

Effect of Extrusion Cooking on the Primary Structure and Water Solubility of β -Glucans from Regular and Waxy Barley

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ABSTRACT

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Water-soluble β -glucan from native and extrusion-cooked barley flours of two barley cultivars, Candle (a waxy starch barley) and Phoenix (a regular starch barley), was isolated and purified. The purity of β -glucan samples was 85–93% (w/w, dry weight basis) for Candle and 77–86% (w/w, dry weight basis) for Phoenix. The water solubility of β -glucan (at room temperature, 25°C) in the native and extruded flours (primary solubility) was different from that of the purified β -glucan samples (secondary solubility). The solubility of β -glucan in the native and extruded Candle flour was substantially higher than that of β -glucan in Phoenix. For both culti-

vars, β -glucan in the extruded flours had solubility (primary solubility) values higher than in their native counterparts. The solubility of β -glucan in the purified β -glucan samples differed depending on the barley cultivar and the extrusion conditions employed. The glycosidic linkage profiles of purified soluble β -glucan from native and extruded barley flours were determined in order to understand the changes in the primary structure of β -glucan and the effect of extrusion on the β -glucan structure-solubility relationship.

The mixed-linkage (1 \rightarrow 3),(1 \rightarrow 4)- β -D-glucan (β -glucan) is the major structural component in the cell walls of barley grain endosperm. β -Glucan is a component of dietary fiber, and the β -glucan content in barley (3–8%, w/w) depends on the cultivar, location, and environment (Henry 1988, Bhatta 1993). Barley β -glucan has received much attention in the past two decades primarily due to its nutritional significance (McIntosh et al 1991, Martinez et al 1992). Martinez et al (1992) have shown that barley β -glucan has significant blood cholesterol lowering effects. Moreover, barley β -glucan increases the viscosity of digesta in the intestinal tract and thereby slows down the rate of digestion and absorption of starch (Anderson et al 1990), which is beneficial to diabetics (Pick 1994, Gosain 1996). Therefore, research and development of β -glucan-enriched barley-based foods will definitely broaden the use of barley in food industry.

Barley β -glucan has been subjected to extensive investigation in terms of extraction, purification, physicochemical properties, and structure (Woodward et al 1983; Klopfenstein and Hosney 1987; Woodward et al 1988; Bhatta 1993, 1992; Vasanthan and Bhatta 1995; Yoon et al 1995). Some of the major primary structural features of β -glucan are 1) the glycosidic linkage profile (the percent ratio between β 1 \rightarrow 4 and β 1 \rightarrow 3 glycosidic linked glucosyl units); 2) the glycosidic linkage sequence; and 3) the degree of polymerization (DP) of linear (β 1 \rightarrow 4 linked) cellulose-like segments that exist between β 1 \rightarrow 3 glycosidic linkages. Among the glucosyl residues in barley β -glucan, \approx 30% are β 1 \rightarrow 3-linked and 70% are β 1 \rightarrow 4-linked (Woodward et al 1983). The physicochemical properties (i.e., viscosity, solubility, shear stability) of barley β -glucan do not depend much on the degree of polymerization or overall asymmetrical conformation but depend heavily on small differences in the structural (primary) features that influence the ability of the molecules and chains to align into relatively stable molecular aggregates (Woodward et al 1988, Gómez et al 1997a–c). Grimm et al (1995) also reported the formation of molecular aggregates in a solution of β -glucan isolated from beer, and compared that to the fringed micelle structure with side-to-side aggregation of chains. The aggregate potential of barley β -glucan was enhanced at higher temperature, which could lead to a decrease in solubility (Gómez et al 1997a). A study (Gómez et al 1997c) on the flow and viscoelastic behavior of β -glucans with varying molecular weights

suggested that the aggregation, existing in both low and high molecular weight samples, relaxes with shearing.

