Carbohydrate Polymers 89 (2012) 1238–1243

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Effects of barley and oat β-glucan structures on their rheological and thermal characteristics

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ARTICLE INFO

Article history:
Received 23 December 2011
Received in revised form 20 March 2012
Accepted 9 April 2012
Available online 17 April 2012

Keywords:
Barley
Oat
β-glucan
Lichenase
DP3:DP4 ratio
Solution viscosity
Thermal property

ABSTRACT

In order to understand the relationship between chemical structure and physical properties of cereal β-glucans, the β-glucans with identical M_w (98.4–99.2 kDa) and R_g (21.1–22.0 nm) were isolated from chal and gwangan barley, and ohl oat, and their linkage structure, flow behavior, and thermal properties were investigated. Previously, we established a purification method of 3-*O*-cellobiosyl-glucose (DP3) and 3-*O*-cellotriosyl-glucose (DP4) (Yoo, Lee, Chang, Lee, & Yoo, 2007) and applied these authentic standards to quantify the ratio of β-(1,4)/(1,3) linkages in cereal β-glucans. β-Glucans isolated from two barley cultivars had greater proportion of DP3 than did the oat, and within barley cultivars chal barley β-glucan had significantly larger amount of DP3 over gwangan cultivar. Thus, chal barley β-glucan had the greatest molar ratio (2.53) of DP3 to DP4, and ohl oat had the lowest (1.51). While all the β-glucan solutions showed strong shear thinning behavior, ohl oat β-glucan with higher proportion of DP4 exhibited the highest viscosity among the β-glucan samples. After 3 freeze-thaw cycles of 3% (w/v) β-glucan samples, chal barley β-glucan had lower onset (T_o) and peak (T_p) temperatures (28.3 and 36.7 °C, respectively) than those of gwangan barley (33.6 and 39.9 °C) and ohl oat (37.9 and 46.9 °C) did, and the heat scan profiles were thermoreversible. The T_o and T_p of inter-chain associations decreased as the DP3:DP4 ratio of the β-glucan increased. From this study, it was suggested that cellotetraosyl units and longer β-(1,4)-linked segments would be a major contributor for improving solution viscosity and gel formation of cereal β-glucans.

1. Introduction

Cereal β-glucans are originated from the subaleurone layers and endosperm cell walls of various grains (Bacic & Stone, 1981; Fincher, 1975). They are a linear biopolymer linked by β-(1,3)/(1,4)-glycosidic bonds. More specifically, it is known that the β-(1,4)-linked glucose chain is interrupted with β-(1,3)-linkages in cereal β-glucan structure. Even though most of consecutive β-(1,4)-linked segments are trimers and tetramers, longer cellulose-like segments up to 14-mers are present in the β-glucan molecules (Cui, 2001; Lazaridou & Biliaderis, 2004). Two major oligomer units, 3-*O*-β-cellobiosyl-D-glucose (DP3) and 3-*O*-β-cellotriosyl-D-glucose (DP4), explain more than 90% of cereal β-glucan structure (Lazaridou & Biliaderis, 2004), and it is known that consecutively distributed cellotriosyl units govern its physical and rheological properties. Typically barley β-glucan causes gelation-induced clogging on the filter media of beer brewing process (Bamforth, 1994) and digestion problems when fed to chickens (Hesselman, Elwinger, Nilsson, & Thomke, 1981). Recently, the beneficial physiologic effects, such as reductions in both blood glucose and cholesterol levels, and constipation relief, have been widely reported due to β-glucan's capability to generate high viscosity in the intestinal tract (Cui, Wood, Blackwell, & Nikiforuk, 2000; Tiwari & Cummins, 2009). A great deal of efforts has been made to characterize the rheological properties of β-glucan, but research focus was mainly placed on the rheological changes of β-glucan from various sources under different processing conditions (Ren, Ellis, & Ross-Murphy, 2003; Böhm & Kulicke, 1999a). Thus, it is very important to find out how the β-glucan structure is related to its physical behavior in aqueous environment.

