fax: +1 780 492 8914.
E-mail address: tv3@ualberta.ca (T. Vasanthan).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.01.056

presence of starch (0.5% w/w) increased by about 2.5-fold. However, the gum mixture separated into two layers following storage at 4 °C. They postulated that the hydrophobicity of the hydrogen-bonded regions of amylose and β-glucan may, in part, be responsible for such destabilization. Carriere and Inglett (1999) investigated the rheological properties of several Oatrim® (β-glucan/amylodextrin) blends. Shear-thickening regions observed in their study were dependent upon the temperature of the suspension and the concentration of β-glucan in the blend. Grimm, Krüger, and Burchard (1995) used rheological methods to investigate the solution properties (behaviour of molecular aggregates) of β-glucan isolated from barley grains and beer. In this study, maltose solutions at different concentrations (2–10%, w/v) were used to simulate a substance that has been found to occur naturally in wort. The above authors reported a decrease in viscosity with increasing maltose concentration (until 5%) and thereafter the viscosity increased with increasing maltose concentration. Using combined static and dynamic light scattering techniques, they also demonstrated that a change (minimum aggregation) in β-glucan structure occurs near 5% maltose. This was attributed to a preferential binding of maltose to β-glucan, which partly breaks up the aggregated β-glucan clusters, as a result of a competitive inhibition mechanism (Grimm et al., 1995; Rees, Morris, Thom, & Madden, 1982).

The effect of barley β-glucan fibre fractions on wheat starch gelatinization and pasting characteristics have been reported by Symons and Brennan (2004). These authors reported that substitution of 5% wheat starch with the barley β-glucan fibre (63.6–73.5% purity) fractions decreased the enthalpy of gelatinization and the Rapid Visco Analyzer parameters (peak viscosity, extent of viscosity breakdown, final viscosity) in comparison to the control starch. They postulated that the reduction in pasting characteristics was due to a decrease in starch granular swelling, resulting from water being withheld from the starch granules by β-glucan and from a general reduction in starch content of the pastes because of replacement with barley β-glucan.

Our previous studies showed that, when a low purity (~50%, w/w) barley β-glucan was mixed into an aqueous solution of gelatinized starch (2.5%, w/w), the viscosity did not develop to an extent equivalent to that of a control solution (β-glucan solution prepared without gelatinized starch at identical concentration) (Faraj, Vasanthan, & Hoover, 2005). Viscosity development of the β-glucan plus gelatinized starch blend solution occurred over a period of time and reached a maximum in approximately 16 h. However, the addition of porcine pancreatic α-amylase into the solution containing β-glucan plus gelatinized starch blend resulted in a rapid viscosity increase with the time of hydrolysis and the viscosity reached a level that was significantly higher than that of a solution containing β-glucan and gelatinized starch (at identical concentrations). This indicated that interaction probably occurs between β-glucan and the products of starch α-amylolysis. Furthermore, the above study showed that complete hydrolysis of β-glucan with lichenase destroyed its ability to interact with the hydrolysed starch products. The objective of the current study was to further understand the mechanism of the aforementioned interaction that was responsible for the enhanced viscosity. The study was performed using both low and high purity β-glucan preparations.

2. Materials and method

2.1. Materials

α-Amylase (EC 3.2.1.1; Porcine pancrease suspension in 2.9 M NaCl containing 3 mM $CaCl_2$), glucose, maltose and maltotriose were purchased from Sigma Chemical Co. (St. Louis, MO). Food grade maltodextrin (Capsul™) was obtained from National Starch and Chemical Company (Bridgewater, NJ). Fungal protease (EC 3.4.21.62; Deerland fungal protease 400,000) was purchased from Deerland Enzymes (Kennesaw, GA). Lichenase (EC 3.2.1.73; from *Bacillus subtilis*) and β-glucosidase (from *Aspergillus niger*) were purchased from Megazyme International (Wicklow, Ireland). A total starch assay kit, that included the enzymes thermostable α-amylase (from *Bacillus licheniformis*) and amyloglucosidase (EC 3.2.1.3; from *Aspergillus niger*), was also purchased from Megazyme International. Termamyl (thermostable α-amylase from *Bacillus licheniformis*) was purchased from Novo Nordisk Biochem, North America Inc. (NC, USA). Sepharose™ CL-6B (in 20% ethanol) was purchased from Amersham Pharmacia Biotech AB (SE-751 84 Uppsala, Sweden). The kit for the determination of total fibre, insoluble and soluble fibre was purchased from Megazyme International (Wicklow, Ireland).

2.2. Isolation and purification of starch and β-glucan from barley flour

Crude (low purity) starch and β-glucan were isolated from pearled hull-less waxy barley flour (CDC-Candle variety, as developed by Dr. Brian Rossnagal at the University of Saskatchewan) according to the procedure of Vasanthan and Temelli (2002). Further purification of crude starch (78%, w/w, purity) and β-glucan (50.7%, w/w, purity) was performed as shown in Fig. 1.

2.3. Chemical analysis

Moisture, lipid and protein contents were determined according to the standard AACC (2000) procedures.

