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Structural characterization and rheological properties of β-D-glucan from hull-less barley (*Hordeum vulgare* L. var. *nudum* Hook. f.)

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ABSTRACT

A high purity of β-D-glucan (80.8%) from hull-less barley (*Hordeum vulgare* L. var. *nudum* Hook. f.) (HBBG) was isolated by alkali extraction and multi-precipitation with ethanol. The molecular weight (Mw) of HBBG was determined as 571.4 kDa with a broad distribution (Mw/Mn = 1.6) by using HPSEC. According to methylation and GC-MS analysis, HBBG was identified to be composed of (1 → 4)- and (1 → 3)-glucopyranosyl (Glc*p*) residues with a ratio of (3.19 ± 0.01). The MALDI-TOF MS and NMR spectroscopy were further conducted to analyze the enzyme hydrolysate released by lichenase digestion on HBBG. The results suggested that HBBG possessed a typical chemical structure of cereal β-D-glucans, namely linear homopolysaccharides formed by β-D-Glc*p* units via (1 → 4)-linkages and occasionally single (1 → 3)-linkage. The trisaccharide and tetrasaccharide of HBBG accounted for 66.6% of total cellulosyl units, accompanying with a ratio of cellotriosyl to cellotetraosyl units = 1.0, which were significant different from those reported for the other cereal β-glucans. Rheological property analysis revealed that HBBG showed a shear-thinning behavior and thermal resilience during heating-cooling process.

1. Introduction

Barley is one of the most ancient and most abundantly cultivated cereal crops. It constitutes 12% of total global cereal production, ranking fourth after wheat, rice and maize (Schulte et al., 2009). Hull-less barley (*Hordeum vulgare* L. var. *nudum* Hook. f. *Poaceae*) describes varieties in which the hull (the inner lemma and outer palea) separates from the grain (caryopsis) during harvest. In Chinese it is referred to as Qingke and it is mainly grown in Qinghai-Tibet Plateau (Du et al., 2014; Tong et al., 2015). In recent years, hull-less barely has attracted increasing attention due to its high content of β-glucan, which is an important water-soluble dietary fiber distributed throughout the whole grain. Consumption of β-glucan confers significant health benefits in humans (Brownlee et al., 2017; Collins et al., 2010). It is reported to aid in alleviation of chronic diseases such as type II diabetes (Tosh and Miller, 2016), and to contribute to protection of intestinal health by showing anti-cancer and anti-inflammatory properties (Aoe et al., 2017; Hussain et al., 2017; Ji et al., 2017; O'Connor et al., 2017; Suchecka et al., 2017). A barley-based food product with high levels of β-glucan is sufficient to satisfy the normal human daily requirement for dietary fiber (Tiwari and Cummins, 2009).

Cereal β-glucans are linear homopolysaccharides constituted of β-D-glucopyranosyl units mainly with (1 → 4) linkages and occasionally with single (1 → 3) linkages. They consist mainly of cellotriosyl and cellotetraosyl units and a minor amount of long β-(1 → 4)-linked cellulosic blocks with 5–14 glucopyranosyl residues (Li et al., 2006). Hull-less barley grains have a higher content of β-glucan (4%–10%) than the other cereal grains (Yang et al., 2014), such as oat (3%–7%) (Skendi et al., 2003), rye (1%–3.4%) (Saastamoinen et al., 1989), and wheat (0.2%–1.2%) (Shen and Jun 2009). The relative contents of cellotriosyl (DP3) units in β-glucans from barley, oat, and wheat grains are of 52–69%, 53–61%, and 67–72%, respectively, accompanying with the cellotetraosyl (DP4) units contents of 25–33%, 34–41%, and 21–24%, respectively. The ratio of DP3 to DP4 is generally considered as the

Abbreviations: DP, degree of polymerization; FT-IR, fourier transform infrared spectroscopy; GC-MS, gas chromatography-mass spectrometry; HBBG, hull-less barley β-glucan; HPSEC, high-performance size exclusion chromatography; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; NMR, nuclear magnetic resonance; PMAA, partially methylated alditol acetate; TFA, trifluoroacetic acid