In two or three decades past, random distribution of β-(1,3) was recognized to be responsible for β-glucan's behavior, but more recently the structural regularity has been more accepted to explain it. Elucidation of the exact length and distribution of linear β-(1,4)-linked portion facilitates the understanding of the fine structure of cereal β-glucans. A high-performance anion-exchange chromatography (HPAEC) assisted by lichenase treatment has

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0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.04.025

been widely used for the quantitative structural analysis of cereal β-glucans (Lazaridou & Biliaderis, 2004; Skendi, Biliaderis, Lazaridou, & Izydorczyk, 2003; Wood, Erfle, Teather, Weisz, & Miller, 1994). In most previous studies, they did not provide accurate quantitative structural data due to the absence of authentic standard oligosaccharides, 3-*O*-β-cellobiosyl-D-glucose (DP3) and 3-*O*-β-cellotriosyl-D-glucose (DP4). Recently, two major lichenase-hydrolyzed products were obtained from a barley β-glucan, and the authentic DP3 and DP4 were separated and highly purified using recycling preparative HPLC (Yoo et al., 2007). Using these structurally confirmed oligosaccharides, the exact amounts of β-glucan lichenase hydrolysates from seven barley and oat cultivars were quantified (Ryu et al., 2009).

A few of studies have been reported about the chemical structure and solution behavior of cereal β-glucans, but the precise mechanism of β-glucan chain association has not been well understood yet. Two possible mechanisms have been proposed mainly focusing on the ways of generating junction zones in the aggregation network. A hypothesis involves randomly distributed DP3 and DP4 on the chain structure and cellulose-like intermolecular association by the longer β-(1,4)-linked segments (Bamforth, 1994; Buliga, Brant, & Fincher, 1986; Staudte, Woodward, Fincher, & Stone, 1983). On the other hand, it has been reported that regularly repeated cellotriosyl segments interrupted by β-(1,3)-linkages make stable junction zones formed by intermolecular hydrogen bondings (Izawa, Kano, & Koshino, 1993; Izydorczyk, Jacobs, & Dexter, 2003); Tvaroska, Ogawa, Deslandes, & Marchessault, 1983.

From the result of our previous study (Ryu et al., 2009), we selected three cereal β-glucans that have identical molecular weight distribution but have distinctively different DP3:DP4 ratio. The aims of this study are to chromatographically quantify the DP3:DP4 ratio of these selected cereal β-glucans using authentic β-glucan oligomers, and to relate this key structural information with the rheological and thermal behaviors of concentrated β-glucan dispersions.

2. Materials and methods

2.1. Materials and reagents

Two barley varieties (gwangan and chal) were harvested on June, 2005, and one oat variety (ohl) were harvested on September, 2005, and they were kindly provided from Honam Agricultural Research Institute (HARI), National Institute of Crop Science (NICS), Iksan, Jeonbuk, Korea. Whole cereal samples were milled with a blender (HR2860; Philips, Korea), and the flour was used for the isolation and analysis of β-glucan. A Megazyme β-glucan (mixed linkage) assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) was applied to determine the content and purity of isolated barley and oat β-glucans. Whatman K6 TLC plate (20 cm × 20 cm) was used for the linkage analysis, and 2,3,4,6-tetra-*O*-methyl-D-glucose (Sigma, S960225), β-1,3-glucan from *Euglena gracilis* (Fluka, 89862), and α-cyclodextrin (Sigma, C4642) were used as reference materials. Lichenase (E-LICHN), a β-(1,3)(1,4)-D-glucan-4-glucanohydrolase (E.C. 3.2.1.73), was purchased from the Megazyme International Co., Ltd.

2.2. Large scale extraction of cereal β-glucan

Water-soluble cereal β-glucan was extracted from the milled cereal flour following the modified method of Wood, Paton, & Siddique (1977) and Tosh, Brummer, Wood, Wang, & Weisz (2004). Cereal flour (1 kg) was dispersed in 5 L of distilled water, and its pH was adjusted to 10.0 with 20% (w/v) sodium carbonate. Then, the β-glucan was extracted for 30 min in a shaking incubator at 45 °C with 125 rpm. After the supernatant was recovered by centrifugation at 15,000 × *g* for 15 min, this extraction process was repeated twice, and then the extract was adjusted to pH 4.0 with 2 M HCl for removing proteins as precipitate form. After removing the protein aggregates by centrifugation, the supernatant was treated with final 50% (v/v) of isopropyl alcohol. Again, the supernatant was discarded by centrifugation at 10,000 × *g* for 15 min, and the resulting precipitates were lyophilized and used as a crude β-glucan sample. Complete dispersion of 0.3% (w/v) crude β-glucan was prepared with boiling, which was added the final 30% (w/v) of ammonium sulfate. The precipitates were separated by centrifugation and were dispersed with 50% (v/v) of isopropyl alcohol. This washing step was repeated three times, and the resulting precipitates were used as a purified β-glucan sample after lyophilization.