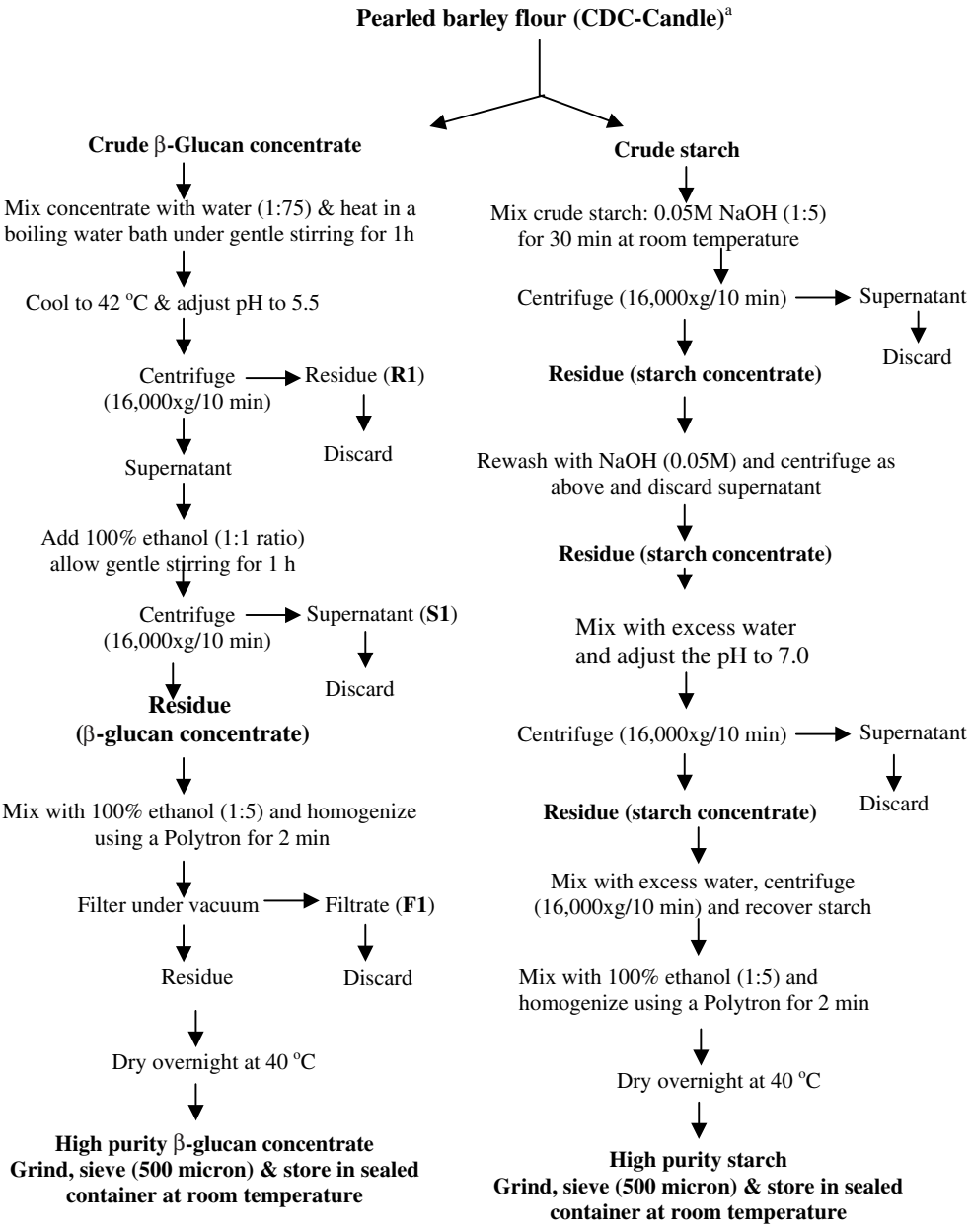

Fig. 1. Isolation and purification of β-glucan and starch. [a]Vasanthan and Temelli (2002).

Total, insoluble and soluble fibres were determined according to the Megazyme procedure provided with their test kits (AACC Method 32-07, 2000). Total starch and β-glucan contents were determined according to the total starch assay (AACC Method 76.13, 2000) and mixed-linkage β-glucan assay (AACC Method 32-23, 2000) procedures provided by Megazyme International Ireland Ltd. (Wicklow, Ireland) with their test kits.

2.4. Fractionation of the hydrolysed products resulting from the action of α-amylase on barley starch

Barley starch (1.0 g) in phosphate buffer (40 ml, pH 9.0) was hydrolysed for different time periods (10 min, 0.5, 1 and 5 h) at 37 °C with porcine pancreatic α-amylase (PPA, 3 U/mg starch). The experiment was carried out in a shaker water bath. At the end of the assay period, the sample was heated in a boiling water bath for 15 min in order to inactivate the enzyme and then cooled to 30 °C. The hydrolysate was centrifuged at (5000*g*) for 10 min, the residue in the centrifuge tube was recovered and freeze-dried. The supernatant was then concentrated in a rotary evaporator to a final volume of about 20 ml. The concentrated sample was chromatographed on a Pharmacia Sepharose™ CL–6B column (2.5 × 75 cm) at room temperature. Degassed MilliQ water was used as the elution medium. Eluent (3 ml) was collected at 6 min intervals using an auto sample collector

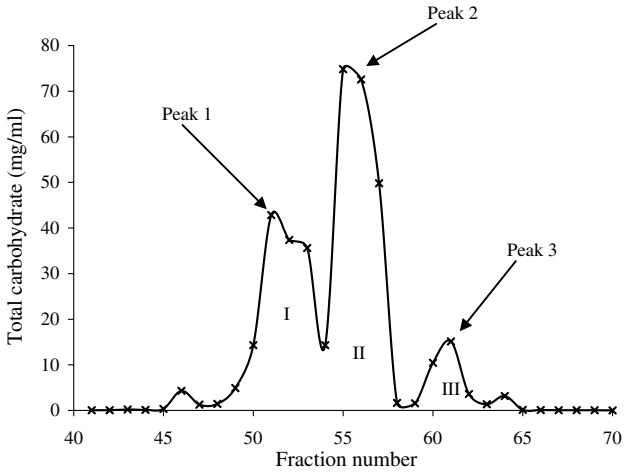

Fig. 2. Gel permeation chromatography of porcine pancreatic α-amylase-hydrolysed barley (CDC-Candle) starch. Peaks 1, 2 and 3 represent the high, medium and low molecular weight fractions, respectively.