Extrusion cooking, which usually involves high shear and temperature, is a popular food-processing technique, especially for the production of fiber-rich products (i.e., breakfast cereals, dextrinized or cooked flour). This technology has many advantages such as the versatility, high productivity, low cost, unique product shapes, and high product quality. The use of barley flour in a variety of extruded products is of interest because of the potential human health benefits of this grain. Extrusion cooking technology provides a variety of temperature, moisture, shear, and mixing combinations that transform fiber-rich barley flour into various products. However, extrusion conditions such as high shear, temperature, and moisture may cause compositional, physicochemical, nutritional, and physiological changes in barley flour, especially in the β -glucan fraction. Karin et al (1989) reported that dietary fiber content of barley increased with extrusion cooking. This was accompanied by a decrease in total starch content. It has been reported that extrusion cooking can cause a shift from insoluble fiber to soluble fiber (Asp and Björck 1989). Similar observations in extruded wheat products (Björck et al 1984) and extruded corn meal (Lue et al 1991) were reported. Further studies on extrusion cooking and its effect on barley flour components such as protein, starch, β -glucan, and other dietary fiber components are important. Very little research has been done on cultivar-dependent structural differences in barley β -glucan that lead to variation in its physicochemical and functional properties. Furthermore, studies on the effect of processing, such as extrusion cooking, on the primary structural characteristics, especially the fragmentation or breakage of the glycosidic linkages, and physicochemical properties (i.e. solubility, viscosity) of barley β -glucan are limited. The present study was intended to address some of these research needs and to further enhance our knowledge of barley β -glucan structure-functionality relationships. It was hypothesized that the fragmentation of barley β -glucan during extrusion processing and the resulting changes in solubility will be influenced by extrusion conditions.

MATERIALS AND METHODS

Materials

Waxy barley grains (CDC Candle) were obtained from Agricore, Calgary, AB. Regular barley grains (Phoenix) were obtained from Jim Helm, Alberta Agricultural, Food and Rural Development, Lacombe, AB. Thermostable α -amylase, protease, amyloglucosidase, and the analytical kits for total dietary fiber and β -glucan were purchased from Megazyme International (Wicklow, Ireland). Biogel P-6 was purchased from Bio-Rad Laboratories (Hercules, CA), and

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Sep-Pak C18 cartridges were purchased from Waters Corp. (Milford, MA). Acetonitrile (HPLC-grade) was obtained from Fisher Scientific (Fair Lawn, NJ). Maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6), and maltoheptaose (DP7) were obtained from Sigma Chemical Co. (St. Louis, MO).

Pearling and Milling of Barley Grains and Extrusion Processing of Barley Flour

Pearling and milling of barley grains was conducted at the POS pilot plant (protein, oil, and starch), Saskatoon, SK. Grains were pearled to 32% in a Satake mill (model RMB 10G, Satake, Houston, TX). The pearled grains were pin-milled (Alpine Contraplex wide chamber mill, type A 250, Hosokawa Micron Systems, Summit, NJ) into a flour at 6,000 rpm and a feed rate of 150 kg/hr. The flour was extruded in a twin-screw extruder (Werner and Pfleiderer ZSK 57W 50P, Stuttgart, Germany). The barrel temperature varied from 90 to 140°C (90, 100, 120, and 140°C), and moisture content varied from 20 to 50% (w/w, dry basis) (20, 35, and 50%). The feed rate, screw speed, and L/D ratio of die nozzle were set at 50 lb/hr, 50 rpm, and 20:1, respectively. Extruded samples were dried overnight in a draft oven at 70°C, ground, and screened (60 mesh). The extruder screw arrangement was an orthogonal design.

Isolation and Further Purification of β -Glucan

β -Glucan was extracted from both native and extruded barley flour samples. The detailed procedure for extraction and purification of β -glucan is presented in Fig. 1. Some steps are adapted from the Megazyme procedure for gravimetric total dietary fiber determination.

Barley flour was mixed with buffer; sequentially treated with three enzymes (thermostable α -amylase, protease, and amyloglucosidase); hydrolyzate centrifuged; filtered; concentrated; and passed through a Sep-Pak and a Bio-Gel P-6 column. The content of total carbohydrates in all column chromatographic fractions (elution fraction) was determined using the method of Dubois et al (1956). The fraction profile showed the presence of two major peaks (Fig. 2). The Megazyme method was used to detect β -glucan in the fractions, and it was determined that the first small peak represents β -glucan. There was convincing evidence that the β -glucan peak did not originate from free glucose, which was absent as determined by glucose oxidase-peroxidase method in fractions 10–28. The β -glucan fractions were pooled and freeze-dried.

