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Physicochemical and Health Beneficial Characteristics of β-Glucans from Jeju Barley

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PART Ⅱ.

Physicochemical and Health Beneficial Characteristics of β-Glucans from Jeju Barley

ABSTRACT

The effect of different temperature (45, 55, 65, and 75°C) on the extraction of β-glucan and the properties of extracted β-glucan were investigated with four different varieties of barley. Jeju naked barley, blue barley, and beer barley and black barley contained 6.85, 5.13, 3.58, and 4.16% of β-glucan, respectively. β-Glucan in barley was extracted in the range of 64.88 to 93.84% depending on the extraction temperature and barley variety. The β-glucans in Jeju naked barley, Jeju blue barley, and black barley were highly extracted at 65°C for 3 h and that in Jeju beer barley was at 75°C. The extracted β-glucan resolubilized to 43.48-81.73% and the ratio of β(1→3) to β(1→4) linkage was in the range of 1:3.8-5.8. These results suggest that purification and properties of β-glucan depended on not only extraction temperature of water but also barley variety.

β-Glucan is a soluble dietary fiber from barley. This study investigated the physicochemical properties of different varieties of barley and their β-glucan extracts and evaluated *in vitro* bile acid binding and starch digestibility for health benefits of barley. β-Glucan concentration of less-hulled barley, beer barley, black barley, waxy naked barley, naked barley, and blue barley were 3.44, 3.46, 6.08, 6.75, 6.45, and 5.91%, respectively. Viscosity of waxy naked barley flour was the highest when performed at room and heat temperature. Yield of β-glcuan from less-hulled barley after extraction process was the lowest as 70.09% and waxy naked barley was the highest as 95.46%. The extracted β-glcuan resolubilized to 29.89-56.56% and ratio of β(1→3) to β(1→4) linkage was in the range of 1.71-2.86. *In vitro* bile acid binding of extracted β-glucan was evaluated with cholestyramin, a positive control, and cellulose, a negative control. As the increase of purification, *in vitro* bile acid

binding was increased. In vitro starch digestibility of barley flour and potato starch was increased by heat treatment and the concentration of β-glucan. The glycemic index (GI) was decreased by increasing the amount of β-glucan. These result suggested that the physicochemical properties of barley were dependent on the variety of barley and especially β-glucan was involved to the barley properties and their health benefits.

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PART I

Effect of Temperature on the Extraction of β-Glucan from Different Jeju Barley Varieties

1. Introduction

Barley has mainly been used for malting and feed purpose and offers a natural fiber in food products (Jadhav et al., 1998). Barley is a rich source for carbohydrates like starch, cellulose, and β-glucan (McClear & Holmes, 1985). Barley β-glucan, a relatively minor fraction (4-7%) in total carbohydrate, has unique properties with both nutritional and technological significance because of their high molecular weight and water solubility (Wood, 2007). β-Glucans are composed of glucoses with β(1→3) and β(1→4) mixed linkage in ratio of about 2.3-3.0 to 1.0 (Stone & Clarke, 1992). β-Glucans from different types of cereals showed the similar molecular structure but exhibited variation in the ratios of β(1→4) to β(1→3) linkages and molecular weights (Autio, 1998). With the structural characteristics, β-glucan can impart high in viscosity to aqueous solution (Izydorczyk et al., 1998) and nutritional effects (Wood et al., 1991). The health benefits of β-glucans include the increase in fecal bulk, the ability of relieve constipation, the reduction of plasma cholesterol, and the control of postprandial serum glucose level in humans (Wood, 2007.; Stone & Clarke, 1992).

About 75% of β-glucans are found in the barley endosperm cell wall and distributed uniformly throughout the endosperm (Miller & Fulcher, 1994). The β-glucans in the endosperm cell wall may be covalently bonded to protein forming large molecules (Baik & Steven, 2008). The extraction and

purification of β-glucans in barley can be affected by flour particle size, temperature, pH, and ionic strength (Wood et al., 1978). The extraction method mostly used is involved in following steps: 1) inactivation of endogenous enzyme, 2) extraction with water or alkali solutions, 3) removal of contaminating protein and starch using hydrolytic enzyme and/or selective adsorption, and 4) precipitation of β-glucans from the purified solutions with alcohol and freeze drying or alternatively drum or spray drying of the extracts (Charles & Louise, 2005.; Izysorczyk et al., 2000). As the steps for extraction were added, the purification of β-glucans were improved (Wood et al., 1978.; Charles & Louise, 2005). Mostly, β-glucan purification was dependent on temperature of solvent and duration of extraction (Izysorczyk et al., 2000).

In this study, the objective was to investigate the effect of temperature on the extraction of β-glucan in different varieties of barley grown in Jeju along with the physicochemical properties of the extracted β-glucans.

2. Materials and Methods

2.1. Materials

Four different varieties of barley were selected for this study. Naked barley (Erie36ho), blue barley (Iksan479ho), and beer barley (Iksan173ho) grown in Jeju were obtained from a local market (Jeju, Korea). Black barley (Iksan100ho) grown in Goesan (Chungbuk, Korea) was purchased in a local market (Jeju, Korea). Dehulled barley grains were ground into flour with a mill (MF10, Ika-Werke GMBH & Co., Staufen, Germany) through a 100 mesh screen.

2.2. Chemical composition of barley flours

AACC method (AOAC, 1995.; AACC, 2000). Moisture content of barley flours was measured with a moisture analyzer (MX-50, AND Ltd. Co., Tokyo, Japan) at 105°C. Crude protein, fat, and ash were determined by Kjeldahl method, Soxhlet extractor, and ashing incineration. Contents of β-glucan, starch, and total dietary fiber were analyzed by following AACC method 32-23.01 and 76.13, and AOAC method 991.43 using assay kits (Megazyme International Co., Wicklow, Ireland).

2.3. Viscosity of barley suspension

The viscosity of barley suspension was determined by modification of the procedure of Jeong et al. (2004). Barley flour suspension was prepared by 1:8, barley flour to water mass ratio and the viscosity was determined by a viscosity analyzer (DV-I, Brookfield Co., Middleboro, MA, USA). A stirring

speed was 100 rpm, spindle No.2 was used at room temperature (22±1°C), and No.4 (black barley, Jeju beer barley), No.5 (Jeju naked barley), and No.6 (blue barley) were used at 75°C for heating.

2.4. Extraction of β-glucan

β-Glucan was extracted from barley using the procedure of Kim & White, (2010) with modifications. Barley flour (15.0 g) was suspended in 150 mL of 82% ethanol and refluxed to remove fat and inactive endogenous enzyme at 80°C for 3 h. Barley suspension was then centrifuged at 3,100 × g for 20 min to remove supernatant. Precipitate was washed with 50 mL of 95% ethanol, centrifuged at 3,100 x g for 15 min twice, and dried at 40°C for overnight. β-Glucan was extracted from the refluxed and dried flour (about 12.0 g) by using 120 mL distilled water at 45 and 55°C, and using 240 mL distilled water at 65 and 75°C for 3 h to optimize extraction temperature. After extraction, suspension was centrifuged at 3,100 × g for 20 min and the process was repeated for two more time. The supernatant was treated with 0.2 mL α-amylase (Sigma-Aldrich, St. Louis, MO, USA) and 36-40 mg calcium chloride (Sigma-Aldrich) in shaking water bath (JSSB-30T, JS Research Inc., Gongju, Korea) at 90°C for 2 h and centrifuged at 3,100 x g for 20 min. The supernatant was then reacted with 15 mg pancreatin (Sigma-Aldrich) and 0.2 mL 10% sodium azide (Sigma-Aldrich) at 40°C for 3 h. Reactant was added with 2-fold 60% ethanol and stayed overnight at 4°C and centrifuged at 3,100 × g for 20 min. The precipitate was freeze dried. Total β-glucan contents of freeze-dried β-glucan were measured for the calculation of extraction yield.

2.5. Resolubility of β-glucans extracted from barley

Resolubility of the extracted β-glucan in water was measured to analyze the physical properties of β-glucan using the method of Lee et al.(2012) with modifications. The β-glucan dispersion in water (1%, w/v) was agitated at 50°C for 12 h and centrifuged at 12,000 x g for 10 min. Precipitate was dried and calculated as the percentage(%) = (weight of β-glucan dissolved in the supernatant/initial weight of β-glucan in the dispersion) x 100.

2.6. Ratio of β(1→3) to β(1→4) linkages in extracted β-glucans

Ratio of β(1→3) to β(1→4) linkages was determined to compare the structure of extracted β-glucan (Woodward et al., 1980). Content of binding β (1→3) glucoses was determined as following: β-glucan (2.0 mg) was added to 1 mL sodium phosphate buffer (20 mM, pH 6.5) with 4U lichenase (Megazyme International Ltd, Co., Wicklow, Ireland) which divided the β(1→ 4) linkage of 3-O-substituted glucose residues in β-glucan at 40°C for 22 h. Reactant was added to 5 mL sodium acetate buffer (200 mM, pH 4.0) and centrifuged at 2,000 x g for 10 min. After obtained 0.1 mL supernatant, content of total glucose was determined as follows: β-glucans (2.0 mg) was added to 1 mL sodium phosphate buffer (20 mM, pH 6.5) with 4U lichenase (Megazyme International Co., Wicklow, Ireland) and incubated at 40°C for 22 h. Reactant was treated with β-glucosidase (0.2U, Megazyme International Ltd, Co., Wicklow, Ireland) at 50°C for 10 min. After the obtained 0.1 mL suspension, glucose content was determined. The β(1→4) linked glucoses was calculated by subtracting β(1→3) linked glucoses from total glucoses and ratio of β(1→3) to β(1→4) linkages was calculated as the amounts of β(1→4) linked glucose / β(1→3) linked glucose.