and the concentration of total carbohydrates in the fractions was determined using the phenol–sulfuric acid procedure (Dubois, Gilles, Hamilon, Rebers, & Smith, 1956). Contents of the tubes were then pooled according to the peaks identified in the fractions profile (Fig. 2). Three main peaks were identified and numbered 1, 2 and 3, depending on their elution time. Peak 1 represented the fragments with the highest molecular weight, peak 2 contained the medium molecular weight fragments, whereas peak 3 contained the fragments with the lowest molecular weight. The pooled fractions representing each peak were then freeze-dried and stored at room temperature in sealed containers until used.

2.5. Collection and recovery of by-products during further purification of crude β-glucan as in Fig. 1

A study was performed to evaluate the effect of by-product addition on the viscosity of the solution containing purified β-glucan and medium molecular weight fraction (Fig. 2 – peak 2) of hydrolysed barley starch. Crude β-glucan was once again purified as shown in Fig. 1. However, this time, the by-products, [residue (R1), supernatant (S1) and filtrate (F1)] were not discarded. The residue (R1) was mixed with milliQ water (250 ml) and homogenized using a Polytron instrument (Type PT 10-35, Brinkmann Instruments, Westbury, NY) at medium speed for 2 min, frozen immediately in liquid nitrogen and then freeze-dried. The supernatant (S1) and filtrate (F1) were combined (as they both would contain water/aqueous ethanol-soluble components) and subjected to rotary evaporation, frozen in liquid nitrogen and then freeze-dried before storage in sealed containers at room temperature.

2.6. Sample preparation for viscosity determination

Hydrolysed starch fraction (0.2 g) was weighed into a screw capped glass tube, mixed with distilled water (8.0 g) and completely dissolved by heating at 80 °C for ~10–15 min. β-Glucan (60 mg, 0.75%, w/w) was then added to the solution at 80 °C while stirring. When all the β-glucan had dissolved (free of clumps), the solution was cooled to room temperature and left to equilibrate for 1 h. Viscosity measurements were then carried out on the solutions (7.0 g) containing the β-glucan and hydrolysed starch fraction. In a parallel experiment, in order to evaluate the effect of heating, solutions containing the β-glucan and hydrolysed starch fractions were heated (80 °C) while stirring for 15 min and then cooled to room temperature.

For the study, which investigated the effect of adding back the by-products generated during further purification of crude β-glucan (Fig. 1), an amount of each by-product equivalent to the amount that may have been present in the crude β-glucan was accurately weighed and added to the solution containing purified β-glucan (0.75%, w/w) and medium molecular weight fraction of hydrolysed barley starch (2.5%, w/w). The final weight of the solution was 8 g. The solution was then gently stirred for 2 h at room temperature before viscosity determination.

2.7. Determination of viscosity

The viscosities of the solutions prepared under various conditions were determined, using a Universal Dynamic Rheometer, Paar Physica UDS 200 (Mebtecknik

Table 1
Proximate composition (% w/w, db) of crude and high purity barley β-glucan

Material	β-Glucan	Starch	Protein	Lipid	Ash	SDF[a]	IDF[b]
Crude β-glucan concentrate	50.7 ± 0.8	8.6 ± 0.11	5.6 ± 0.01	0.07 ± 0.0	0.43 ± 0.01	51.9 ± 1.2	31.9 ± 1.3
High purity β-glucan concentrate	87.5 ± 1.1	0.97 ± 0.01	1.8 ± 0.0	0.05 ± 0.0	0.32 ± 0.01	89.3 ± 2.1	8.2 ± 1.1

Values are means of duplicate determination ± standard deviation.
[a] SDF, soluble dietary fibre.
[b] IDF, insoluble dietary fibre.

Table 2
The influence of porcine pancreatic α-amylase-hydrolysed (at different time intervals) barley starch fractions[A] on the viscosity of high purity (87.5%, w/w, purity) β-glucan solution (0.75%, w/w)

Time of hydrolysis	Shear rate (1/s)	Viscosity (mPa s)									
		Control[B]		BG + HMWSF[C]		BG + MMWSF[C]		BG + HMWSF[C]		BG + residue[C]	
		20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
10 min	1.29	1070^{a}	583^{e}	1035^{b}	560^{f}	972^{c}	519^{g}	883^{d}	527^{g}	1035^{b}	506^{h}
	12.9	676^{b}	420^{e}	659^{b}	407^{f}	630^{c}	383^{g}	591^{d}	411^{ef}	699^{a}	395^{f}
	129	309^{a}	220^{b}	304^{a}	216^{bc}	296^{a}	208^{c}	288^{a}	197^{cd}	292^{a}	190^{d}
0.5 h	1.29	1070^{a}	583^{e}	969^{c}	476^{g}	958^{c}	464^{g}	1040^{b}	514^{f}	910^{d}	453^{gh}
	12.9	676^{b}	420^{d}	668^{b}	375^{f}	660^{b}	369^{f}	702^{a}	396^{e}	657^{bc}	365^{f}
	129	309^{a}	220^{c}	262^{b}	182^{d}	262^{b}	181^{d}	270^{b}	188^{d}	263^{b}	182^{d}
1 h	1.29	1070^{a}	583^{e}	959^{d}	509^{g}	1040^{b}	552^{f}	IS[D]	IS	999^{c}	485^{h}
	12.9	676^{a}	420^{d}	628^{c}	381^{e}	664^{b}	406^{de}	IS	IS	679^{a}	383^{e}
	129	309^{a}	220^{c}	298^{ab}	209^{c}	310^{a}	219^{c}	IS	IS	265^{b}	184^{d}
5 h	1.29	1070^{a}	583^{e}	1016^{c}	552^{f}	1031^{b}	539^{g}	875^{d}	452^{h}	NS[E]	NS
	12.9	676^{a}	420^{d}	627^{b}	382^{e}	636^{b}	354^{f}	555^{c}	297^{g}	NS	NS
	129	309^{a}	220^{c}	303^{a}	217^{c}	254^{b}	175^{d}	240^{b}	160^{e}	NS	NS
5 h	1.29	1150^{a}	691^{e}	1045^{c}	580^{f}	1110^{b}	590^{f}	1018^{d}	538^{g}	NS	NS
(Heated @ 80 °C)	12.9	754^{a}	512^{d}	636^{c}	384^{f}	684^{b}	395^{e}	630^{c}	352^{g}	NS	NS
	129	281^{a}	221^{c}	256^{b}	176^{e}	266^{b}	218^{d}	257^{b}	176^{e}	NS	NS