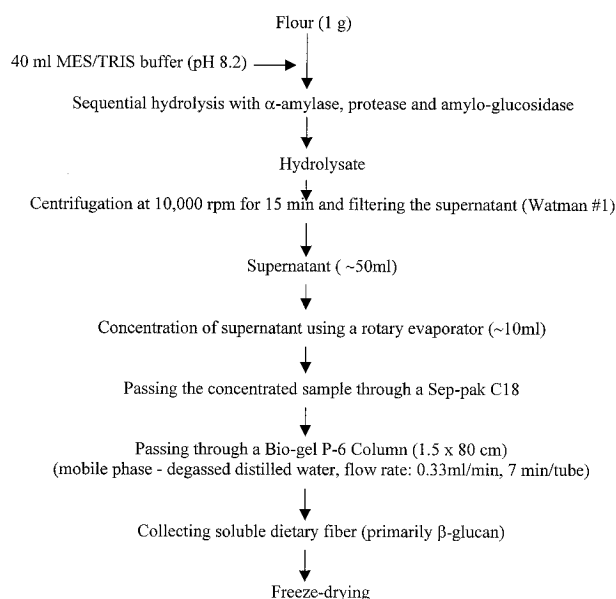


Fig. 1. Schematic representation of the protocol used for isolation and purification of β -glucans.

Purity and Solubility of β -Glucan Samples

The purity of β -glucan samples was determined using the Megazyme method. The solubility of β -glucan (unpurified) in the native and extruded flours (primary solubility) was determined by mixing 100 mg of flour with 10 mL of distilled water and subjecting it to continuous shaking for 2 hr at room temperature (25°C), followed by centrifugation at 10,000 rpm for 5 min. β -Glucan content in an aliquot (0.1 mL) of the supernatant was determined. The solubility was calculated as a percentage of the total β -glucan content in the flour. The solubility of purified β -glucan (secondary solubility) was determined using the same procedure, but the weight of the sample and volume of distilled water added were 10 mg and 1 mL, respectively.

Lichenase Hydrolysis of Purified Native β -Glucan and Determination of Oligosaccharides

Lichenase hydrolysis of purified β -glucans from Candle and Phoenix barley was conducted using a modified assay procedure of mixed-linkage β -glucan (Steps 1–6) (Megazyme International). The hydrolyzate of purified β -glucan was centrifuged at 10,000 rpm for 5 min, and the supernatant was used for oligosaccharide determination.

An HPLC system, equipped with a Shimadzu Ezchrom chromatography data system, a solvent delivery system (Varian 9010, Sunnyvale, CA), a HP Series 1050 autosampler, a polyamine column (250 mm length \times 4.6 mm) (Jordi Gel DV 13 Bellingham, MA), and an evaporative light-scattering detector (Alltech 500 ELSD, Mandel Scientific, Guelph, ON) was used for oligosaccharide determination. Different combinations of distilled water (A) and acetonitrile (B) were used as mobile phases. The solvent gradient used was A 10% (v/v) and B 90% (v/v) at the beginning; A 40% and B 60% after 25 min; A 0% and B 100% after 26 min; A 10% and B 90% after 30 min. The detector temperature was set at 125°C, and a flow rate of 1.0 mL/min was maintained. A series of authentic oligosaccharide mixtures (glucose, maltose, DP3–7) were used for the quantification of oligosaccharides in the barley β -glucan hydrolyzates.

Methylation of Purified β -Glucan of Barley

The glycosidic linkage profile of β -glucan was determined using the methylation-acetylation procedure of Harris et al (1984). The acetylated alditols of methylated sugars were analyzed using a HP 5890 series II gas chromatograph equipment with a J&W Scientific DM-5MS (25 mm \times 30 m) column and a HP 5971A mass selective detector. Ultra high-purity helium was used as the carrier gas. After injection of sample, the oven temperature was maintained at 80°C for 0.5 min and then raised to 230°C at a rate