2.7. Statistical analysis

All analyses were done in triplicate. Data were analyzed by the analysis of variance (ANOVA), followed by the Duncan's multiple range test ($p<0.05$) using statistical soft ware SPSS (Statistics Package for the Social Science, Ver. 18.0, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Chemical composition and viscosity of barley flour

Chemical composition including crude protein, fat, ash, starch, total dietary fiber, and β-glucan contents of four different varieties of barley are shown in Table 1. Protein concentrations of black barley and Jeju naked barley were greater than those of Jeju blue and beer barley ($p<0.05$) but these values were within the range of common barley reported as 4-9% (Baik & Steven, 2008). Fat and ash contents of the four different barley varieties were not different from each other. Starch contents of four different barley varieties ranged from 72.86 to 77.44%. According to Lee (1992), total dietary fiber content of naked barley was 9-10% which were similar result with the current study. Total dietary fiber content of Jeju naked barley contained great amount as 10.55%. Other three varieties of barley contained 5.69, 7.41, and 7.53% of total dietary fiber. Significant differences were observed in the total β-glucan content among four varieties of barley. Jeju naked barley contained 6.85% β-glucan, which is greater than black barley and Jeju blue and beer barley. The concentration of β-glucan was positively related to the amounts of total dietary fiber. Jeju beer barley typically used for beer brewing was the lowest content of β-glucan as 3.58%. The barley for beer brewing is genetically modified to reduce the content of β-glucan which causes a problem like cloudiness of beer (Bamforth, 1982). MacGregor & Fincher (1993) reported a 4.0-7.0% of β-glucan in barley. And Batty (1993) reported the chemical composition of barley like protein 12.7%, fat 2.5%, ash 1.8%, starch 74%, total dietary fiber 8.7%, and β-glucan 4.5% which were similar result with this study.

Viscosity is one of the most important physical characteristics of food

components affecting their functionality in the food system (Izysorczyk, 2000). The viscosity of barley suspension was measured at room temperature and heating temperature and those are in Table 1. The viscosity of Jeju naked, Jeju blue barley, Jeju beer barley, and black barley were 39.70, 33.00, 22.62, and 21.10 cP at room temperature, respectively. The viscosity of Jeju naked barley, which β-glucan was the highest as 6.85%, was the highest. Aastrup (1979) reported that viscosity was highly correlated with the extractable β-glucan contents. The highest concentrations of total dietary fiber and β-glucan in Jeju naked barley appeared the highest viscosity at room temperature. In addition, viscosity of Jeju naked, Jeju blue, Jeju beer barley, and black barley suspensions increased after heating 75°C. Viscosity of barley suspension increased with heating temperature and this was possibly related to starch gelatinization (McCleary, 1988). According to Izydorczyk et al.(2000) reported, a β-glucan degrading enzyme, like lichenase and β-glucanase, caused an immediate decline in viscosity before α-amylase and protease worked. β-Glucan degradation directly affected the viscosity of barley suspension.

Table 1. Chemical composition[1)] and viscosity of barley flours

Barley	Protein (%)	Fat (%)	Ash (%)	Starch (%)	Total dietary fiber (%)	β-glucan (%)	Viscosity (cP) at room temperature	Viscosity (cP) at heating
Jeju naked barley	12.81±0.09[a]	2.43±0.16[a]	1.14±0.09[ab]	77.44±1.62[a]	10.55±1.21[a]	6.85±0.40[a]	39.70±0.71[a]	433.00±12.73[b]
Jeju blue barley	7.70±0.07[d]	2.45±0.17[a]	1.29±0.21[ab]	72.86±3.18[a]	7.53±2.09[b]	5.13±0.37[b]	33.00±0.28[b]	1116.00±8.49[a]
Jeju beer barley	9.10±0.08[c]	2.46±0.06[a]	1.05±0.12[b]	73.73±0.96[a]	5.69±0.59[b]	3.58±0.38[c]	22.62±0.28[c]	85.50±1.84[c]
Black barley	12.00±0.09[b]	2.11±0.21[b]	1.34±0.09[a]	73.53±2.76[a]	7.41±0.84[b]	4.16±0.25[c]	21.10±0.42[d]	75.00±0.28[c]

[1)] All chemical contents were calculated as dry weight basis, %.

[2)] Each value is mean±standard deviation.

[3)] Means with the different letter in a column indicate significant difference ($p<0.05$) by Duncan's multiple range test.

3.2. Effect of temperature on β-glucan extraction

To obtain highly purified β-glucan, barley was extracted with water at 45, 55, 65, and 75°C and the concentration of β-glucans ranged from 64.88 to 93.84% after extraction (Fig. 1). As the extraction temperature increased from 45 to 65°C, yields of purified β-glucans were increased. Especially, the yield of β-glucan extracted from Jeju naked barley was 93.84% when extracted at 65°C and Jeju beer barley extracted highly with 91.15% of β-glucan when extracted at 75°C. These results indicated that concentration of purified β-glucans depended on variety of barley and extraction temperature. Wood et al.(1978) reported a similar increase in extraction yield from oat flour with temperature when the starch gelatinization commenced above 63°C and they concluded that the optimum extraction temperature to avoid contamination with starch was 45°C. However, McCleary (1988) showed that sequential water extraction at 40, 65, and 95°C increased the extraction rate of barley β-glucans to 90% and minimized the contamination from starch. Also, Saunier et al. (1994) used a hot water extraction procedure in the presence of thermostable a-amylase to remove starch. In this study, β-glucan of Jeju naked barley was properly separated from gelatinized starch which was effectively removed by α-amylase and centrifuge process so that obtaining highly purified β-glucan.

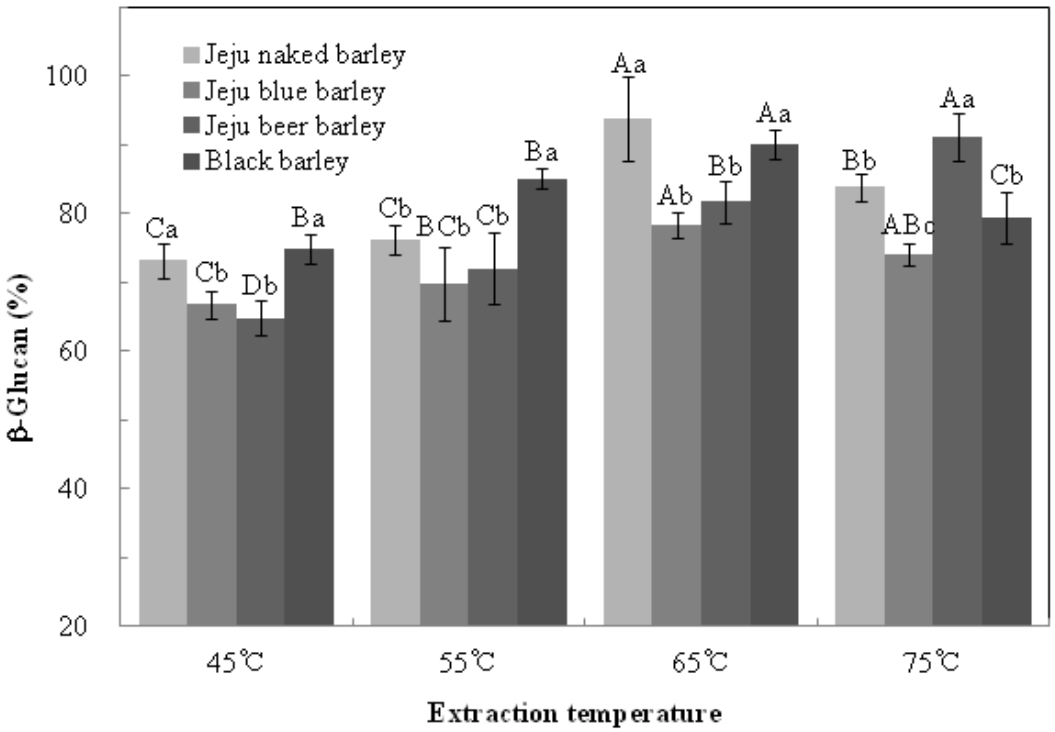

Fig. 1. β-Glucan concentration after extraction of different barley varieties at 45, 55, 65, and 75°C. Data are expressed as mean±standard deviation. Means with different capital letters on the bars indicate significant difference ($p<0.05$) within the same barley variety and means with small letters indicate significant difference ($p<0.05$) within the same extraction temperature by Duncan's multiple range test.