The standard deviations of all determinations were within ±10; Viscosity values followed by different superscripts in each row are significantly different ($p < 0.05$) by Tukey's HSD test.

[A] Concentration of starch fraction in the solution was 2.5%, w/w.

[B] Control solution containing β-glucan (0.75%, w/w) plus gelatinized starch (2.5%, w/w).

[C] Test solutions containing β-glucan (0.75%, w/w) and gel chromatographed starch fractions (2.5%, w/w) or residue (2.5%, w/w). HMWSF, MMWSF, LMWSF = high, medium and low molecular weight starch fractions, respectively.

[D] IS, insufficient sample.

[E] NS, no residue.

GmnH Stuttgart, Germany). Six measurements were taken at shear rates of 1.29, 6.45, 12.9, 25.8, 64.5 and 129 s^{-1}, which corresponded to 1, 5, 10, 20, 50 and 100 rpm, respectively. The solutions were weighed (7 g) directly into the rheometer measuring cup. All measurements were made in duplicate and performed mostly at room temperature (20 °C) and in some instances at 37 °C. The temperature of the samples was controlled using a Peltier plate, which enabled the temperature of the viscometer to be controlled to within ±0.1 °C.

2.8. Effects of glucose, maltose and maltodextrins on the viscosity of low and high purity β-glucan concentrates

Robyt and French (1970) and Robyt (1984) have shown that the products of PAA hydrolysis are mainly maltose, maltotriose and maltotetraose. Therefore, in order to further understand the influences of glucose, maltose and low molecular weight dextrins on the solution viscosities of low and high purity β-glucan concentrates, authentic samples (glucose, maltose, maltotriose and maltodextrins) were obtained and investigated according to the same procedures and identical concentrations as that used for the PAA hydrolysed starch fractions.

2.9. Statistical analysis

All trials were performed at least in triplicate and chemical analyses were performed in duplicate. Analysis of variance of the data was performed using the general linear model (GLM) procedure of SAS Statistical Software Version 8 (SAS Institute, 2000). Multiple comparisons of the means were performed using Tukey's test at $P \leqslant 0.05$ level.

3. Results and discussion

3.1. Proximate composition

Most purification protocols for β-glucan (Burkus & Temelli, 1998; Dawkins & Nnanna, 1993; Lazaridou et al., 2003; Vaikousi et al., 2004; Westerlund, Andersson, & Åman, 1993; Wood, Siddiqui, & Paton, 1978) start with grain flour or bran as raw material and employ treatments with thermostable α-amylase and protease, followed by dialysis or alcohol precipitation, to obtain a high purity β-glucan gum concentrate. In this study, the preparation of high purity β-glucan concentrate was carried out using the low purity β-glucan concentrate (as raw material) obtained as described earlier (Vasanthan & Temelli, 2002). The high purity β-glucan thus obtained had a purity of 87.5% (db), as shown in Table 1. Preliminary studies in our laboratory showed that the use of termamyl (a thermostable α-amylase) in the preparation of low purity β-glucan concentrate (50.7%, w/w) resulted in a β-glucan concentrate with low residual starch (~5%, w/w). Also, α-amylase activity in the concentrate was not detected, as the process involved an enzyme inactivation step (boiling the fibre slurry after acidifying to pH 3–3.5 for 15 min). In this study, in order to ensure minimal starch content in the high purity β-glucan, the low purity β-glucan was treated with amyloglucosidase, which can be totally inactivated by a simple heat treatment. The amyloglucosidase treatment resulted in a gum with less than 1% starch. The soluble (SDF) and insoluble (IDF) dietary fibre content of the low purity β-glucan concentrate was 51.9% and 31.9%, respectively. The purification procedure used in this study increased the amount of SDF to 89.3% (w/w), most of which was β-glucan (87.5%, w/w).