β-Glucan content of cereal flours and the purity of β-glucan extracts were determined by the AOAC method 995.16 that was adopted by the Megazyme β-glucan (mixed linkage) assay kit with some modifications. Either cereal flour (100 mg) or its β-glucan extract (10 mg) was dispersed in 0.2 mL of 50% (v/v) ethanol, and 4.0 mL of 20 mM sodium phosphate buffer (pH 6.5) was added. The mixture was fully dispersed in a boiling water bath for 3 min and incubated with 10 U of lichenase at 50 °C for 1 h. Sodium acetate buffer (200 mM, pH 4.0) was added to make 10 mL of total volume. After centrifugation at 10,000 × *g* for 10 min, the supernatant (0.1 mL) was incubated with 0.2 U of β-glucosidase at 50 °C for 10 min, and the amount of released glucose was assayed by the Megazyme β-glucan (mixed linkage) assay kit.

2.3. Determination of weight-average molecular weight of β-glucans

The β-glucan solution was obtained by dissolving β-glucan polymers (6 mg) in 1 mL of 0.1 M NaOH and diluted with distilled water (1 mL), followed by neutralization with 1 mL of 0.1 M HCl. The solution was heated in a microwave oven (RE-552W, SamSung, Seoul, Korea) using a microwave bomb (#4782, Parr Instrument Co., Moline, IL, USA) for 30 s. For measuring the *weight*-average molecular weight (M_w) and the radius of gyration (R_g) of β-glucans, the β-glucan solutions were filtered through cellulose acetate membranes (3.0-μm pore size, Whatman International Ltd.), and then injected into the high performance size exclusion chromatography coupled to multi-angle laser light scattering and refractive index detection (HPSEC-MALLS-RI) system. The HPSEC-MALLS-RI system consisted of a pump (model 321, Gilson, Middleton, WI, USA), an injector valve with a 200-μL sample loop (model 7725i, Rheodyne, Rohnert Park, CA, USA), a guard column (HyperGel AP, Thermo Fisher Scientific Inc., Waltham, MA, USA), a SEC column (HyperGel AP50, 7.8 mm × 300 mm, Thermo Fisher Scientific Inc.), a multi-angle laser light scattering detector (HELEOS, Wyatt Technology Corp, Santa Barbara, CA, USA), and a refractive index detector (RI-150, Thermo Electron Corp., Yokohama city, Japan). The aqueous solution of 0.15 M $NaNO_3$ and 0.02% NaN_3 was used as a mobile phase at a flow rate of 0.4 mL/min. The normalization of MALLS detector and the determination of volume delay between MALLS and RI detectors were carried out with bovine serum albumin (BSA). The *dn*/*dc* value was set to 0.145 for β-glucan polymers (Knuckles, Yokoyama, & Chiu, 1997). The M_w and R_g of β-glucan polymers were calculated from the data collected from MALLS and RI detectors using ASTRA 5.3 software.

2.4. Compositional and linkage analysis of barley and oat β-glucans

To determine the sugar composition of isolated β-glucans, TLC analysis was conducted after acid hydrolysis of β-glucan samples. The sample (10 mg) was dissolved with 2 mL of 2 M TFA in a

reaction vial, which was heated for 2 h at 121 °C in a heating block. The hydrolyzate was dried with gentle nitrogen gas flow, and the dried residue was dissolved in water. Aliquots (1–3 μL) were placed on the Merck SI 50000 TLC plate using a micro-pipet; the TLC plate was developed in a glass chamber at room temperature with n-propanol–water–triethylamine–30% ammonia water (80:20:0.2:4, v/v/v/v). The separated sugar components on the air-dried plate in a chemical hood was sprayed with the solution of 0.3 g of *N*-(1-naphthyl)ethylenediamine and 5 mL of concentrated sulfuric acid in 100 mL of methanol. After drying the plate, the black spots were visualized by heating in an oven at 120 °C for 10 min. In order to complete qualitative glycosidic linkage analysis, the β-glucan samples were derivatized and pre-treated by following the Hakomori method (1964). The full description of this methodological procedure was described in our previous study (Yoo et al., 2007).