3.3. Resolubility and ratio of β(1→3) to β(1→4) linkages in β-glucan extract

Resolubility of the extracted β-glucan is shown in Fig. 2(A). Resolubility of the β-glucans extracted from Jeju naked and blue barley at 45 and 65°C were the greatest. Resolubility of Jeju beer barley β-glucan extracted at 65°C was the highest as 81.73%. Resolubility of β-glucan extracted from black barley at 45°C was 73.14%. When extracted at high temperature, the resolubility of β-glucan was getting low. The β-glucan with high molecular weight had a low diffusion rate in solution (Buliga et al., 1986) so the β-glucan with high resolubility possibly contained low molecular weight β-glucan. Resolubility was also influenced by the ratio of β(1→3): β(1→4) linkages (Autio, 1996). Since the β(1→3) linkage broke up the regularity of the β(1→4) linkage in β-glucan molecules, the molecule could be more soluble and flexible to be high resolubility.

The ratio of β(1→3) and β(1→4) linkages in β-glucan is an important factor in determining the physical properties, such as solubility, viscosity and gel formation (Miller & Fulcher. 1994). The ratio of β(1→3) to β(1→4) linkages in β-glucan extracts is shown in Fig. 2(B) and ranged from 3.44 to 4.98. Izydorczyk et al. (1998) reported that the ratio of β(1→3) to β(1→4) linkages of β-glucan in plants was usually 1:2.3-3.0 which were much lower than those ratio in this study. The report of Phillip and Stone (1988) indicated that the more β-glucan purified, the more ratio of β(1→3): β(1→4) linkages increased. The β-glucan concentrations after extraction from barley in our study were greater than those shown in the report of Izydorczyk et al. (1998). Thus, the extraction procedure in this study was optimized to purify β-glucan highly.

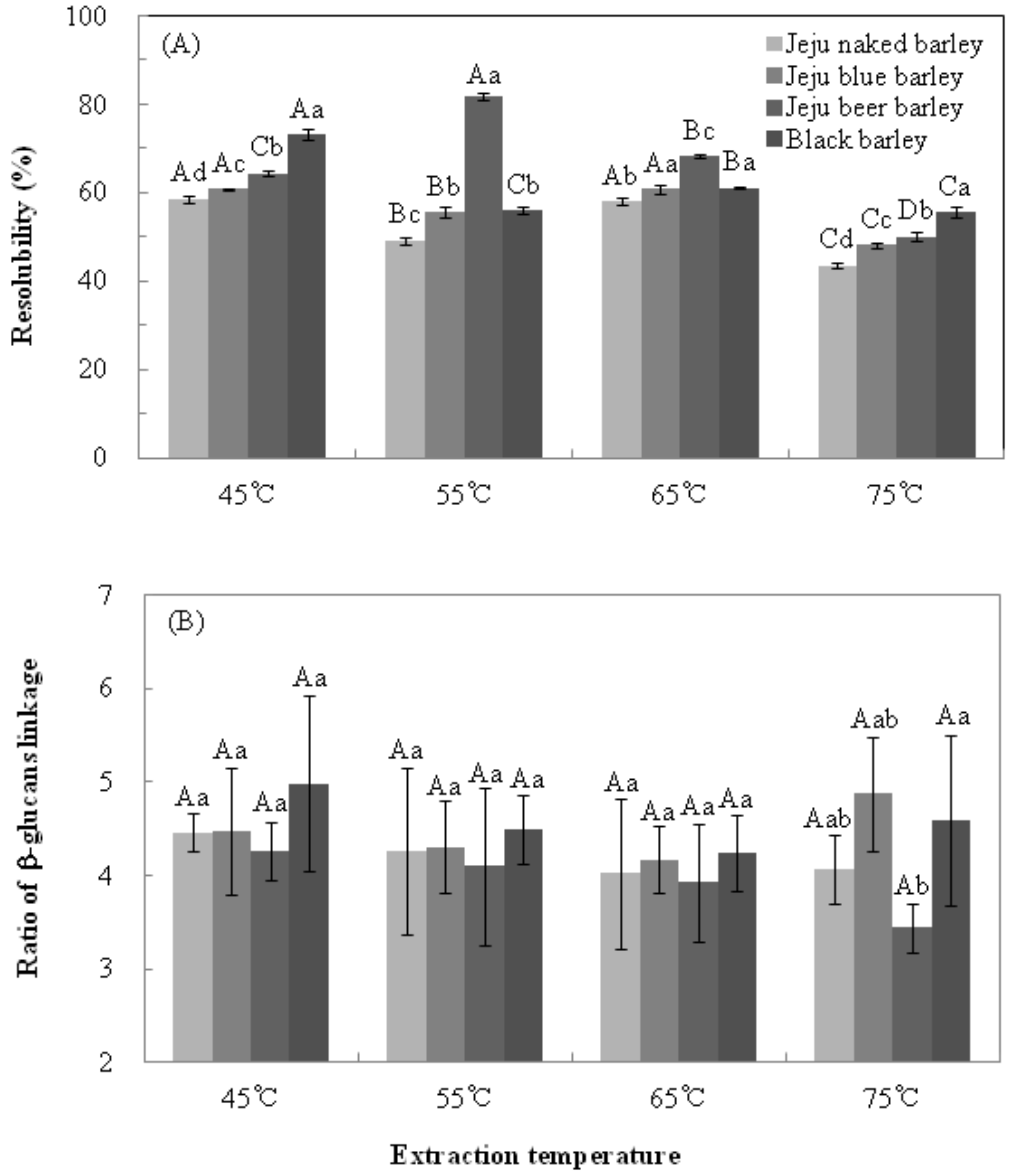

Fig. 2. Resolubility (A) and ratio of β(1→3) to β(1→4) linkages (B) of extracted β-glucan from different barley varieties at 45, 55, 65, and 75°C. Data are expressed as mean±standard deviation. Means with different capital letters on the bars indicate significant difference ($p<0.05$) within the same barley variety and means with small letters indicate significant difference ($p<0.05$) within the same extraction temperature by Duncan's multiple range test.

4. Conclusions

Chemical compositions including β-glucan concentration were dependent on barley variety grown in Jeju. Viscosity of barley suspension was increased with β-glucan content and increased after heating. Barley β-glucans differently extracted at 45, 55, 65, and 75°C and contained 64.88 to 93.84% of β-glucan after extraction. The optimum temperature for the β-glucan extraction from Jeju naked barley, Jeju blue barley, and black barley was 65°C and that from Jeju beer barley was 75°C. Resolubility of extracted β-glucan was dependent on barley varieties and extraction temperature. Ratio of β(1→3) to β(1→4) linkages ranged from 3.44 to 4.98 which were high as indicating highly purified. These results indicate that the purification and physicochemical properties of β-glucan were dependent on the varieties of barley and the temperature of water for extraction. It is possible to produce highly purified and uniform β-glucan which can impact nutritional and textural properties of food developed with barley.

PART Ⅱ

Physicochemical and Health Beneficial Characteristics of β-Glucans from Jeju Barley

1. Introduction

Cereal grains have become staple foods providing protein, carbohydrate, and dietary fiber (Susanne et al., 2007). Barley, an ancient and important cereal grain crop, was presumably first used as human food but evolved primarily into a feed, malting, and brewing (Newman & Newman, 2006). Barley can be classified into various types: spring or winter, two-row or six-row, hulled or hulless, and malting or feed (Baik & Steven, 2008). Many different barley types show various properties and difference in the concentration of dietary fiber including β-glucan (Izydorczyk et al., 2000). Hulless barley is absence of hull tightly adhering to the grain that is composed of flour and bran yields of about 70 and 30%, respectively (Batty, 1992). Hulled barley requires removing the hull and would be more suitable for processing and human consumption than hulled barley. On the other hand, hulled barley is preferred to hulless barley for malting and brewing (Quinde et al., 2004). Some hulless barley recently developed is the genotype, which not only exhibits unusual characteristics, such as waxy endosperm and high-amylose starch, but also contains high levels of β-glucans (Jadhav et al., 1998). Waxy starch genotypes give unique physical properties to food products and contain higher contents of protein and β-glucan than normal starch genotype barley. Waxy cultivars are usually associated with high β-glucan content (Izydorczyk et al., 2005). The mixed linkage (1→3, 1→4)-β-D-glucans (β-glucan) are usually

found in barley (Kalra & Jood, 2000).