Table 3
The influence of PAA-hydrolysed (5 h) starch fractions on the solution[A] viscosity of crude low purity β -glucan concentrate[B]

Shear rate (1/s)	Viscosity (mPa s)							
	Control[C]		BG + HMWSF[D]		BG + MMWSF[D]		BG + HMWSF[D]	
	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
1.29	7520 ± 60^{b}	5970 ± 42^{d}	7960 ± 11^{b}	5770 ± 40^{d}	9840 ± 41^{a}	6840 ± 70^{c}	7920 ± 13^{b}	6040 ± 16^{d}
6.45	3700 ± 27^{b}	2740 ± 18^{d}	3630 ± 10^{b}	2680 ± 20^{d}	4340 ± 40^{a}	3060 ± 10^{c}	3600 ± 35^{b}	2630 ± 34^{d}
12.9	2340 ± 16^{a}	1860 ± 11^{b}	2320 ± 7^{a}	1830 ± 5^{b}	2470 ± 30^{a}	2000 ± 30^{b}	2300 ± 13^{a}	1800 ± 17^{b}
25.6	1410 ± 10^{a}	1180 ± 8^{b}	1420 ± 6^{a}	1170 ± 5^{b}	1470 ± 1^{a}	1240 ± 2^{b}	1410 ± 4^{a}	1160 ± 6^{b}
64.5	710 ± 4^{a}	610 ± 4^{b}	710 ± 3^{a}	600 ± 2^{b}	730 ± 1^{a}	620 ± 1^{b}	710 ± 0^{a}	600 ± 1^{b}
129	410 ± 3^{a}	350 ± 2^{b}	420 ± 2^{a}	360 ± 2^{b}	420 ± 0^{a}	370 ± 1^{b}	420 ± 0^{a}	360 ± 0^{b}

The viscosity values followed by different superscripts in each row are significantly different ($p < 0.05$) by Tukey's HSD test.
[A] The β-glucan concentration of the solution is 0.75%, w/w.
[B] The β-glucan content of the concentrate is 50.7%, w/w.
[C] Control solution containing β-glucan (0.75%, w/w) plus gelatinized starch (2.5%, w/w).
[D] HMWSF, MMWSF, LMWSF = High, medium and low molecular weight starch fractions, respectively.

3.2. Gel permeation chromatography of hydrolysed starch fractions and their interaction with high- and low purity β-glucan

The starch hydrolysates collected at 10 min, 0.5 and 1 h gave a residue upon centrifugation, which was collected separately and freeze dried. However, the hydrolysate collected at 5 h, did not have any residue. The effect of the hydrolysed starch fractions on the viscosity of gelatinized starch plus high purity β-glucan solution is presented in Table 2. The viscosity measurement at 37 °C, and at low shear rates, mimics the viscosity anticipated in the human intestinal tract. While the measurement at 20 °C (generally represents room temperature in many places) mimics the expected viscosity in a food product.

The viscosity of the solutions was higher at 20 °C than at 37 °C (Table 2). This was expected as temperature inversely affects viscosity of most liquid materials (Dawkins & Nnanna, 1995). The viscosity of the samples containing the hydrolysed starch fractions (low, medium and high molecular weights) and/or residue plus high purity β-glucan was generally lower than that of the control which contained gelatinized non-hydrolysed starch plus high purity (87.5%, w/w) β-glucan. In addition, the effects of heating (80 °C for 15 min) of the samples containing high purity (87.5%, w/w) β-glucan and 5 h hydrolysate (no insoluble residue) fractions were investigated. Heating of the samples containing fractions from the 5 h hydrolysate slightly increased the viscosity in all samples but did not influence the above trend, i.e., the viscosity of the samples containing high purity (87.5%, w/w) β-glucan plus 5 h hydrolysate-isolated fractions was still lower than of the control. The small increase in viscosity due to heating may be attributed to the improved solubilization of and interactions among hydrolysed starch and β-glucan molecules. The low and high molecular weight fractions resulting from the action of α-amylase on barley starch for 5 h did not significantly influence the solution viscosity of low purity (50.7%, w/w) β-glucan concentrate (Table 3). However, significant increase in viscosity at low shear rates was observed when the medium molecular weight fraction was added to the low purity (50.7%, w/w) β-glucan concentrate. For instance, at a shear rate of 1.29 s^{-1} the slurry containing the medium molecular weight fraction had viscosities of 6840 and 9840 mPa s at 37 and 20 °C, respectively. The corresponding values for the controls at 37 and 20 °C were 5970 and 7520 mPa s, respectively.

The slight reduction in viscosity upon addition of hydrolysed starch fractions to high-purity (87.5%, w/w) β-glucan (Table 2) may be attributed to the influence of sugar and/or dextrin molecules on network formation, which is essential for viscosity development of β-glucan in aqueous systems. The increase in viscosity

Table 4
The effects of glucose, maltose and dextrins on the viscosities of high purity β-glucan concentrate (0.75%, w/w: 87.51% purity)[A]

Material	Shear rate (1/s)	Viscosity (mPa s)									
		BG control[B]		BG + glucose		BG + maltose		BG + maltotriose		BG + maltodextrins	
		20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
Unheated	1.29	1070^{a}	583^{d}	995^{b}	530^{e}	974^{e}	514^{e}	957^{e}	523^{e}	991^{b}	534^{e}
1% w/w	12.9	676^{a}	420^{d}	654^{b}	351^{de}	641^{bc}	357^{d}	635^{e}	353^{d}	692^{a}	344^{e}
Fractions	129.0	309^{a}	220^{e}	215^{d}	167^{f}	253^{e}	175^{f}	252^{e}	174^{f}	269^{b}	194^{de}
Heated[C]	1.29	1150^{a}	691^{d}	1104^{bc}	641^{f}	1090^{e}	630^{fg}	1080^{bc}	625^{g}	1130^{ab}	660^{e}
1% w/w	12.9	754^{a}	512^{c}	701^{b}	401^{d}	725^{ab}	411^{d}	716^{ab}	406^{d}	740^{a}	400^{d}
Fractions	129.0	281^{a}	221^{b}	275^{a}	189^{b}	275^{a}	192^{b}	272^{a}	190^{b}	295^{a}	197^{b}

Means at the specific temperatures (20 and 37 °C) within a row with different letters are significantly different ($P < 0.05$).
[A] The standard deviations of all determinations were within ±10.
[B] Control solution-β-glucan (0.75%, w/w) plus gelatinized starch (2.5%, w/w).
[C] Heated to 80 °C for 15 min.