2.5. Structural analysis of barley and oat β-glucans

Approximately 10 mg of the isolated β-glucan was dissolved in 20 mM sodium phosphate buffer (10 mL, pH 5.0), and the sample solution was incubated with lichenase (8 units) at 40 °C for 1.5 h. The filtrate from the reaction mixture, using a 0.45-μm membrane filter, was directly injected into an HPAEC system (DX300 series, Dionex Corp., Sunnyvale, CA, USA) (Tosh et al., 2004). A Carbopac™ PA1 (4 mm × 20 mm) analytical column was adopted, and two eluents A (150 mM NaOH) and B (150 mM NaOH + 600 mM sodium acetate) were applied at a flow rate of 1.0 mL/min. The proportion of eluent B was linearly changed until it reached 50% from 0.01 to 50 min after either equilibration or regeneration of the HPAEC system for 15 min with 100% eluent A. The potentials (E) and duration time (t) of the PAD (pulsed amperometric detector) was set at $E_1 = 0.05$ V ($t_1 = 300$ ms), $E_2 = 0.6$ V ($t_2 = 120$ mV), and $E_3 = -0.15$ V ($t_3 = 300$ ms). The released amount of oligosaccharides generated by the lichenase treatment was quantitatively analyzed using the standard curves of authentic DP3 and DP4 to identify the linkage structure of β-glucan (Yoo et al., 2007).

2.6. Rheological analysis of cereal β-glucan suspension

The sample suspension was prepared by dispersing cereal β-glucan in distilled water at a concentration of 3% (w/v) for the rheological property test (Lazaridou & Biliaderis, 2004). It was heated at 80 °C for 30 min, cooled down to room temperature, and then transferred to a controlled-stress rheometer (AR1500ex, TA Instruments, DE, USA) with a 40-mm parallel plate. Shear rates in the range of 1–1000 s^{-1} under steady shear conditions were applied and the resulting shear stress was measured at 25 °C. The samples were covered with a thin layer of mineral oil to prevent dehydration during the rheological testing. The rheological curves reported in this study are the mean values of at least two measurements.

2.7. Thermal properties of cereal β-glucans

Thermal properties of β-glucans were analyzed by a differential scanning calorimeter (DSC-200, Netzsch, Germany) that was calibrated with indium (156.6 °C, 28.591 J/g). β-Glucan samples in distilled water (3%, w/w) were prepared and heated at 95 °C for 1 h. They were then stored in a freezer at −20 °C for 24 h and allowed to thaw in a refrigerator at 4 °C for 24 h. After 3 freeze-thaw cycles, β-glucan samples were freeze-dried for 48 h. The lyophilized sample (5 mg) was exactly weighed in the aluminum pan, 38.5 mg of distilled water was added, and the pans were hermetically sealed. After equilibrated for 6 h at room temperature, the samples were heated at a rate of 5 °C/min over a temperature range of 10–100 °C, using an empty reference pan. After the first heat scanning, the sample pan was cooled down to 10 °C with the same rate (5 °C/min), and this heating-cooling cycle was repeated.

Table 1
Extractability and purity of β-glucans from whole grains of selected 3 cereal varieties.

Cereal cultivar	Extractable amount (g/kg)	Purity of β-glucan (%)
Chal barley	21.06	81.1 (3.1)
Gwangan barley	19.21	76.2 (2.5)
Ohl oat	18.76	78.8 (2.0)

Note: The content and purity of β-glucan were determined by using Megazyme β-glucan (mixed linkage) assay kit, AOAC Method 995.16.

2.8. Statistical analysis

Statistical analysis was performed by using the SAS for Window version 8.1. Statistical significance in the difference among the values was evaluated by the Duncan's test. The significance level was $p < 0.05$.