β-Glucans, non-starch polysaccharides found in walls of endosperm and aleurone cells of barley. They constitute 2-11% of the weight of the total kernel carbohydrates but usually ranged between 4 and 7% (Papageorgiou et al., 2005). Because β-glucans are enclosed with starch matrix of protein, and lipid in the grain, it is difficult to extract β-glucan from barley (Tosh et al., 2003). The extraction and purification of β-glucan in barley can be affected by flour particle size, temperature, pH, and ionic strength (Wood et al., 1978). A typical extraction process involves three steps: inactivation of endogenous enzymes, extraction of β-glucans, and a purification-isolation stage (Charles & Louise, 2005). β-Glucans are linear homopolymers of D-glucopyranosyl residues linked mostly via two or three consecutive β(1→4) linkages that are separated by a single β(1→3) linkages (Cui et al., 2000). β-Glucans are composed of glucoses with β(1→3) and β(1→4) mixed linkages in the ratio of about 2.3-3.0 to 1.0(Stone & Clarke, 1992). β-Glucans from different types of cereals showed the similar molecular structure but exhibited variation in the ratios of β(1→4) to β(1→3) linkages and molecular weights (Autio, 1996). The chain conformation of β-glucans is attributed to their ability to occupy large hydrodynamic volumes and to form solution with high intrinsic viscosity (Izydorczyk & Dexter, 2008). The rheological properties of β-glucan solution depend mainly on the ability of β-glucan chains to associate, which can be determined by the proportion of cellotrisyl/cellotetraosyl units and their arrangements (Tudorica et al., 2002). In addition, solubility of β-glucan is influenced by decreasing or increasing molar ratio of cellotrisyl/cellotetraosyl units within the β-glucan chains (Jiang & Vasanrhan, 2000). The ability of β -glucans to form viscous solutions is a key physicochemical property responsible for physiological effects of consuming soluble dietary fiber (Wood, 2004). When consumed soluble dietary fiber of barley, β-glucan increases small intestinal viscosity because of its low molecular weight and its

tendency to form viscous solution, and results in reducing cholesterol levels (Kalra & Jood, 2000). In addition, β-glucan help to control blood cholesterol and glucose level (Izydorczyk & Dexter, 2008).

Serum cholesterol and lipoprotein concentrations can be reduced by β-glucan from barley. A reason for the cholesterol-lowering effects of β-glucan resulted from the great excretion of bile acids (Kahlon & Woodruff, 2003). Bile acids are acidic steroids synthesized in the liver from cholesterol. Circulation by being converted to bile acids in the liver can reduce cholesterol (Kahlon & Smith, 2007). Generally, bile acid almost reabsorbs and transports to the liver. Elimination of bile acids increased synthesis of bile acid, which in turn consumed cholesterol (Sayar et al., 2005).

Glycemic Index (GI) is a classification of the blood glucose-raising potential of carbohydrates in foods. In general, low-GI foods are defined as having a GI of less than 55, medium-GI foods a GI of 56-69, and high-GI foods a GI of over 70. The large differences in GI is the rate of digestion or absorption of the carbohydrates and low-GI foods are differentiated from other foods by the reduced rate at which they are digested and release glucose to the blood. Low-GI diets are associated with improvement of insulin sensitivity and increase of colonic fermentation. β-Glucans from barley and oat grains have been shown to influence human glycemic control such as the starch digestibility and help lower GI of foods. β-Glucan is required to produce a low glycemic index food product. The physicochemical properties of β-glucan which influences the viscosity of foods, is attributed reduce starch digestibility in foods (Kim & White, 2013; Bjorck et al., 2000; Chillo et al., 2001; Kim & White, 2012).

In this study, the physicochemical properties of β-glucan from different varieties of barley were investigated and the health beneficial characteristics of β-glucan extract were evaluated for an attempt to better understand the characteristics of β-glcuan in barley.

2. Materials and Methods

2.1. Materials

Barley can be classified with and without adhering hulls. It is called hulled barley with adhering hull. Hulless barley has few hulls that are composed of flour and bran. Six different varieties of barley including barley produced in Jeju, Korea were selected for this study. Hulled barley varieties were less-hulled barley (Iksan168ho) and beer barley (Iksan173ho. Hulless barley varieties were black barley (Iksan100ho), waxy naked barley (Suwon236ho), naked barley (Erie36ho), and blue barley (Iksan479ho). Barley varieties grown in Jeju were obtained from a local market (Jeju, Korea). All barley grains were ground into flour with a mill (MF10, Ika-Werke GmbH & Co., Staufen, Germany) through a 100 mesh screen.

2.2. Chemical composition of barley flours

Chemical composition of barley flours were determined by AOAC (1995) method and AACC (2000) method. Moisture content of barley flours was measured with a moisture analyzer (MX-50, AND Ltd. Co., Tokyo, Japan) at 105°C. Crude protein, fat, and ash were determined by Kjeldahl method, Soxhlet extractor, and ashing incineration, respectively. Contents of β-glucan, starch, and total dietary fiber were analyzed by following AACC method 32-23.01 and 32-76.13, and AOAC method 991.43 using assay kits (Megazyme International Co., Wicklow, Ireland). All analyses were run in triplicate and the averages were reported on a dry-weight basis.

2.3. Viscosity of barley suspension

The viscosity of barley suspension was determined by modification of the procedure of Jeong et al. (2004). Barley flour suspension was prepared by 1:8, barley flour to water mass ratio and the viscosity was measured by a viscosity analyzer (DV-I, Brookfield Co., Middleboro, MA, USA). A stirring speed was 100 rpm, spindle No.2 was used at room temperature (25±5°C), and No.4 (less-hulled barley), No.5 (beer barley), No.6 (black barley), and No.7 (waxy naked, naked, and blue barley) were used at 70°C for heating.

2.4. Extraction of β-glucan

β-Glucan was extracted from barley using the procedure of Kim & White (2010) with modifications. Barley flour (15.0 g) was suspended in 150 mL of 82% ethanol and refluxed to remove fat and inactive endogenous enzyme at 80°C for 3 h. Barley suspension was then centrifuged at 3,100 × g for 20 min to remove supernatant. Precipitate was washed with 50 mL of 95% ethanol, centrifuged at 3,100 × g for 15 min twice, and dried at 40°C for overnight. β-Glucan was extracted from the refluxed and dried flour (about 12.0 g) by using 240 mL distilled water at 65°C for 3 h. After extraction, suspension was centrifuged at 3,100 × g for 20 min and the process was repeated for two more time. The supernatant was treated with 0.2 mL α-amylase (Sigma-Aldrich, St. Louis, MO, USA) and 36-40 mg calcium chloride (Sigma-Aldrich) in a shaking water bath (JSSβ-30T, JS Research Inc., Gongju, Korea) at 90°C for 2 h and centrifuged at 3,100 × g for 20 min. The supernatant was then reacted with 15 mg pancreatin (Sigma-Aldrich) and 0.2 mL 10% sodium azide (Sigma-Aldrich) at 40°C for 3 h. Reactant was added with 2-fold 60% ethanol and stayed overnight at 4°C and centrifuged at 3,100 × g for 20 min. The precipitate was freeze dried. Total β-glucan contents of freeze-dried β-glucan were measured for the calculation of β-glucan

concentration.

2.5. Resolubility of β-glucans extracted from barley

Resolubility of the extracted β-glucan in water was measured to analyze the physical properties of β-glucan according to the method of Lee et al.(2012) with modifications. The β-glucan dispersion in water (1%, w/v) was agitated at 50°C for 12 h and centrifuged at 12,000 x g for 10 min. Precipitate was dried and calculated as the percentage(%) = (weight of β-glucan dissolved in the supernatant/initial weight of β-glucan in the dispersion) x 100.

2.6. Ratio of β(1→3) to β(1→4) linkages in extracted β-glucans

Ratio of β(1→3) to β(1→4) linkages was determined to compare the structure of extracted β-glucan (Woodward et al., 1980). Content of binding β(1→3) glucoses was determined as following: β-glucan (2.0 mg) was added to 1 mL sodium phosphate buffer (20 mM, pH 6.5) with 4U lichenase (Megazyme International Ltd, Co., Wicklow, Ireland) which broke all β(1→4) linkages of 3-O-substituted glucose residues in β-glucan at 40°C for 22 h. Reactant was added to 5 mL sodium acetate buffer (200 mM, pH 4.0) and centrifuged at 2,000 x g for 10 min. After obtained 0.1 mL supernatant, the content of β(1→3) linked glucoses was determined. Total glucose was determined as followed: β-glucans (2.0 mg) was added to 1 mL sodium phosphate buffer (20 mM, pH 6.5) with 4U lichenase (Megazyme International Co., Wicklow, Ireland) and incubated at 40°C for 22 h. Reactant was treated with β-glucosidase (0.2U, Megazyme International Ltd, Co., Wicklow, Ireland) at 50°C for 10 min. After the obtained 0.1 mL suspension, total glucose content was determined. The β(1→4) linked glucoses was calculated by

subtracting β(1→3) linked glucoses from total glucoses and ratio of β(1→3) to β(1→4) linkages was calculated as the amounts of β(1→4) linked glucose / β(1→3) linked glucose.

2.7. *In vitro* bile acid binding

In vitro bile acid binding of β-glucans from barley was determined by the modified methods of Kim & White (2012). The bile acid mixture was prepared with sodium cholate (35%, Sigma-Aldrich), sodium deoxycholate (35%, Sigma-Aldrich), sodium glycocholate (15%, Sigma-Aldrich), and sodium taurocholate (15%, Sigma-Aldrich) in 10 mL of sodium phosphate buffer (50 mM, pH 6.9). The first total amount of bile acid was 11.2 μmol/100 mg of bile acid mixture. Cholestyramine (Sigma-Aldrich) as a positive control and cellulose (Sigma-Aldrich) as negative control were used. The stimulated gastric digestion was performed that a 50 mg of cholestyramin and cellulose, and a 10 mg of β-glcuan were digested with 1 mL of 0.01N hydrochloric acid and incubated in a shaking water bath at 37 °C for 1 h. The pH of mixture was adjusted to 6.9 with 0.1N sodium hydroxide. The bile acid mixture (4 mL) and porcine pancreatin (5 mL, Sigma-Aldrich; 6.25 mg/mL in a 50 mM phosphate buffer, pH 6.9) were added and incubated in a shaking water bath at 37°C for 1 h. After centrifugation at 2,700 x g for 15 min, the supernatant was taken. The precipitate was added a 5 mL of sodium phosphate buffer (50 mM, pH 6.9) and centrifuged again to remove the residue. The supernatant was taken and combined with former supernatant. The supernatant that contained unbound bile acid was analyzed by using a Bile Acid Diagnostic Kit (Trinity Biotech, Bray Co., Wicklow, Ireland). The standard curve was developed from the different concentrations of bile acid mixture (1.4, 0.7, 0.35, 0.175, 0.07, 0.035 μmole/mL) and the concentration of the bile acid bound were calculated based on this standard curve.