(Table 3) in the sample that contained medium molecular weight hydrolysed starch fraction (resulting from the action of α-amylase on barley starch for 5 h) and low-purity (50.7%, w/w) β-glucan suggested that there may be a non-β-glucan component in the low purity (50.7%, w/w) β-glucan concentrate that enhances β-glucan network formation.

3.3. Effects of glucose, maltose and dextrins on the viscosities of low and high purity β-glucan concentrates

The effects of glucose, maltose, maltotriose and maltodextrins on the solution viscosities of high purity β-glucan concentrate are presented in Table 4. The viscosity of high purity β-glucan concentrate decreased on the addition (2.5%, w/w) of glucose, maltose and the dextrins. However, the viscosity decreased only slightly when the control [β-glucan concentrate (0.75%, w/w) plus gelatinized starch (2.5%, w/w)] and the β-glucan concentrate containing the added sugars were heated for a short period of time (80 °C/15 min) and then cooled to room temperature before viscosity measurements.

Autio et al. (1987) and Dawkins and Nnanna (1995) reported an increase in viscosity of aqueous β-glucan solution with addition of sucrose. The sucrose concentrations (25% and 50%, w/v) used in their study were much higher than that used in this study (2.5% w/w). However, at higher sucrose concentrations (>65%), they observed a decrease in viscosity of the β-glucan solutions. Similar effects were reported by Elfak, Navarro, Manzanares, Horta, and Carbonell (1977) for guar and locust bean gum solutions, who suggested that the added sugar at higher polymer concentration restricts the hydration and extension of polymer molecules, causing a decrease in viscosity.

In this study, the viscosity of low purity (50.7%, w/w) β-glucan concentrate increased slightly on the addition (2.5%, w/w) of glucose, maltose and dextrins (Table 5). However, the extent of this increase was not significant (at $P < 0.05$).

3.4. The influence of isolated by-products on the solution viscosity of purified β-glucan in the presence of the medium molecular weight fraction of hydrolysed barley starch

During the purification of crude β-glucan (Fig. 1), the product (pure β-glucan and by-products) recovery, based on the original weight of raw material (crude β-glucan), was 88% (w/w). The yields of purified β-glucan and by-products [residue (R1), supernatant (S1) and filtrate (F1)] were 54.5% (w/w), 26.2% (w/w) and 7.4% (w/w) (supernatant and filtrate were collected together), respectively. The purified β-glucan concentrate had a β-glucan content of 87.5% (w/w). Proportionate amounts of by-products (i.e. 26.2% for R1 and 7.4% for S1 + F1) were added back to the solution containing purified β-glucan and medium molecular weight fraction of 5 h hydrolysed barley starch. The effect of by-product addition on solution viscosity is presented in Table 6.

The control and the test solutions containing the by-product fractions displayed pseudoplastic/shear-thinning behaviour over the shear rates of 1.29–129 s^{-1}. Adding and mixing of the S1 + F1 fraction into the control solution (during its preparation) increased the viscosity (determined at 1.29 s^{-1}) significantly from 1031 to 1865 mPa s. Viscosity of the control solution was not influenced by the addition of R1 fraction. However, a moderate but significant increase in viscosity (from 1110 to 1360 mPa s) was observed when the R1 and S1 + F1 fractions, were added together to the control solution during its preparation. Similar trends were also observed at a shear rate of 12.9 s^{-1}. Interestingly, at a higher shear rate (129 s^{-1}), none of the byproducts influenced the viscosity of the control solution. These results indicated

Table 5
The effects of glucose, maltose and dextrins on the viscosities of low purity β-glucan concentrate (at 0.75%, w/w: 50.7% purity)[a]

Shear rate (1/s)	Viscosity (mPa s)									
	Control[b]		BG + glucose[c]		BG + maltose[c]		BG + maltotriose[c]		BG + maltodextrin[c]	
	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
1.29	7940 ± 6	6270 ± 8	8100 ± 9	6360 ± 10	8160 ± 21	6520 ± 10	8050 ± 14	6250 ± 10	8200 ± 10	6660 ± 10
6.45	3880 ± 3	2860 ± 5	3730 ± 3	2840 ± 2	3790 ± 6	3030 ± 5	3670 ± 28	2870 ± 6	3960 ± 20	3020 ± 5
12.9	2450 ± 2	1940 ± 5	2370 ± 2	1900 ± 1	2520 ± 2	2020 ± 5	2310 ± 2	1870 ± 3	2510 ± 4	2030 ± 4
25.8	1480 ± 1	1230 ± 2	1450 ± 0	1200 ± 2	1530 ± 1	1280 ± 2	1390 ± 6	1170 ± 5	1520 ± 1	1280 ± 5
64.6	730 ± 1	620 ± 1	730 ± 0	620 ± 0	770 ± 0	660 ± 0	700 ± 0	600 ± 5	760 ± 0	650 ± 1
129	430 ± 0	370 ± 0	430 ± 0	370 ± 0	450 ± 0	390 ± 0	410 ± 0	350 ± 0	450 ± 0	390 ± 10

At a given shear rate and specific temperature the viscosities of the interaction between β-glucan and glucose, maltose and dextrins were not significantly different ($P \geqslant 0.05$).

[a] Means of duplicate determinations ± standard deviation.