3. Results and discussion

3.1. Large-scale extraction of water-soluble cereal β-glucans

In an effort to determine the inter-relationships between β-glucan structure and its rheological and thermal properties, we chose three cereal β-glucan samples, previously showing distinctly different ratio of DP3:DP4 but having similar molecular weight distribution. Water-extractable β-glucan contents of two barley (chal and gwangan) and one oat varieties (ohl) were 21.06, 19.21, and 18.76 g in 1-kg basis of cereals, respectively (Table 1). The purity of the extracted β-glucans was determined to be 81.1 (chal), 76.2 (gwangan), and 78.8% (ohl). Chal barley had the greatest amount of water-extractable fraction and the greatest purity of β-glucan among the cereal samples tested in this study.

3.2. Molecular weight distribution of barley and oat β-glucans

The molecular weight distribution of cereal β-glucans was previously known to be scattered over a wide range from 2.1×10^4 (rye) to 3.1×10^6 (oat), depending on the methods of isolation and heat treatment, cereal cultivars, and the method of molecular weight determination (Doublier & Wood, 1995; Lazaridou & Biliaderis, 2007; Roubroeks, Andersson, & Aman, 2000). To confirm that the molecular mass and size of selected barley and oat β-glucans were in the nearly identical range, the M_w of the β-glucans extracted from chal and gwangan barley, and ohl oat was measured by using an HPSEC-MALLS-RI system. The *weight*-average M_ws of these cereal β-glucans were within 9.8×10^4 to 9.9×10^4 kDa (Table 2), and the distribution patterns of M_w were almost identical with the polydispersity (M_w/M_n) of 1.72–1.85. Even the molecular size represented by the radius of gyration (R_g) was exactly identical within 21–22 nm.

Table 2
Weight-average molecular weight (M_W), peak molecular weight, and radius of gyration (R_g) of β-glucans measured with HPSEC-MALLS-RI system.

Cereal β-glucans	M_W (kDa)	M_{Wp} (kDa)	R_g (nm)	Polydispersity
Chal barley	98.4 ± 2.8	59.4 ± 0.4	21.9 ± 2.3	1.85 ± 0.04
Gwangan barley	99.2 ± 0.5	61.8 ± 0.6	21.1 ± 3.2	1.74 ± 0.02
Ohl oat	98.1 ± 0.3	61.3 ± 3.3	22.0 ± 1.6	1.72 ± 0.00

Table 3
Quantification of lichenase-hydrolyzates derived from water-extractable cereal β-glucans.

Cereal β-glucans	DP3 (%)	DP4 (%)	Total amount of DP3 + DP4 (%)	Weight ratio of DP3/DP4	Molar ratio of DP3/DP4
Chal barley	55.30 ± 0.35a	28.91 ± 0.71c	84.21 ± 1.06a	1.91 ± 0.02a	2.53 ± 0.02a
Gwangan barley	51.11 ± 0.24b	32.20 ± 0.81b	83.31 ± 1.05ab	1.58 ± 0.02b	2.10 ± 0.02b
Ohl oat	43.53 ± 0.35c	38.03 ± 0.42a	81.56 ± 0.77b	1.14 ± 0.01c	1.51 ± 0.01c

Note: The values within the same column with different letters are significantly different ($P < 0.05$).

3.3. Structural identification of barley and oat β-glucans

In the sugar composition analysis using TLC, it was shown that all the large-scale extracted β-glucans were composed exclusively of glucose (Fig. 1). This result suggested that the isolated β-glucans were not linked by any other types of sugars in their structure. The partial structural information of the purified cereal β-glucans was obtained by thin layer chromatography (TLC) analysis. Compared to the structurally confirmed methyl-derivatized glucoses, only two distinct spots on the TLC plate were identified to be 2,3,6-tri-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose. This result indicated that the isolated β-glucans was exclusively composed of two types of β-(1,3) and β-(1,4) linkages (Fig. 2). From all the information obtained above, it was confirmed that the extracted β-glucans had very similar purity, M_w distribution, and sugar composition. Therefore, the distinguishable difference in their structure would be mainly derived from the proportion of β-(1,3) to β-(1,4) linkages in their glucan chains.