2.8. Preparation of barley flour and the mixtures of potato starch and β-glucans for *in vitro* starch digestibility

To evaluate *in vitro* starch digestibility of β-glucan, barley flour (5% in distilled water) and the mixture of potato starch with β-glucan are prepared by the method of Kim & White (2013) with modification. Eight different mixtures with potato starch and β-glucan extract were prepared as followings : (1) 5% potato starch solution (S, non-heated), (2) 5% potato starch solution with heat (S, heated), (3) potato starch and β-glucan from less-hulled barley (S + Less-hulled), (4) potato starch and β-glucan from beer barley (S + Beer), (5) potato starch and β-glucan from black barley (S + Black), (6) potato starch and β-glucan from waxy naked barley (S + Waxy), (7) potato starch and β-glucan from naked barley (S + Naked), and (8) potato starch and β-glucan from blue barley (S + Blue). The ratio of potato starch and β-glucan weight was 5:1 which was established after preliminary study. All solutions were adjusted to the same volume. All solution except barley flours (non-heated) and S (non-heated) were heated at 90°C for 10 min to gelatinize starch and cooled to room temperature.

2.9. *In vitro* starch digestibility

In vitro starch digestibility of barley flours (non-heated and heated) and the mixtures of potato starch and β-glcuans were determined by the method of Kim & White (2013). The enzyme for digestion was prepared with 0.9 g of pancreatin (Sigma-Aldrich) in 8 mL of distilled water and centrifuged at 1,500 x g for 10 min. The supernatant (5.4 mL) was mixed with 0.8 mL of diluted amyloglucosidase that prepared with 0.64 mL of amyloglucosidase and 0.8 mL of distilled water. The enzyme solution was added with 0.5 mL of

distilled water.

Barley flour, heated barley flour, the mixture of potato starch and β-glcuans, potato starch, heated potato starch, and white bread was prepared mixture in a 50 mL tube was added with 10 glass beads (5 mm diameter), 2 mL of 0.05 M hydrochloric acid, and 10 mg of pepsin. The mixture incubated at 37 °C in a shaking water bath for 30 min. Four milliliter of sodium acetate buffer (0.5M, pH 5.2) and 1 mL of enzyme solution were added to the tube at 1 min intervals while the mixtures were incubated at 37 °C in a shaking water bath. Aliquots (100 μL) from the tube were taken at 0, 10, 20, 30, 60, 90, 120, and 180 min and 1 mL of 50% ethanol was added to each tube. These tubes were centrifuged at 800 x g for 5 min. The glucose concentration of the supernatants (100 μL) was measured using D-glucose assay kit (K-GLUC, Megazyme International Ltd, Co., Wicklow, Ireland). Total starch hydrolysis was calculated as following: Total starch hydrolysis (%) = {{released glucose weight x 160/182}/(total starch weight in prepared solutions)} x 100.

2.10. Estimated glycemic index

The glycemic index (GI) was calculated by the equation of Goni et al. (1997). Hydrolysis curve for each product was described as follows: $C=C_\infty(1-e^{-kt})$, where C is the concentration at time t (min), C_∞ is the equilibrium estimated by above equation. The area under the hydrolysis curve (AUC) was calculated for all products. $AUC=C_\infty(t_f-t_0)-(C_\infty/k)[1-\exp^{-k(k_t-t_f)}]$, where t_f is the final time (180 min) and t_0 is the initial time (0 min). A hydrolysis index (HI) was predicted by dividing the AUC of each treatment by the AUC of control (white bread). The GI was then estimated as followed: GI=3.971+0.549(HI)

2.11. Statistical analysis

All analyses were done in triplicate. Data were analyzed by the analysis of variance (ANOVA), followed by the Duncan's multiple range test ($p<0.05$) using statistical software SPSS (Statistics Package for the Social Science, Ver. 18.0, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Chemical composition of barley flours

Chemical compositions of different varieties of barley are indicated in Table 2. Protein contents of black barley were greater than other barley varieties as showing 14.88%. Fat contents of black barley, waxy naked barley, naked barley, and blue barely were greater than those of less-hulled barley and beer barley. Six varieties of barley were consisted of similar amounts of ash and starch as 1.05-1.31% and 69.53-76.53%, respectively. Total dietary fiber in barely was ranged from 10.07 and 15.64% (Table 2), which was greater than those reported by Lee (1992) indicated that total dietary fiber in barley were 9-10%. Waxy naked barley, naked barley and blue barley contained high amount of total dietary fiber. β-Glucan contents of black barley, waxy naked barley, naked barley, and blue barley were ranged from 5.91 to 6.75%, which were greater than those of less-hulled barley and beer barley. According to Skendi et al. (2003) report, β-glucan content was 5-11% in barley. The less-hulled barley and beer barley were low in β-glucan because these barley varieties were genetically modified to reduce the content of β-glucan for beer brewing (Bamforth, 1982). The study of Baik (2008) was reported that barley grain was usually composed of about 65-68% starch, 10-17% protein, 4-9% β-glucan, 2-3% lipid, and 1.5-2.5% ash, which were similar results with the current study.

Table 2. Chemical composition of different varieties of barley

Barley	Protein[1)]	Fat	Ash	Starch	Total dietary fiber	β-Glucan
Less-hulled barley	9.22±0.42[b,2),3)]	1.26±0.23[c]	1.05±0.12[a]	72.24±2.77[a]	10.07±2.56[a]	3.44±0.14[c]
Beer barley	9.41±0.63[b]	1.22±0.10[c]	1.15±0.10[a]	74.65±3.92[a]	10.74±2.99[a]	3.46±0.06[c]
Black barley	14.88±0.84[a]	3.98±0.56[a]	1.26±0.21[a]	70.60±5.34[a]	10.86±2.24[a]	6.08±0.23[b]
Waxy naked barley	8.87±0.87[b]	2.70±0.10[b]	1.31±0.09[a]	76.53±5.23[a]	15.64±2.82[a]	6.75±0.07[a]
Naked barley	7.99±0.70[b]	4.08±0.52[a]	1.16±0.07[a]	69.53±6.99[a]	14.10±2.26[a]	6.45±0.42[ab]
Blue barley	9.32±0.96[b]	2.39±0.95[b]	1.22±0.27[a]	72.83±5.80[a]	14.09±4.18[a]	5.91±0.53[b]

1) All chemical contents were calculated as dry basis, %.

2) Each value is mean±standard deviation.

3) Means with the different letter in a column indicate significant difference ($p<0.05$) by Duncan's multiple range test.

3.2. Viscosity of barley suspension

The viscosity of barley suspensions was measured at room and heating temperature shown in Fig 3. Viscosity of waxy naked barley at room temperature was 73.80 cP which was the highest among other varieties and the barley grains as shown in the study of Izydorczyk et al. (2000). Other barley viscosities were in the range of 30.13 to 55.20 cP. After heating, the viscosity of waxy naked barley increased to 4960.00 cP, which was the highest viscosity among other barley varieties. In addition, the viscosities of black barley, waxy naked barley, naked barley, and blue barley as (973.33-2333.33 cP) were greater than those of less-hulled and beer barley (428.67-493.33 cP). Viscous solution generally contains water soluble polysaccharides (Guleria et al., 2015). Viscosity, which determined the physical characteristics of barley, was affected by not only β-glucan concentration but also endogenous β-glucan degrading enzymes (Izydorczyk et al., 2000). According to Wood (2004), β-glucan as soluble dietary fiber was able to form high viscous solutions. Black barley, waxy naked barley, naked barley, and blue barley containing high amount of β-glucan showed the higher viscosity than less-hulled barley and beer barley did (Fig 3). The viscosity of naked barley at room temperature was the lowest among the less-hulled barley and beer barley. As heating, the viscosity of naked barley was increased. This result can explain that viscosity of barley suspension increased with heating and this possibly related to starch gelatinization (Wood et al., 1978). As the insoluble cell wall of barley was destroyed by heating, soluble dietary fiber was increased to affect in viscosity (Brandt et al., 1984).