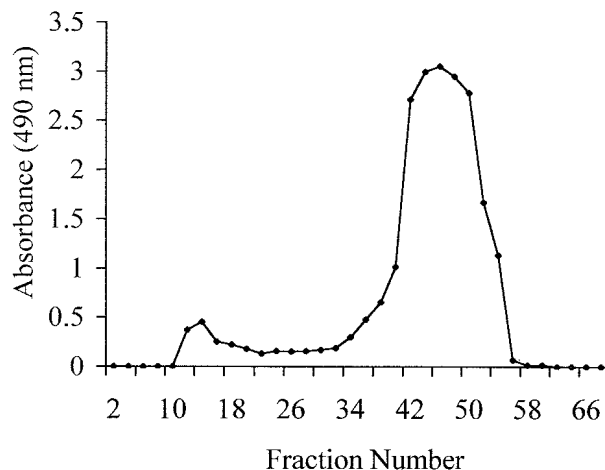


Fig. 2. Chromatography fraction profile of native or extruded barley hydrolyzate. Degassed distilled water eluant with flow rate of 0.33 mL/min (7 min/tube). Total carbohydrate content measured as in Dubois et al (1956).

of 20°C/min. The temperature of the injection port and detector were set at 260 and 280°C, respectively. The total run time was 28 min.

RESULTS AND DISCUSSION

Isolation and Further Purification of β -Glucans

Laboratory isolation of β -glucan has been traditionally conducted using water or aqueous NaOH. Further purification of β -glucan included treatment with heat-stable α -amylase (hydrolyzes starch into maltose and soluble oligosaccharides) and the subsequent use of 95% ethanol to precipitate β -glucan and to wash out the products of starch hydrolysis (Klopfenstein and Hosney 1987, Bhatti 1993). However, the purity of such β -glucan preparations remained inadequate for molecular structural characterization, where there is a need for a complete removal of contaminant starch and protein. This study developed an improved methodology for isolation and further purification of β -glucan from barley (Fig. 1). β -Glucan from native and extruded barley flour was purified by this method, freeze-dried, and used for further investigation. The purified samples were, in fact, water-soluble dietary fiber in which β -glucan was a major component. However, other nonstarch polysaccharides such as pentosans, hemicellulose, and arabinoxylans may be present (Henry 1988, Jadhav et al 1998) in the crude β -glucan fraction.

The content of β -glucan in native and extruded flours of Candle were generally similar but small differences were observed in Phoenix (Table I). Karin et al (1989) reported that the extrusion cooking increased the dietary fiber content of barley products. Lue et al (1991) found that the content of soluble dietary fiber (SDF) in sugar beet samples increased with extrusion cooking. In this

study, the extractability (extraction efficiency) of β -glucan was >80% (w/w, dry basis). The β -glucan content (purity) in purified β -glucan samples was 84–93% (w/w, dry basis) for Candle and 77–86% (w/w, dry basis) for Phoenix.

Effect of Extrusion on β -Glucan Solubility

In this study, the primary solubility refers to the solubility of β -glucan in the native or extruded flours. The solubility of purified β -glucan is referred to as the secondary solubility. The primary and secondary solubilities of native and extruded β -glucan determined at room temperature are presented in Table I. The primary and secondary solubilities of native Candle β -glucan (42 and 48%, respectively) were higher than those of Phoenix (27 and 32%, respectively). For both barley cultivars, the primary solubility of extruded β -glucan was greater than that of the native counterparts. The secondary solubility (at 100, 120, and 140°C) of extruded β -glucan from Candle was lower than that of the native counterpart. It was evident that the solubility decreased with the increasing extrusion-moisture level at each temperature. In contrast, the secondary solubility of extruded Phoenix β -glucan was greater than that of the native counterpart, where the solubility increased with increasing extrusion moisture level at each temperature. In addition, among the extruded samples, the secondary solubility of Candle is substantially lower than the primary solubility. However, an opposite trend was observed for Phoenix.

The differences in the primary and secondary solubilities of native β -glucans from Candle and Phoenix may be attributed to cultivar-dependent primary structural differences. Woodward et al (1988) reported that the solubility of β -glucan (40 and 60°C soluble fractions) was largely dependent on the small differences in the structural features that alter the ability of chains to align into relatively stable molecular aggregates. Woodward and Fincher (1983) reported that irregular spacing of (β 1 \rightarrow 3)-linked β -glucosyl residues in barley β -glucan chains was responsible for the overall irregularity in the conformation of the polymer. The authors further indicated that higher percentages of (β 1 \rightarrow 3)-linked β -glucosyl residues (high irregularity) in barley β -glucan chains reduce its tendency to aggregate, which in turn, enhances the solubility.