[b] Control solution containing β-glucan (0.75%, w/w) plus gelatinized starch (2.5%, w/w).

[c] Glucose, maltose and dextrins concentrations – 2.5%, w/w.

Table 6
The influence of by-products isolated during purification of β-glucan[A] on the viscosity of purified β-glucan[B] in the presence of the medium molecular weight PAA-hydrolysed barley (CDC Candle) starch fraction

Shear rate (1/s)	Viscosity (mPa s)			
	By-product fractions[A]			
	Control[C]	Supernatant	Residue	Residue + supernatant
1.29	1031 ± 10[c]	1865 ± 20[a]	1165 ± 20[c]	1360 ± 23[b]
12.9	636 ± 5[e]	795 ± 5[d]	648 ± 8[e]	717 ± 7[d]
129	254 ± 1[f]	262 ± 1[f]	260 ± 2[f]	265 ± 2[f]

Values are means of duplicate determinations ± standard deviations.
[A] See Fig. 1
[B] 0.75%, w/w.
[C] Control = high purity β-glucan concentrate (0.75%, w/w) plus medium molecular weight fraction of 5 h hydrolysed barley starch (2.5%, w/w).

that the third non-β-glucan component that enhances solution viscosity mainly exists in the S1 + F1 fraction of the byproducts. In addition, the viscosity-enhancing property of this component disappeared at shear rates exceeding 12.9 s^{-1}.

Carriere and Inglett (1999) investigated the rheological properties of several β-glucan/amylodextrin blends (Oatrim®). The Oatrim® blend, with 10% β-glucan by weight, exhibited shear-thickening behaviour at 25 °C above a critical shear rate of 30 s^{-1}; however, an Oatrim® with 5% β-glucan did not show any shear-thickening. Furthermore, when the β-glucan content was increased to 24%, two regions of shear-thickening were observed in the Oatrim® blend, with critical shear rates of 30 and 100 s^{-1}. The authors concluded that the shear-thickening regions are due, in part, to a complex interaction between β-glucan and amylodextrin components of Oatrim® at or above a critical level of β-glucan. Their results support our findings that low purity β-glucan interacts with α-amylase hydrolytic products to enhance solution viscosity.

4. Conclusions

The influence of PAA-hydrolysed barley starch fractions (low, medium and high molecular weight) on the viscosity of low and high purity β-glucan was studied. Significant changes in viscosity were not observed when the hydrolysed fractions were added to high purity β-glucan. However, the viscosity of the low purity β-glucan increased significantly in the presence of the medium molecular weight hydrolysed fraction, but remained unchanged in the presence of the low and high molecular weight fractions. This suggested that some non-β-glucan components (removed during further purification) in the low purity β-glucan may have been the causative factor responsible for the increase in viscosity observed on the addition of medium molecular weight hydrolysed starch fraction to low purity β-glucan.

Acknowledgements

This research was funded by the Natural Sciences and Engineering Research Council of Canada and the Alberta Funding Consortium for agricultural research. The Canadian Commonwealth Fellowship and Scholarship programme granted a scholarship to Mr. Abdul Faraj to pursue his doctoral study at the University of Alberta, Canada.

References

AACC. (2000). Approved methods of the AACC. American Association of Cereal Chemists, St. Paul, MN.

Anderson, J. W., Story, L., Seiling, B., Chen, W., Petro, M., & Story, J. (1984). Hypercholesterolemic effects of oat bran or bean intake for hypercholesterolemic men. *American Journal of Clinical Nutrition, 40*, 1146–1155.

Autio, K., Myllymäki, O., & Mälkki, Y. (1987). Flow properties of solutions of oat beta-glucans. *Journal of Food Science, 52*, 1364–1366.

Bohm, N., & Kulicke, W. –M. (1999). Rheological studies of barley (1 → 3) (1 → 4)-B-glucan in concentrated solution: investigation of the viscoelastic flow behaviour in the sol-state. *Carbohydrate Research, 315*, 293–301.

Bourdon, I., Yokoyama, W., Davis, P., Hodson, C., Backus, R., Richter, D., et al. (1999). Postprandial lipid, glucose and cholecystokinin responses in men fed barley pasta enriched with β-glucan. *American Journal of Clinical Nutrition, 69*, 55–63.

Braaten, J. T., Wood, P. J., Scott, F. W., Wolynetz, M. S., Lowe, M. K., Bradley-White, P., et al. (1994). Oat β-glucan reduces serum cholesterol concentration in hypercholesterolemic subjects. *European Journal of Clinical Nutrition, 48*, 465–474.

Burkus, Z., & Temelli, F. (1998). Effect of extraction conditions on yield, composition and viscosity stability of barley β-glucan gum. *Cereal Chemistry, 75*, 805–809.

Burkus, Z., & Temelli, F. (1999). Gelation of barley β-glucan concentrate. *Journal of Food Science, 64*, 198–201.

Burkus, Z., & Temelli, F. (2000). Stabilization of emulsions and foams using barley beta-glucan. *Food Research International, 33*, 27–33.

Carriere, C. J., & Inglett, G. E. (1999). Nonlinear viscoelastic solution properties of oat-based β-glucan/amylodextrin blends. *Carbohydrate Polymers, 40*, 9–16.

Cui, W., & Wood, P. J. (2000). Relationship between structural features, molecular weight and rheological properties of cereal

beta-glucan. In K. Nishinari (Ed.), *Hydrocolloids – Part 1* (pp. 159–168). Amsterdam: Elsevier Science BV.

Dawkins, N. L., & Nnanna, I. A. (1993). Oat gum and β-glucan extraction from oat bran and rolled oats: temperature and pH effects. *Journal of Food Science, 58*, 562–566.