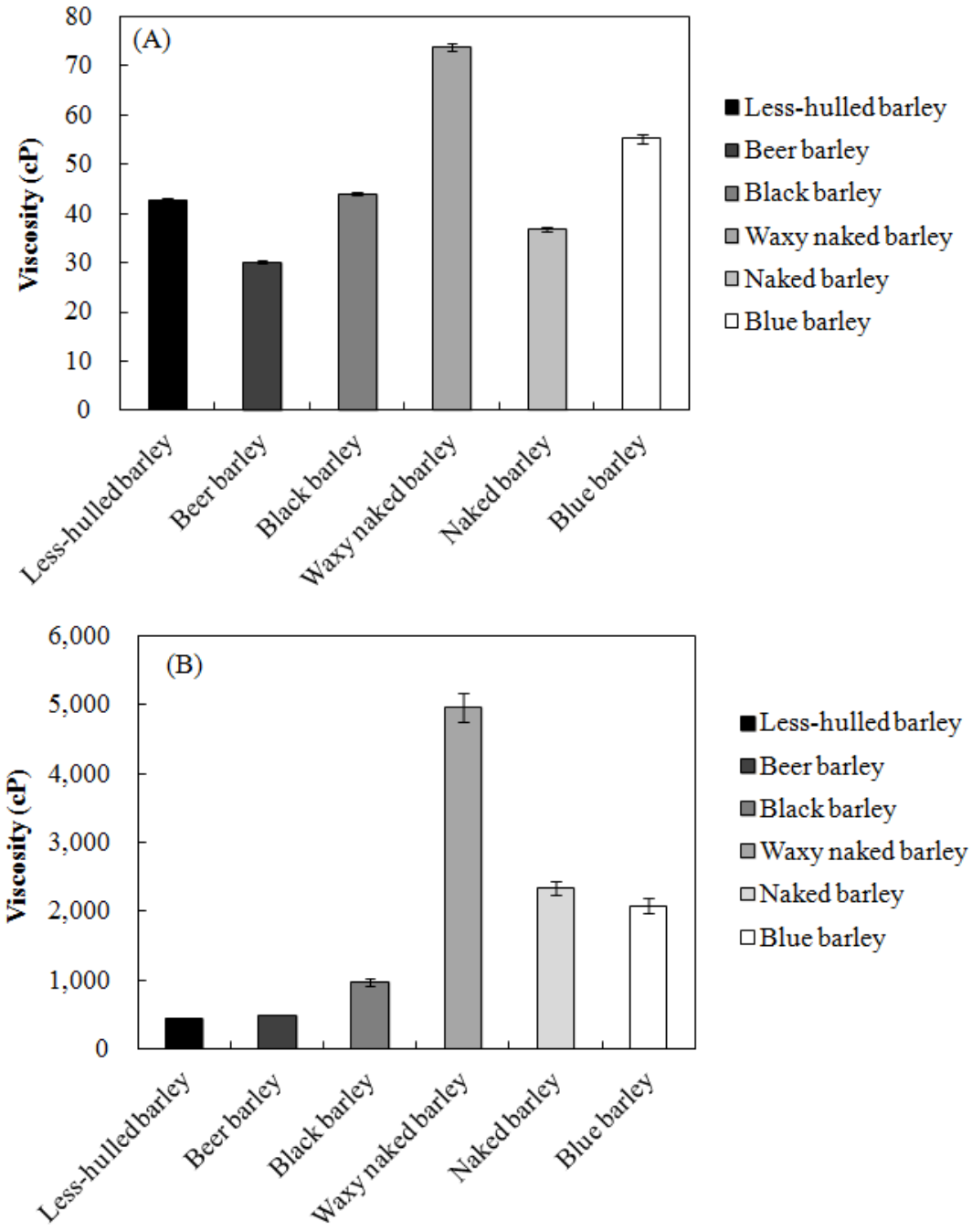

Fig 3. Viscosity of barley suspensions at room temperature (A) and heating (B).

3.3. Characteristics of extracted β-glucan from barley

β-Glucans extracted from different varieties of barley contained the β-glucan in the range of 70.09 to 95.46% (Table 3). β-Glucan extracted from less-hulled barley was the lowest as 70.09%. Izydorczyk et al. (2003) reported that β-glucan extraction was affected by barley genotype and milling rate. Although barley had high β-glucan concentration, β-glucan extraction was difficult because β-glucan was enclosed to starch, matrix protein, and lipid (Brennan & Cleary, 2005). The less-hulled barley was not completely milled, thus the yield of β-glucan was possibly low. However, beer barley was comparatively high in β-glucan content as 85.47%. The extraction yield of β-glucan depended on the extraction processing conditions, such as solvents, temperature, pH, and time, as well (Kim & White. 2011). The β-glucan extracts contained 2.57-7.48% of starch likely as most of the starch in the barley flour was removed during treatment with a-amylase and pancreatin. β-Glucans was mostly included soluble β-glucan. Other complex carbohydrates might not be measured by the methods of total starch and β-glucans analysis McCleary (2010).

Resolubility of β-glucans extracted from barley is shown in Table 3. Resolubility of the β-glucans from waxy naked barley was the greatest as 56.56%. According to Izydorczyk et al. (2003), the waxy type barley showed slightly higher solubility of β-glucan than other type barley. Although β-glcuan extracted from beer barley was high content of β-glucan, resolubility was the lowest as 29.89%. This result indicated that beer barley β-glucan contained insoluble components. In addition, the previous research was demonstrated that high molecular weight β-glucan has a lower solubility. Based on the results, β-gluans with low resolubility were possible to have high molecular weight (Li et al., 2010).

The ratio of β(1→3) and β(1→4) linkages in β-glucan is in Table 3. The

ratio of β(1→3) and β(1→4) linkages is able to determine the physical properties. According to Stone & Clarke (1992) study, the ratio of β(1→3) and β(1→4) linkages of β-glucan was usually 2.3-3.0 to 1.0. The ratio of β(1→3) and β(1→4) linkages of β-glucan was determined as 1.71-2.86 to 1.0 which were similar results with Stone & Clarke (1992) study. The report of Phillip and Stone (1988) indicated that the more β-glucan purified, the more ratio of β(1→3):β(1→4) linkages increased. The ratio of less-hulled barley resulted from lowest yield of β-glucan. β-Glucan concentration can affect in the ratio of β(1→3) and β(1→4) linkages. However, although yield of β-glucan was low, the ratio of β(1→3) and β(1→4) linkages has not similarity between β-glucan extracted from various barley.

Table 3. Chemical composition and characteristics of β-glucan extracted from different varieties of barley

Barley	β-Glucan (%)	Starch (%)	Resolubility (%)	Ratio of β(1→3):β(1→4) linkages
Less-hulled barley	70.09±5.78[c1),2)]	7.25±0.39[a]	39.67±1.44[bc]	1.71±0.11[a]
Beer barley	85.47±4.42[b]	6.7±0.78[ab]	29.89±3.00[d]	2.86±1.47[a]
Black barley	89.17±2.95[ab]	3.29±1.29[c]	45.93±0.82[b]	2.37±0.02[a]
Waxy naked barley	95.46±3.10[a]	2.57±1.38[c]	56.56±8.70[a]	2.25±0.34[a]
Naked barley	88.85±5.42[ab]	4.32±1.24[bc]	47.17±3.85[b]	2.02±0.13[a]
Blue barley	83.06±1.53[b]	7.48±2.72[a]	36.13±1.04[cd]	2.15±0.11[a]

1) Each value is mean±standard deviation.

2) Means with the different letter in a column indicate significant difference ($p<0.05$) by Duncan's multiple range test.

3.4. *In vitro* bile acid binding

In vitro bile acid binding of β-glucan and control (positive and negative) are shown in Table 4. The positive control, cholestyramine, was bound to 8.96 μmol of bile acid/100 mg of cholestyramine. The negative control, cellulose, was calculated as 0.66% binding when cholestyramine was bound to 100% of bile acids. These values of two controls were similar to the results shown in Kim & White (2011). The *in vitro* bile acid binding of β-glucan from different varieties of barely was calculated on the basis of 100% bile acid binding of cholestyramine. The *in vitro* bile acid binding of β-glucan extracted from waxy naked barley was the highest as 27.16%. This result was possibly attributed to the high amount of β-glucan in the extract. Other barley β-glucan extracts were calculated as 24.39-25.74% of bile-acid binding. Although the less-hulled barley contained low β-glucan content, the *in vitro* bile acid binding was measured as similar as other barley varieties. Kim&White (2011) reported that the bile acid binding values was influenced by the β-glucan concentration. β-Glucan is able to lower the cholesterol level by removing bile acid (Yao et al. 2008). Kahlon & Smith (2007) was shown that food fractions prevented the reabsorption of bile acid and stimulated plasma and liver cholesterol conversion to additional bile acids. Eliminating bile acids consumes cholesterol and reduces the serum cholesterol level. *In vitro* bile acid bindings of β-glucans extracted from different varieties of barley were similar to each other but black barley β-glucan was significantly low. Solubility of β-glucan affected bile acid binding (Kim & White, 2011). The result in this study suggested that β-glucan was not strictly influenced by only solubility. Kahlon & Smith (2007) confirmed that bile acid binding was affected by the structure of carbohydrate, and the presence of flavonoids, and polyphenols. The bile acid binding of black barley β-glcuan was possibly influenced by flavonoids because it contained anthocyanins (Siebenhandl et al.,

2007). In addition, the high bile-acid binding value of the less-hulled barley containing low β-glucan concentration might be suggested that the other types of dietary fiber present in the β-glcuan extract was bound to bile acids. This study suggested that further investigation of the composition of β-glucan extract and its structure is needed.