Lichenase Hydrolysis of Purified Water-Soluble Native β -Glucan

Lichenase is an enzyme that selectively hydrolyzes the β 1 \rightarrow 4 glycosidic linkages of the 3-*O*-substituted β -glucose of β -glucan. The purified β -glucans (water-soluble) from native flours were hydrolyzed using lichenase, and the resulting segments of linear oligosaccharides were identified and quantified (relative%, Table II). DP3 and DP4 segments made up >90% (w/w, dry basis) of the β -glucan hydrolyzate. The Phoenix β -glucan had nearly 4.5% more DP4 segments than Candle. The relative contents of DP5 and DP6 of both cultivars were generally similar; DP7 was not detected in Phoenix. Wood et al (1994) also reported similar trends in the relative% of oligosaccharides in barley β -glucan. Higher relative% of DP4 in Phoenix β -glucan suggests that the molecules may have a higher tendency for interchain aggregation (hydrogen bonding). These aggregates may influence the water solubility of β -glucan because the hydroxyl groups in the aggregated linear β 1 \rightarrow 4 linked regions are not available for hydration as they are already engaged in hydrogen bonding. Furthermore, it is also plausible that these aggregates would enhance the strength of the region and thereby improve the resistance of β -glucan against process-induced (i.e., extrusion cooking) molecular fragmentation.

The solubility (primary and secondary) of native Phoenix β -glucan was lower than that of Candle (Table I), and the effect could be attributed to a higher content of DP4 linear oligosaccharide in Phoenix (Table II) that would have promoted a higher degree of interchain aggregation. The higher primary and secondary solubilities of extruded Phoenix β -glucan and the high primary solubility

TABLE I
Content and Water Solubility (25°C) of Crude and Purified β -Glucans from Native and Extruded Barley Flour

Cultivar Conditions ^a	β -Glucan Content (% w/w)		β -Glucan Solubility	
	Flour	Purified Sample	Primary ^b	Secondary ^c
Candle				
Native ^d	6.43 \pm 0.11 ^e	86.17 \pm 0.12	41.52 \pm 0.17	47.95 \pm 0.35
Extruded				
90/20	6.63 \pm 0.06	92.03 \pm 0.09	61.92 \pm 1.20	54.05 \pm 1.48
90/35	6.53 \pm 0.02	92.51 \pm 0.15	62.90 \pm 0.92	50.70 \pm 0.57
90/50	6.36 \pm 0.05	84.77 \pm 0.56	76.37 \pm 1.41	48.32 \pm 0.71
100/20	6.61 \pm 0.07	87.00 \pm 0.26	86.68 \pm 1.65	47.55 \pm 0.92
100/35	6.38 \pm 0.09	83.80 \pm 0.58	91.57 \pm 1.32	43.85 \pm 1.48
100/50	6.27 \pm 0.02	93.30 \pm 0.92	94.10 \pm 0.81	41.10 \pm 0.14
120/20	6.57 \pm 0.03	88.26 \pm 1.13	89.50 \pm 1.36	45.55 \pm 0.49
120/35	6.34 \pm 0.00	93.07 \pm 0.35	93.57 \pm 0.94	41.19 \pm 0.78
120/50	6.15 \pm 0.09	87.85 \pm 0.76	95.32 \pm 1.26	40.45 \pm 1.25
140/20	6.38 \pm 0.16	92.02 \pm 0.46	74.94 \pm 1.15	41.15 \pm 0.21
140/35	6.41 \pm 0.15	87.02 \pm 0.68	84.24 \pm 1.41	35.70 \pm 0.75
140/50	6.55 \pm 0.28	88.86 \pm 0.12	89.55 \pm 1.62	25.25 \pm 0.21
Phoenix				
Native	3.87 \pm 0.06	80.17 \pm 0.57	26.84 \pm 0.46	32.00 \pm 0.14
Extruded				
90/20	4.29 \pm 0.09	79.30 \pm 0.43	26.97 \pm 1.01	39.14 \pm 0.43
90/35	4.31 \pm 0.08	77.34 \pm 0.27	28.09 \pm 1.25	42.47 \pm 0.25
90/50	3.85 \pm 0.11	81.24 \pm 0.91	39.33 \pm 0.16	47.34 \pm 0.44
100/20	4.12 \pm 0.01	85.23 \pm 0.32	29.49 \pm 0.30	39.15 \pm 0.57
100/35	3.81 \pm 0.01	79.41 \pm 0.28	32.62 \pm 1.27	43.35 \pm 1.12
100/50	4.06 \pm 0.28	82.36 \pm 0.37	40.03 \pm 1.58	47.34 \pm 0.44
120/20	4.35 \pm 0.02	83.34 \pm 0.46	31.56 \pm 1.51	39.25 \pm 1.05
120/35	4.22 \pm 0.09	85.50 \pm 0.67	33.79 \pm 1.15	46.82 \pm 1.03
120/50	4.18 \pm 0.09	86.08 \pm 0.83	40.96 \pm 1.07	47.07 \pm 0.86
140/20	3.99 \pm 0.05	79.37 \pm 0.25	36.03 \pm 0.98	40.65 \pm 0.65
140/35	3.77 \pm 0.04	81.82 \pm 0.56	38.90 \pm 0.30	46.30 \pm 0.87
140/50	3.60 \pm 0.12	84.44 \pm 0.72	41.05 \pm 1.37	48.12 \pm 0.58