When the lichenase-hydrolyzates of these 3 cereal β-glucans were analyzed using the HPAEC, almost identical chromatographic profiles were obtained from all the samples but the proportion of DP3 and DP4 were quite different among them. Two major peaks on the HPAE chromatogram were clearly identified to be β-(1,3)/(1,4)-linked DP3 and DP4 (data not shown). As shown in Table 3, the β-(1,3)/(1,4)-linked DP3 and DP4 weight fractions of chal and gwangan barleys and ohl oat were in the ranges from 55.30% to 43.53% and from 28.91% to 38.03%, respectively. These two major hydrolyzed products accounted for 81.56–84.21% of total β-glucan content and these values are somewhat lower than expected from the previous study (Ryu et al., 2009). β-Glucans isolated from two barley cultivars had greater proportion of DP3 than did the oat, and even within the same barley crops chal barley β-glucan had significantly larger amount of DP3 over the other gwangan cultivar. It was reported that the PAD signal intensity of 3-*O*-cellobiosyl-glucose was lower than those of malto-DP3 and cello-DP3 in HPAEC analysis (Yoo et al., 2007). This discrepancy in the PAD signal intensity was more obvious when 3-*O*-cellotriosyl-glucose was compared with malto-DP4 and cello-DP4. Thus, the actual amounts of DP3 and DP4 were underestimated when the malto- and cello-oligosacharide-based standard curves were used for quantifying the β-(1,3)/(1,4)-linked DP3 and DP4 produced by lichenase hydrolysis of cereal β-glucans. When we used the standard curves of highly purified authentic β-(1,3)/(1,4)-linked DP3 and DP4, the molar ratio of the DP3 to DP4 were significantly lower for both barley (2.1–2.5) and oat β-glucans (1.5) than the results from the previous studies (Lazaridou & Biliaderis, 2004; Lazaridou & Biliaderis, 2007). From this result, it is obvious that cereal β-glucan structures have more DP4 and even longer β-(1,4)-linked chain segments than previously reported. Skendi et al. (2003) reported that the proportion of DP3 + DP4 accounted for 90.9–92.3% of total oat β-glucan oligomers and the molar ratio of DP3:DP4 was 1.99–2.11. The weight fraction of DP3 + DP4 in barley β-glucan was determined to be 90.9% with the DP3:DP4 ratio of 3.04 (Lazaridou & Biliaderis, 2004). However, in our study, barley and oat β-glucan chains have only 83–84% and 82% of DP3 + DP4, respectively, possibly resulting in their more prominent structural irregularity than expected.

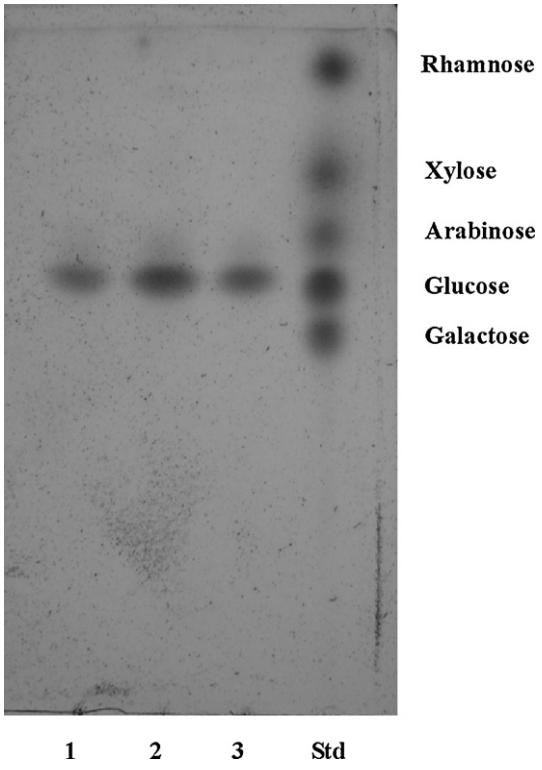

Fig. 1. TLC analysis of acid-hydrolyzed cereal β-glucans for the sugar composition determination. (1) Acid-hydrolyzed ohl oat β-glucan; (2) acid-hydrolyzed chal barley β-glucan and (3) acid-hydrolyzed gwangan barley β-glucan.