Table 4. *In vitro* bile acid binding of extracted β-glucan from different varieties of barley

Barley	Bile acid bound	
	Relative % to cholestyramine	μmol/100 mg total weight
Less-hulled barley	25.74±0.18[b1),2)]	2.31±0.02[b]
Beer barley	25.07±0.28[bc]	2.25±0.02[bc]
Black barley	24.39±0.19[c]	2.18±0.11[c]
Waxy naked barley	27.16±0.43[a]	2.43±0.04[a]
Naked barley	25.40±0.40[bc]	2.27±0.04[bc]
Blue barley	25.26±0.62[bc]	2.26±0.06[bc]
Cholestyramin	100	8.96±0.53
Cellulose	0.66	0.06±0.02

[1)] Each value is mean±standard deviation.

[2)] Means with the different letter in a column indicate significant difference ($p<0.05$) by Duncan's multiple range test.

3.5. *In vitro* starch digestibility

In vitro starch digestibility of barley flour is shown in Fig 4. The starch digestibility of raw barley flours and heated barley flour were ranged about 2-10% and 15-35%. The starch hydrolysis of all barley flour increased as increasing the digestion time. Heat treatment increased the starch digestibility of barley flour greater than that of raw barley flour. The starch digestibility of all raw barley flour was similar. Barley that contained low β-glucan was in low starch digestion. Although total dietary fiber and β-glucan content were low, they might affect starch digestion. The starch digestibility of heated barley flour was distinguished. Especially, the starch digestibility of heated blue barley flour was the highest. The starch digestibility was influenced by structure of starch, cultivar, β-glucan content, and sensitivity of enzyme (Zhou et al., 1998). Barley was known as having starch of low amylose content. Thus, the previous study suggested that rice, potato, and bean starch with high amylose content were more easily digested than barley did (Zheng & Sosulski, 1998; Goni et al., 1997).

In vitro starch digestibility of potato starch and the mixture of potato starch and β-glucan are shown in Fig 5. The starch digestibility of mixture of potato starch and β-glucan were 15.21-34.54%. The starch digestibility of the mixture of potato starch and β-glucans were lower than those of heated potato starch and control (white bread) in Fig 5. These results showed that β-glucan possibly reduced starch digestion and affected the starch digestibility of barley flour. The mixture of waxy naked barley β-glucan (S+Waxy) was high in starch digestibility when compared with other β-glucan extracts. According to Kim & White (2013), low molecular weight β-glucan impacted to low starch digestion. Also, other β-glucan having low β-glucan content might have impurity such as amylopectin. The indigestible amylopectin during starch digestion process could cause low rate of starch digestion. Therefore,

these results were possibly influenced by not only β-glucan concentration but also molecular weight of β-glucan and structural features of starch.

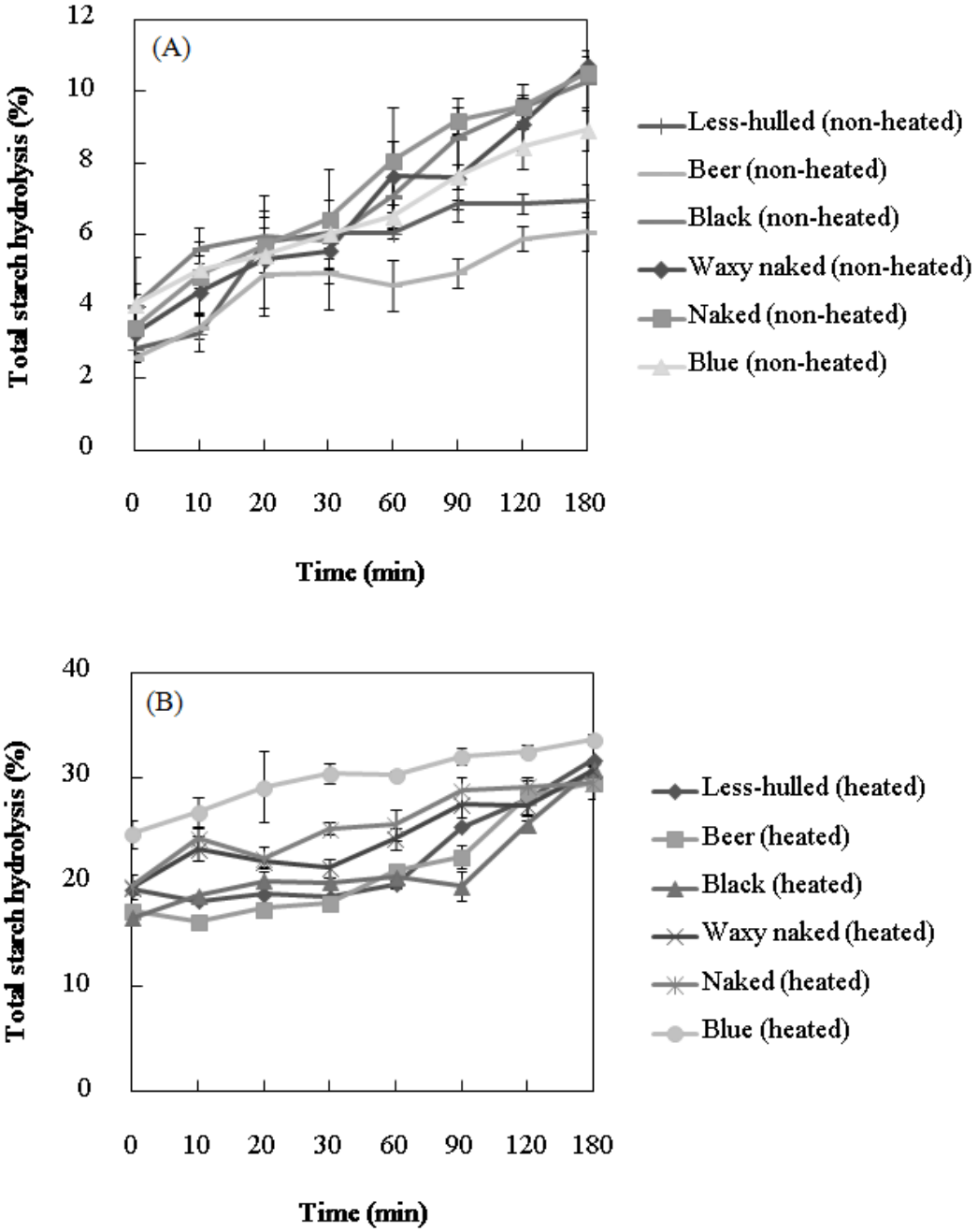

Fig 4. *In vitro* starch hydrolysis of barley flour solution non-heated (A) and heated (B).

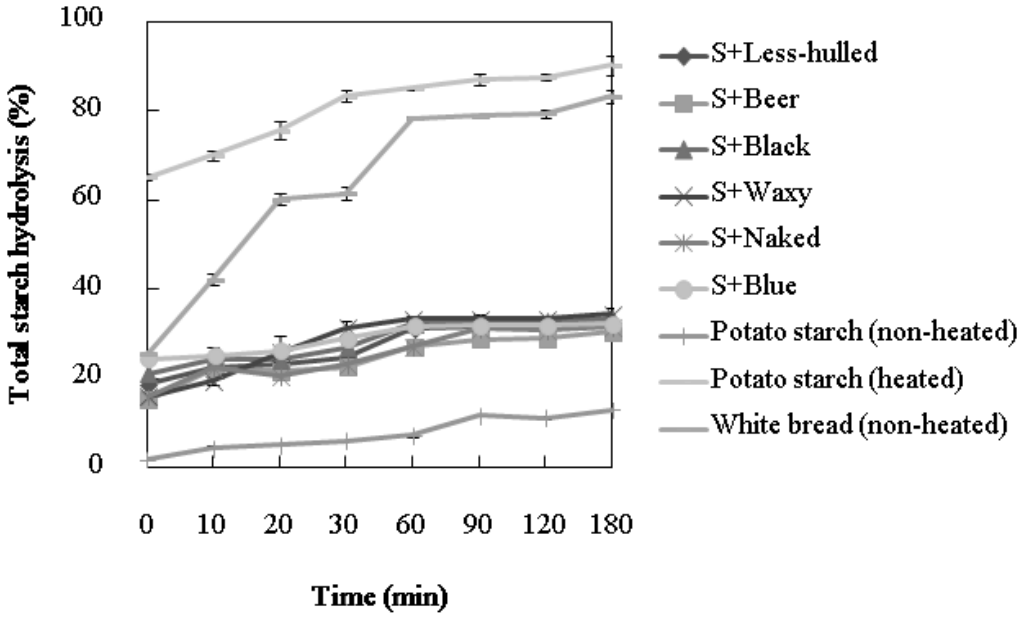

Fig 5. *In vitro* starch hydrolysis of potato starch (non-heated and heated), white bread, and the mixture of potato starch with β-glucan extracted from different varieties of barley.