^a Temperature and moisture (°C/%) used to produce extruded barley flour.

^b Solubility of crude β -glucan in native or extruded flour.

^c Solubility of purified β -glucan.

^d Barley flour produced by pin-milling of pearled (30–32%) grains.

^e \pm Standard deviation.

TABLE II
Relative% of Oligosaccharides in Lichenase-Hydrolyzed Water-Soluble β -Glucans^a

Source	DP3	DP4	DP5	DP6	DP7
Candle	60.66 ± 0.69	30.44 ± 0.13	5.18 ± 0.76	2.27 ± 0.44	1.44 ± 0.37
Phoenix	56.74 ± 0.91	34.95 ± 0.31	5.84 ± 0.48	2.47 ± 0.08	nd ^b

^a Means of triplicate determinations (% w/w) ± standard deviation. DP = degrees of polymerization

^b Not detected.

TABLE III
Glycoside Linkage Profile of Purified Barley β -Glucans

Conditions ^a	% (1→)	% (1→3)	% (1→4)
Candle			
Native ^b	2.51 ± 0.06 ^c	28.00 ± 0.29	70.14 ± 0.58
Extruded			
90/20	6.26 ± 0.33	27.04 ± 0.67	66.71 ± 0.35
90/35	4.95 ± 0.04	27.02 ± 0.26	68.04 ± 0.29
90/50	4.35 ± 0.25	26.94 ± 0.49	69.72 ± 0.74
100/20	4.89 ± 0.09	26.76 ± 0.34	67.36 ± 0.25
100/35	6.06 ± 1.19	26.90 ± 0.49	67.06 ± 1.69
100/50	3.73 ± 0.85	26.17 ± 0.62	70.60 ± 0.93
120/20	4.53 ± 0.13	26.98 ± 0.08	66.50 ± 0.06
120/35	3.87 ± 0.21	26.47 ± 0.29	65.67 ± 0.50
120/50	3.70 ± 0.13	25.23 ± 0.38	68.08 ± 0.52
140/20	5.86 ± 0.04	26.53 ± 1.75	65.52 ± 1.70
140/35	6.13 ± 0.05	25.90 ± 0.88	64.98 ± 0.83
140/50	11.85 ± 1.0	21.13 ± 0.13	67.02 ± 0.91
Phoenix			
Native	9.57 ± 0.63	27.63 ± 0.54	62.80 ± 1.20
Extruded			
90/20	5.58 ± 0.18	25.98 ± 0.38	68.45 ± 0.20
90/35	5.91 ± 0.54	25.28 ± 0.92	68.81 ± 0.90
90/50	5.88 ± 0.85	26.82 ± 0.17	67.30 ± 1.00
100/20	5.88 ± 1.12	24.63 ± 0.88	69.49 ± 0.20
100/35	5.21 ± 0.64	25.21 ± 0.70	69.60 ± 1.31
100/50	5.34 ± 0.83	26.42 ± 0.10	68.25 ± 0.73
120/20	6.42 ± 0.91	26.56 ± 0.06	69.02 ± 0.96
120/35	3.49 ± 0.20	26.29 ± 0.25	70.23 ± 0.05
120/50	3.72 ± 0.26	26.01 ± 0.64	70.28 ± 0.38
140/20	5.09 ± 0.13	27.09 ± 0.31	70.82 ± 0.19
140/35	4.69 ± 0.44	26.69 ± 1.34	70.13 ± 0.43
140/50	2.23 ± 0.23	26.26 ± 0.93	71.52 ± 0.71

^a Temperature and moisture (°C/%) used to produce extruded barley flour.