3.4. Rheological properties of cereal β-glucan solutions

The flow behaviors of β-glucan suspensions at a concentration of 3% (w/v) were investigated. Fig. 3 exhibits the relationship between

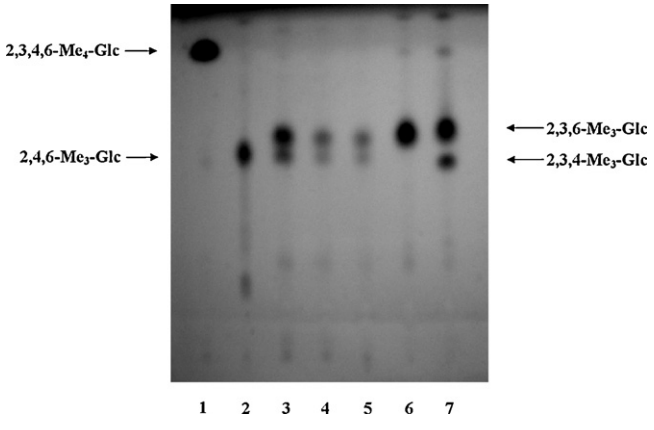

Fig. 2. TLC analysis of methylated and acid-hydrolyzed β-glucans for the glycosidic linkage determination Lane 1, 2,3,4,6-Me_4-glucose; Lane 2, 2,4,6-Me_3-glucose from β-1,3-glucan; Lane 3, methylated and acid-hydrolyzate from chal barely β-glucan; Lane 4, methylated and acid-hydrolyzate from gwangan barley β-glucan; Lane 5, methylated and acid-hydrolyzate from ohl oat β-glucan; Lane 6, 2,3,6-Me_3-glucose from α-CD; Lane 7, 2,3,6-Me_3-glucose and 2,3,4-Me_3-glucose from pullulan.

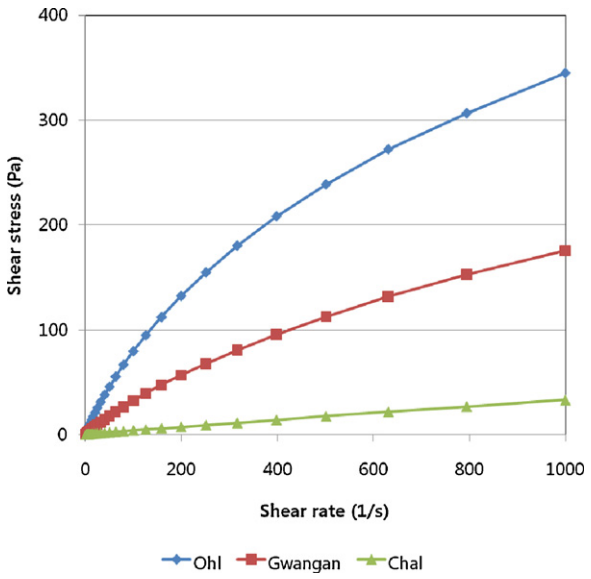

Fig. 3. Flow curves of chal barley, gwangan barley, and ohl oat β-glucan solutions at a concentration of 3% (w/v).

Table 4
Power-law parameters of cereal β-glucan solutions at a concentration of 3% (w/v).

Cereal β-glucans	K	n	R^2
Chal barley	0.06	0.92	1.00
Gwangan barley	1.14	0.73	1.00
Ohl oat	4.02	0.65	1.00

shear stress and shear rate when subjected to steady-shear conditions. While the shear stress appeared to be proportional to the shear rate in chal barley β-glucan, the plots of shear stress versus shear rate were nonlinear for ohl oat and gwangan barley β-glucan solutions, thus showing non-Newtonian behaviors (Bourne, 2002). Specifically, it looked like that they behaved like shear-thinning fluids due to a convex curvature with respect to the shear rate axis. Also, since viscosity is defined as the ratio of shear stress to shear rate, the ohl oat β-glucan solution was shown to have the highest viscosity while the lowest one was observed in the chal barley β-glucan sample. Moreover, the flow curves were satisfactorily fitted to the well-known mathematical relationship between shear stress (σ) and shear rate ($\dot{\gamma}$) which is the Power-law equation (Lee & Inglett, 2007).

$$\sigma = K(\dot{\gamma})^n$$

where K and n are the flow consistency index and flow behavior index, respectively.