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structural fingerprint of cereal β-glucans, following the order of wheat (4.2–4.5) > barley (2.8–3.3) > oat (2.0–2.4) (Cui and Wood, 2000). A higher DP3/DP4 ratio is generally associated with a better water solubility and faster gelation tendency (Cui, 2000; Cui and Wood, 2000), since that β-(1 → 3) linkages are able to hinder the crystallization of long cellulosic blocks and endow a good water solubility to β-glucans (Grimm et al., 1995). Additionally, the molecular weight of β-glucan is also crucial to its physical properties and physiological functions by influencing the mobility and possibility to collide each other of β-glucan chains (Böhm and Kulicke, 1999; Cui et al., 2000; Lazaridou et al., 2004; Li et al., 2006; Vaikousi et al., 2004). These solubility, viscosity, and gelling properties of β-glucans make a great contribution to healthy benefits for human, especially reducing glucose and cholesterol absorption by increasing digestive viscosity in the small intestine (Behall et al., 2006; Grundy et al., 2017; Rieder et al., 2017).

It is generally believed that barley β-glucan appears mainly in hulled barley grains. However, β-glucan from hull-less barley flour has been rarely studied till now. The objectives of the present work are to: (1) establish a cost-effective method to improve the extraction and purification efficiency of β-glucan from hull-less barley flour (HBBG), (2) characterize the fine structure and molecular weight of HBBG, and (3) study the rheological properties of HBBG.

2. Results and discussion

2.1. Purity and molecular properties of HBBG

Hull-less barley flour (HBF) was firstly treated with thermal stable α-amylase suspension at 95 °C for 30 min to remove starch, and then extracted with 0.5 M NaOH aqueous solution to obtain raw hemicellulose extract (HBHE). HBHE was then subjected to purification by ethanol precipitation for three times, giving purified β-glucan sample (designated as HBBG) at a yield of 4.0% (w/w, on dry HBF basis). After β-glucan content analysis according to the AOAC Method 991.43, β-glucan content was found 4.9% (w/w, on dry flour basis) for HBF, increasing to 40.8% (w/w, on dry extract basis) for HBHE, and 80.8% (w/w, on dry purified sample basis) for HBBG. Generally, the β-glucan recovery was 82.1% (w/w) from barley flour. In addition to β-glucan, the other components in HBBG determined were moisture (9.6%) and protein (2.1%).

The molecular weight (Mw) and distribution of HBBG were determined by using HPSEC method. The results showed that the Mw, intrinsic viscosity ([η]), and radius of gyration (Rg) were (571.4 ± 0.7) kDa, 3.96 dL/g, and 53.5 nm, respectively, in good agreement with the results of Wu et al. (2017). The reported Mw values for cereal β-glucans vary widely in the range of 150–2500 kDa due to the diversity in biological origin and different extraction methods used (Izydorczyk and Dexter, 2008). The polydispersity index (PDI, Mw/Mn) of 1.6 indicated a relative broad distribution of HBBG.

2.2. Monosaccharide composition analysis

HBBG was firstly hydrolyzed by TFA to obtain the monosaccharide hydrolysate, then derived with 1-phenyl-3-methyl-5-pyrazolone (PMP) before subjected to HPLC system. Seven kinds of neutral monosaccharides and two kinds of uronic acids were used as standards to identify the monosaccharide composition of HBBG. It is indicated that the monosaccharide standards were separated efficiently under the chromatographic condition applied (Fig. 1a). The profile of HBBG hydrolysate (Fig. 1b) revealed a main peak of glucose and two tiny peaks of xylose and arabinose at a molar ratio of 41.3: 1.3: 1.0. This suggests that HBBG was a high purity of glucan, accompanying with trace of arabinoxylan which was composed of xylose and arabinose and usually appeared with β-glucan in the endosperm of cereals. The trace of arabinoxylan in HBBG had been tried to be removed by traditional ammonium sulfate precipitation method (Preece and Hobkirk, 2013) but was unsuccessful.