^b Barley flour produced by pin-milling of pearled (30–32%) grains.

^c ± Standard deviation.

of extruded Candle β -glucan as compared with the low solubilities of the native counterparts could be due to extrusion-induced structural changes in β -glucan. One possible explanation would be that the high temperature and shear during extrusion cooking could break the interchain aggregations in β -glucan and enhance solubility. However, the lower secondary solubility of extruded Candle β -glucan was unexpected.

Effect of Extrusion on Glycosidic Linkage Profile of Water-Soluble Barley β -Glucan

Methylation-acetylation of carbohydrates and analysis of the resulting acetylated alditols of methylated sugars using gas chromatography and mass spectroscopy (GC-MS) provide valuable information on the type and content (relative%) of glycosidic linkage. A GC profile of acetylated alditols of methylated sugars from a purified barley β -glucan sample is shown in Fig. 3. A mass selective detector indicated that peaks at 7.89, 8.39, and 8.47 min originated from 2,3,4,6-tetra-*O*-methyl-glucose (represents terminal reducing-end glucose, β 1→), 2,4,6-tri-*O*-methyl-glucose (represents β 1→3 glycosidic linkages), and 2,3,6-tri-*O*-methyl-glucose (represents β 1→4 glycosidic linkages), respectively. Determination of the relative% of glycosidic linkage types provides useful data for the interpretation of some primary structural aspects of β -glucan. For example, a higher percent of β 1→ (terminal) would indicate lower

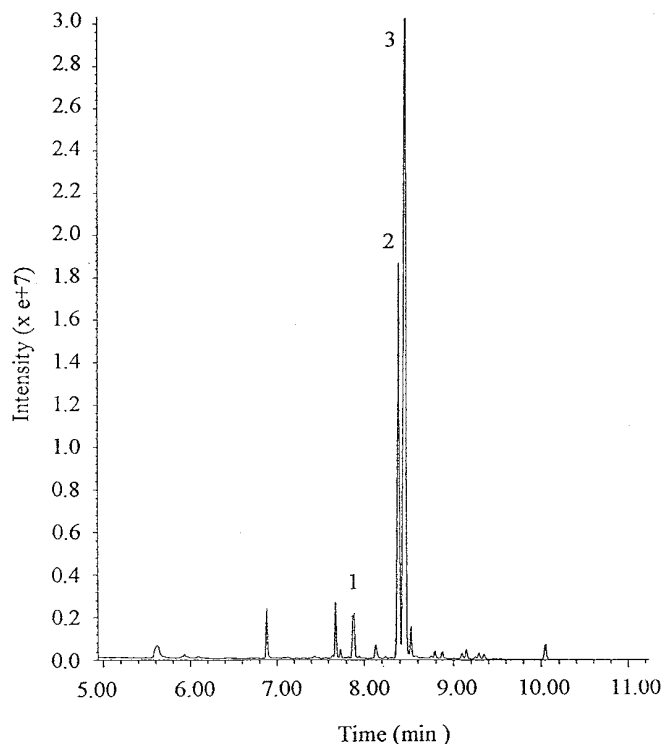
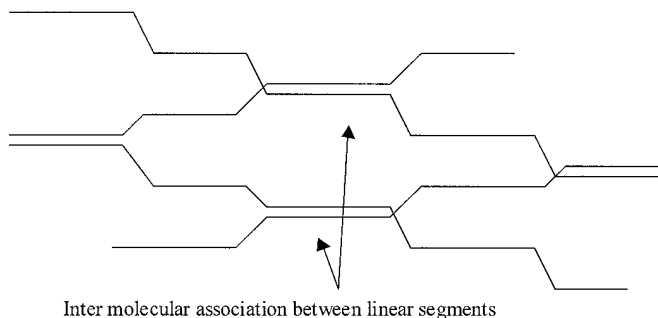


Fig. 3. Gas chromatogram of acetylated alditols of methylated sugars from barley of β -glucans. Peaks 1–3: terminal, reducing-end glucose; β 1→3 linked glucose; β 1→4 linked glucose.

molecular size of β -glucan; a higher percent of β 1→3 linkages would indicate a high number of kinks in the primary structure of β -glucan; and a higher percent of β 1→4 linkages would indicate a high number of linear oligosaccharide segments (Fig. 4). Furthermore, changes in the relative% of glycosidic linkage types could be used to understand the nature of β -glucan fragmentation (whether the fragmentation is random or specific to β 1→3 or β 1→4 linkages) during processing (i.e., extrusion cooking).