As shown in Table 4, the values of the flow behavior index were less than unity ($n < 1$), confirming that all β-glucan solutions exhibited shear-thinning behaviors. The flow behavior index decreased in the following order: chal, gwangan, and ohl β-glucan solutions. Also, the ohl β-glucan had the highest consistency index, followed by gwangan and chal β-glucan solutions. The β-(1,4)-linked segments in a β-glucan structure are regarded as semi-flexible ones while the presence of β-(1,3)-bonds gives rise to kinks in the chain (Izydorczyk, Macri, & MacGregor, 1998; Woodward, Fincher, & Stone, 1983). Therefore, β-glucans with greater ratio of DP4/DP3 appears to have a greater tendency of association since the linearity of polymer chains can give rise to greater molecular interactions. That is, more intermolecular junction zones by the existence of β-(1,4) linkage blocks can cause stronger bonding among the β-glucan chains, consequently providing more resistance against flow and giving high viscosity. Hence, the ohl oat β-glucan containing the high ratio of glycosidic β-(1,4)- over the β-(1,3)-linkages exhibited the highest viscosity over the shear rate range tested in this study while the chal barley β-glucan had the lowest viscosity. Even though Böhm and Kulicke (1999b) proposed that the associated consecutive cellotriose units by β-(1,3)-linkages play an important role in forming extended junction zones, two barley β-glucans may have more irregular and cellulose-like chain segments in the structure than ohl oat β-glucan in our study. The proportion of cellotetraosyl and longer β-(1,4)-linked segments in the β-glucan structure increased in the order of chal barley, gwangan barley, and ohl oat β-glucans aforementioned. Thus, the driving force involved in the β-glucan aggregation may be derived from high content of β-(1,4) linkages rather than structural regularity by repeated cellotriosyl units.

3.5. Thermal properties of cereal β-glucans

Thermal properties of the β-glucans isolated from chal barley, gwangan barley, and ohl oat were investigated and compared each other to find the relationship between the chemical structure and thermal transition behavior. After three freeze-thaw cycles of 3% β-glucans, chal barley β-glucan had lower onset (T_o) and peak (T_p) temperatures (28.3 and 36.7 °C, respectively) than did those of gwangan barley (33.6 and 39.9 °C) and ohl oat (37.9 and 46.9 °C) (Table 5). The T_o and T_p of β-glucan chain associations decreased as the DP3:DP4 ratio of the β-glucan increased. In the second heat scanning, all 3 cereal β-glucans displayed almost identical thermogram profiles and endothermic enthalpy change (ΔH) when compared to those obtained from the first heat scanning. Thus, the gelation of β-glucan chains is thermally reversible and the greater proportion of DP4 than DP3 may contribute on more linear segments on β-glucan backbone, leading to easier β-glucan inter-chain associations. From this study, it could be concluded that β-(1,4) linked linear segments provided by cellotetraosyl units and longer β-(1,4)-linked segments would be a major contributor for the stable gel formation of cereal β-glucans. There was an opposite result obtained from the similar thermal analysis conditions. The ΔH and T_o of cereal β-glucan cryogel increased with increasing the amount of DP3 units (Lazaridou & Biliaderis, 2004). In this same study, the authors also tested the strength of cryogels and showed that the gel strength increased with decreasing DP3. However, lots of factors affecting β-glucan gelation and flow behavior should be considered like cereal cultivar, β-glucan concentration, gelling condition, molecular weight range, and so on. It is not intended

Table 5
Thermal properties of β-glucans after 3 freeze-thaw cycles.

Cereal β-glucans	No. of heat scanning	T_{onset} (°C)	T_{peak} (°C)	T_{end} (°C)	ΔH (J/g)
Chal barley	1	28.3 ± 4.4	36.7 ± 2.5	49.9 ± 1.1	6.2 ± 0.3
	2	29.1 ± 6.3	36.4 ± 5.8	47.1 ± 5.1	6.0 ± 1.1
Gwangan barley	1	33.6 ± 4.4	39.9 ± 2.3	49.2 ± 4.9	4.9 ± 0.6
	2	36.3 ± 0.8	42.4 ± 2.8	52.7 ± 4.1	4.6 ± 1.6
Ohl oat	1	37.9 ± 0.4	46.9 ± 0.8	56.1 ± 1.0	6.1 ± 0.8
	2	37.2 ± 4.1	44.9 ± 3.9	56.1 ± 5.6	5.4 ± 0.7

to directly compare our results with those from other studies. At least we obtained consistent rheological and thermal results, and those data clearly explained each other under our experimental conditions.

Acknowledgments

This study was supported by the BioGreen 21 Program (No. 20070301034008), Rural Development Administration (RDA), Republic of Korea.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.04.025.

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