Dawkins, N. L., & Nnanna, I. A. (1995). Studies on oat gum [(1 → 3, 1 → 4)-β-D-glucan]: composition, molecular weight estimation and rheological properties. *Food Hydrocolloids, 9*, 1–7.

Doublier, J. L., & Wood, P. J. (1995). Rheological properties of aqueous solutions of (1 → 3, 1 → 4)-β-D-glucan from oats (*Avena sativa* L.). *Cereal Chemistry, 72*, 335–340.

Dubois, M., Gilles, K. A., Hamilon, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for the determination of sugars and related substances. *Anal. Chem., 28*, 350–356.

Elfak, A. M., Navarro, A., Manzanares, P., Horta, A., & Carbonell, J. V. (1977). The viscosity of dilute solutions of guar gum and locust bean gum with and without added sugars. *Journal of the Science of Food and Agriculture, 28*, 895–899.

Faraj, A. K., Vasanthan, T., & Hoover, R. (2005). The influence of beta-glucan concentration on the in vitro hydrolysis of barley starch by porcine pancreatic α-amylase. Food Chemistry (under review).

Grimm, A., Krüger, E., & Burchard, W. (1995). Solution properties of β-D-(1 → 3) (1 → 4)-glucan isolated from beer. *Carbohydrate Polymers, 27*, 207–214.

Hallfrisch, J., & Behall, K. M. (2000). Mechanisms of the effect of grains on insulin and glucose responses. *Journal of the American College of Nutrition, 19*, 320S–325S.

Inglett, G. E. (1990). USDA's Oatrim replaces fat in many food products. *Food Technology, 44*, 100.

Keagy, P. M., Knuckles, B. E., Yokoyama, W. H., Kahlon, T. S., & Hudson, C. A. (2001). Health-promoting properties of a high beta-glucan fraction. *Nutrition Today, 36*, 121–124.

Lazaridou, A., Biliaderis, C. G., & Izydorczyk, M. S. (2003). Molecular size effects on rheological properties of oat β-glucans in solution and gels. *Food Hydrocolloids, 17*, 693–712.

Mälkki, Y., & Virtanen, E. (2001). Gastrointestinal effects of oat bran and oat gum: A review. *Lebensmittel-Wissenschaft und Technologie, 34*, 337–347.

Morin, L. (2001). Development of a reduced-fat breakfast sausage utilizing barley β-glucan as a fat replacer. M.Sc. Thesis. University of Alberta, Edmonton, AB.

Pszczola, D. E. (1996). Oatrim finds application in fat-free, cholesterol-free milk. *Food Technology, 50*, 80–81.

Rees, D. A., Morris, E. R., Thom, D., & Madden, J. H. (1982). Shapes and interactions of carbohydrate chains. In G. O. Aspinall (Ed.). *The polysaccharides* (vol. 1, pp. 195–290). New York: Academic Press.

Robyt, J. F. (1984). Enzymes in hydrolysis and synthesis of starch. In R. L. BeMiller & E. L. Paschall (Eds.), *Starch chemistry and technology* (pp. 87–124). New York: Academic Press.

Robyt, J. F., & French, D. (1970). The action patterns of porcine pancreatic α-amylase in relation to the substrate site of the enzyme. *Journal of Biological Chemistry, 245*, 1917–1927.

SAS Institute Inc. (2000). SAS/STAT User's Guide, Version 6, forth ed., Vol. 2. Cary, NC: SAS Institute Inc.

Skendi, A., Biliaderis, C. G., Lazaridou, A., & Izydorczyk, M. S. (2003). Structure and rheological properties of water soluble β-glucans from oat cultivars of *Avena sativa* and *Avena bysantina*. *Journal of Cereal Science, 38*, 15–31.

Symons, L. J., & Brennan, C. S. (2004). The effect of barley β-glucan fiber fractions on starch gelatinization and pasting characteristics. *Journal of Food Science, 69*, 257–261.

Temelli, F. (2001). Potential food applications of barley beta-glucan concentrate. *Barley Country, Alberta barley Commission, 10*.

Vaikousi, H., Biliaderis, C. G., & Izydorczyk, M. S. (2004). Solution flow behavior and gelling properties of water-soluble barley (1-3, 1-4)-β-glucans varying in molecular size. *Journal of Cereal Science, 39*, 119–137.

Vasanthan, T., & Temelli, F. (2002). Grain fractionation: methods and production. International Patent No. WO 02/27011 A2.

Westerlund, E., Andersson, R., & Åman, P. (1993). Isolation and chemical characterization of water-soluble mixed-linkage β-glucans and arabinoxylans in oat milling fractions. *Carbohydrate Polymers, 20*, 115–123.

Wood, P. J. (1986). Oat β-glucan: structure, location and properties. In F. H. Webster (Ed.), *Oats: Chemistry and technology* (pp. 121–152). St. Paul, MN: Am. Assoc. Cereal Chemists.

Wood, P. J., Braaten, J. T., Scott, F. W., Riedel, K. D., Wolynetz, M. S., & Collins, M. W. (1994). Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *British Journal of Nutrition, 72*, 731–735.

Wood, P. J., Siddiqui, I. R., & Paton, D. (1978). Extraction of high viscosity gums from oats. *Cereal Chemistry, 55*, 524–533.

Yokoyama, W. H., Hudson, C. A., Knuckles, B. E., Chiu, M. C., Sayre, R. N., Turnlund, J. R., et al. (1997). Effect of barley β-glucan in Durum wheat pasta on human glycemic response. *Cereal Chemistry, 74*, 293–296.