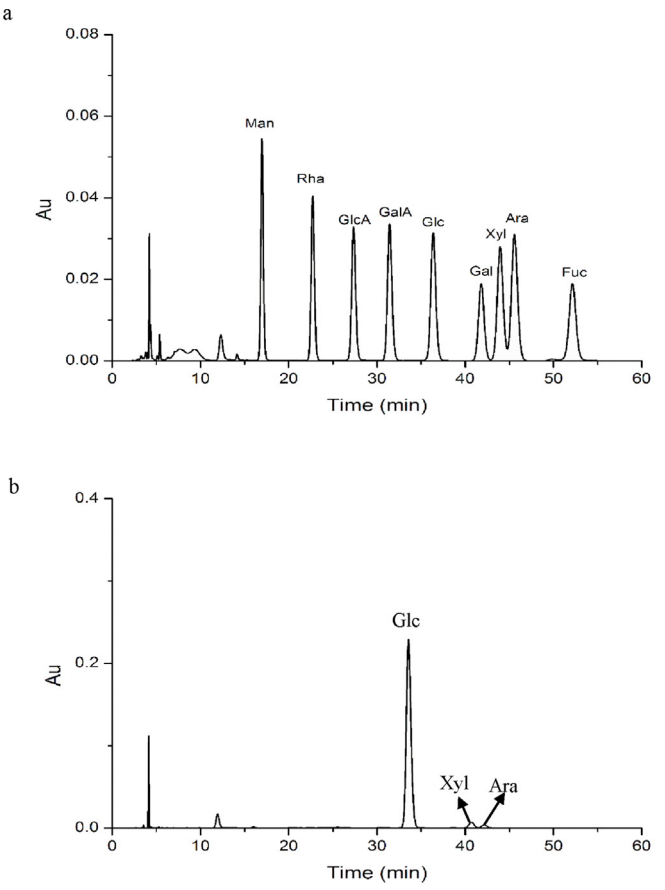

Fig. 1. HPLC Chromatograms of 1-phenyl-3-methyl-5-pyrazolone (PMP) derivatives of monosaccharide standards (a) and HBBG hydrolysate (b). Ara = arabinose; Fuc = fucose; GalA = galacturonic acid; Gal = galactose; Glc = glucose; GlcA = glucuronic acid; Man = mannose; Rha = rhamnose; Xyl = xylose.

2.3. Linkage pattern analysis of HBBG

Analysis of partially methylated alditol acetate (PMAA) by GC-MS showed the presence of 2,3,6-Me_3-Glc*p* and 2,4,6-Me_3-Glc*p* derivatives, pointing to that HBBG was mainly consisted of →3)-Glc*p*-(1→ and →4)-Glc*p*-(1→ residues. According to the peak area percentages of PMAAs, the ratio of (1 → 4)/(1 → 3) linkages was calculated as (3.19 ± 0.01), which was higher than the ratio of oat β-glucan (2.54–2.69) (Wu et al., 2017). This result implies that HBBG possessed a higher amount of β-(1 → 4)-linked cellulosic blocks than did oat β-glucan, possibly due to the differences in cereal biological species and the extraction methods employed.

2.4. Oligosaccharide analysis

Lichenase (EC 3.2.1.73) can digest β-(1 → 4)-glycosidic bonds in 3-*O*-substituted glucose residues specifically in β-glucans, releasing the oligosaccharides hydrolysate with different degrees of polymerization (DP). The resultant hydrolytic fragments of HBBG by lichenase were subjected to MALDI-TOF MS analysis. As shown in Fig. 2, most of the oligosaccharide fragments were trisaccharide (DP3) and tetrasaccharide (DP4), associated with a relative low content of cellulosic block with the DP ranging from 5 to 9. The DP3 and DP4 accounted totally for 66.6% of total cellulosyl units, according to the peak height percentage. The molar ratio of DP3/DP4 was calculated as 1.0, consistent with the results of linkage pattern analysis mentioned above.