Purified β -glucan from native and extruded barley (Candle and Phoenix) flours was subjected to methylation-acetylation analysis to study the extrusion-induced changes in the relative% of glycosidic linkage types. The relative% of glycosidic linkages in native and extruded Candle and Phoenix barley β -glucan is given in Table III. A substantially lower percentage of β 1→ (terminal glucose) in native Candle (2.5%) than in native Phoenix (9.6%) suggests that the molecular size of β -glucan from Candle (waxy barley type) is substantially larger than that of regular (nonwaxy) Phoenix barley. Bengtsson et al (1990) reported that molecular weight and concentration of β -glucan are higher in waxy barley than in non-waxy starch. In the present study, the ratio between the contents of β 1→3 and β 1→4 glycosidic linkages was 1:2.5 for Candle and 1:2.3 for Phoenix. This also indicates that the average length of β 1→4 glycosidic linked linear segments existing between β 1→3 glycosidic linkages is \approx 2–3. This data is in close agreement with Woodward et al (1988), who reported (β 1→3)-glucosyl and (β 1→4)-glucosyl contents of 28 and 72%, respectively, in Clipper barley β -glucan fractions extracted at 40°C.

In general, extruded samples of Candle had a higher percentage of β 1→ content and lower β 1→3 and β 1→4 content than native samples. These trends became highly pronounced at >120°C. The increase in relative% of β 1→ and a simultaneous decrease in the relative% of β 1→3 and β 1→4 suggest that Candle β -glucan has fragmented during extrusion cooking. The extent of β 1→4 depletion was higher in a low moisture environment regardless of the extrusion temperature. The decrease in percentage of β 1→3 was relatively high when extrusion temperatures were \geq 120°C, especially



Inter molecular association between linear segments

Fig. 4. Illustration of β -glucan network, showing interchain aggregations.

at 50% moisture level. The substantially high primary solubility of extruded Candle β -glucan compared with its native counterpart may be attributed to molecular fragmentation during extrusion cooking because in general, the solubility of plant polysaccharides increases with decreasing molecular size. However, despite molecular fragmentation, the lower secondary solubilities of extruded Candle β -glucan processed at $>100^\circ\text{C}$ may be due to the fragmentation of the molecule mostly at $\beta 1\rightarrow 3$ glycosidic linkages (Table III). The resulting improved structural regularity would have enhanced inter-chain aggregation and thereby suppressed solubility.

Phoenix β -glucan appeared to be relatively resistant to extrusion-induced fragmentation. The extruded Phoenix samples had generally similar percentages of $\beta 1\rightarrow 3$ as in the native counterparts. However, the percentage of $\beta 1\rightarrow 4$ decreased and the percentage of $\beta 1\rightarrow 4$ increased with extrusion, suggesting that some changes (other than molecular fragmentation) must have occurred in the aggregated linear segments ($\beta 1\rightarrow 4$ linked) of β -glucan. Further research is required to understand the nature of these changes and to explain the data. A possible explanation is that the methylation-acetylation reaction may be incomplete at the aggregated linear $\beta 1\rightarrow 4$ linked segments (Fig. 4) of native Phoenix β -glucan. These aggregated segments might have separated by the action of high temperature and shear during extrusion cooking.

CONCLUSIONS

Extrusion cooking at higher temperatures and higher moisture levels influenced the water solubility of β -glucan at room temperature. Earlier studies have shown that the human health benefits of barley β -glucan rely on both content and solubility. Therefore, a proper selection of barley cultivars is important in the production of extrusion processed barley-based functional foods to optimize human health benefits. However, more research is needed to investigate the implications of process-induced β -glucan fragmentation on the nutritional quality of barley in human diet.

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