3.6. Estimated glycemic index

Based on the starch digestibility, the estimated GI was calculated and shown in Table 5 and 6. The estimated GI of raw and heated barley flour belonged to low-GI food and medium-GI food as 43.14-46.06 and 55.36-61.97, respectively (Table 5). The GI of Jeju barley was lower than the GI of barley as shown in Foster-Powell et al. (2014). According to Zhang et al., (1996), normal and waxy type cereals had 20-30% and 0-5% amylose content, respectively. Barley was mostly constituted low amount of amylose and high amount of amylopectin. Amylopectin was not completely hydrolyzed, thus barley possibly indicated low GI. Although less-hulled barley and beer barley were normal type barley, GI was lower than that of other barley. These results could be predicted that starch structure of less-hulled barley and beer barley was more complex, which possibly mixed with indigestible carbohydrate because these varieties were not completely polished. The GI of heated barley flour was higher than raw barley flour. The gelatinized barley flour formed swelling amylopectin and amylose. Swelling degree influenced increasing digestibility (Singh et al. 2010). By heating barley flour, barley was gelatinized and increased starch digestibility with the result of increasing GI.

The estimated GI of potato starch was reduced by addition of β-glucan extract from various barley varieties (Table 6). The GI of potato starch (heated) was high as 100.50 and the estimated GI of all mixtures with β-glucan were lower than potato starch (heated) as 59.71-63.19. These results indicated that β-glucan helped to reduce potato starch (heated) GI. The GI was first introduced as a mean for identifying carbohydrate-rich foods based on their ability to raise postprandial blood glucose levels (Thondre & Henry, 2009). According to Kim & White (2013), high molecular weight β-glucan showed low GI value. High-molecular weight β-glucan formed highly viscous solution. The viscous property of the mixture in the current study retarded

the susceptibility of enzyme to digest starch. Therefore, extracted β-glucan might be high molecular weight to have high viscosity. Black barley, waxy naked barley, naked barley, and blue barley were shown as higher viscosity (Fig 3) than less-hulled barley and beer barley and they indicated lower GI values. This study demonstrated that starch digestibility was affected by heating, starch structure, and presence of β-glucan, thus dietary fiber including β-glucan from barley can retard starch digestion and reduce GI value.

Table 5. Estimated glycemic index (GI) for different barley flour

Barley	GI	
Barley flour	Non-heated	Heated
Less-hulled barley	44.56±0.14[c1),2)]	57.26±0.29[d]
Beer barley	43.14±0.41[d]	55.40±1.13[e]
Black barley	45.75±1.11[ab]	55.36±1.36[f]
Waxy naked barley	45.05±1.22[bc]	58.66±1.22[c]
Naked barley	46.06±0.35[a]	59.85±1.26[b]
Blue barley	44.99±0.35[c]	61.97±0.78[a]

1) Each value is mean±standard deviation.

2) Means with the different letter in a column indicate significant difference ($p<0.05$) by Duncan's multiple range test.

Table 6. Estimated glycemic index (GI) for the mixture of potato starch and β-glucan extracted from varieties barley

Heated mixture	GI
S+Less-hulled	62.14±0.35[a1),2)]
S+Beer	59.71±1.44[b]
S+Black	62.68±1.62[a]
S+Waxy naked	63.19±0.27[a]
S+Naked	62.21±0.80[a]
S+Blue	62.47±0.39[a]
Potato starch (non-heated)	47.98±0.19
Potato starch (heated)	100.50±1.43
White bread	94.61±0.25

[1)] Each value is mean±standard deviation.

[2)] Means with the different letter in a column indicate significant difference ($p<0.05$) by Duncan's multiple range test.

4. Conclusions

The chemical composition, viscosity, β-glucan composition, β-glucan characteristics, *in vitro* bile-acid binding, and estimated GI of barley produced in Jeju were determined. The concentration of β-glucan in hulless barley flours was high as 5.91-6.75%. Viscosity of barley flour was increased with β-glucan contents and heating. β-Glucan was extracted from barley at 65°C with water and β-glucan purity of waxy naked barley was high as 95.46%. Resolubility of β-glucan extract was similar to each other but ratio of β(1→3): β(1→4) linkages was statistically significantly different. *In vitro* bile-acid binding was mostly influenced by β-glucan concentration. *In vitro* starch hydrolysis of barley flour and potato starch was affected by total dietary fiber, β-glucan concentration, and heat treatment. Estimated GI was increased by starch gelatinized and decreased by addition of β-glucans. These results indicated that β-glucan was influenced in *in vitro* bile acid binding and starch digestibility. β-Glucan is considered as an important component showing potential health impact. However, β-glucan concentration was not strictly influenced in β-glcuan characteristics and health benefit. Thus, it needs further investigation of the composition of β-glucan extract and structural characteristics of starch and β-glucan

국문요약

본 연구에서는 제주 지역에서 재배 및 생산되는 보리의 수용성 식이섬유인 β-glucan을 추출하는 방법을 확립하였다. 또한 추출된 β-glucan의 이화학적 특성을 분석하고 bile acid binding과 starch digestibility를 측정한 것을 토대로 glycemic index 수치를 추정하여 제주 보리가 기능성을 갖춘 제품으로 활용될 수 있는 자료를 제시하고자 하였다. β-glucan 추출은 45, 55 ,65 75℃ 에서 추출되었고, 추출 수율과 β-glucan의 특성 모두 65℃ 에서 우수하였다. 선행 연구를 토대로 겉보리, 맥주보리, 검정보리, 찰쌀보리, 쌀보리, 청보리를 이용하여 65℃에서 β-glucan 추출을 실시하였다. 겉보리가 70.09% 추출 수율이 가장 낮았으며 이는 도정이 완전히 되지 않기 때문에 수율이 낮았던 것으로 판단되었다. 찰쌀보리의 추출 수율이 95.46%로 가장 높았다. β-glucan 수율이 높았던 찰쌀보리가 재용해율이 가장 높았고 β(1→3): β(1→4) 결합비는 모든 보리에서 통계적으로 차이가 없게 나타났다. 추출된 β-glucan을 가지고 bile acid binding을 측정하였는데, β-glucang 함량이 가장 높았던 찰쌀보리가 bile acid binding 수치가 높았다. 도정 된 보리 현탁액의 GI를 측정했을 때 가열하지 않은 보리 현탁액은 low-GI에 속했고 가열한 보리 현탁액은 medium-GI에 속한 것으로 보아, 제주 보리가 다른 곡물보다 GI수치가 낮은 것을 알 수 있었다. 또한 감자 전분에 β-glucan을 첨가하였을 때 제주 보리 β-glucan에서 전분 소화율과 GI 수치가 낮아졌다. 감자 전분의 GI 수치는 100.50 이였지만 β-glucan을 첨가하였을 때에는 59.71-63.19로 떨어지는 것을 확인하여 전분의 소화율이 β-glucan의 영향을 받는 것을 입증하였다. 하지만 β-glucan 농도에 따른 차이는 보이지 않아 β-glucan의 구조나 추출된 β-glucan에 섞여있는 전분이 영향을 미쳤을 것이라 판단되어, β-glucan의 구조와 보리에 함유되어있는 전분의 구조 및 특성을 연구하는 것이 필요하다고 생각된다. 본 연구를 토대로 제주에서 재배된 보리의 일반성분과 온도에 따른 점도 특성, 추출된 β-glucan의 이화학적 특성 및 건강기능성을 바탕으로 제주 보리를 활용한 새로운 기능소재 및 제품개발에 이용 가능성이 높을 것으로 기대된다.

REFERENCES

AACC International. Approved Method of the AACC, 10th ed. Method 21-23.01, 76.13. Association of Cereal Chemists, St. Paul, MN, USA (2000)

Aastrup S. The relationship between the viscosity of an acid flour extract of barley and its β-glucan content. Carlsberg Res. Common. 44: 289-304 (1979)

AOAC. Official methods of analysis of AOAC Intl. 16th ed. Method 991.43. Association of Official Analytical Chemists, Arlington, VA, USA (1995)

Autio K. Functional aspects of cereal cell wall polysaccharides. pp. 227-264. In: Carbohydrate in Food. Eliasson AC (ed). Marcel Dekker, New York, NY, USA (1996)

Baik BK and Steven EU. Barley for food: Charcteristics, improvement, and renewed interest. J. Cereal Sci. 48: 233-242 (2008)

Bamforth CW. Barley β-glucans. Their role in malting and brewing. Brew. Dig. 57: 22-27 (1982)

Batty RS. β-glucan content and viscosities of barleys and their roller-milled flour and bran products. Cereal Chem. 69: 469-471 (1992)

Batty RS. Physicochemical properties of roller-milled barley bran and flour. J. Cereal Chem. 70: 397-402 (1993)

Bjorck I, Lijeverg H, Ostman E. Low glycemic-index foods. Br J Nut 83: s149-55 (2000)

Brandt LM, Jeltema MA, Zabik ME, Jeltema BD. Effects of cooking in solutions of varying pH on the dietary fiber components of vegetables. J. Food Sci. 49: 133-141 (1984)

Brennan CS and Cleary LJ. The potential use of cereal (1→3, 1→4)-β-D-glucans as functional food ingredients. J. Cereal Sci. 42: 1-13 (2005)

Buliga GS, Brant DA, Fincher GB. The sequence statistics and solution

conformation of a barley (1→3, 1→4)-β-glucan. Carbohyd. Res. 157: 139-156 (1986)

Charles SB and Louise JC. The potential use of cereal (1→3,1→4)-β-D-glucans as functional food ingredients. J. Cereal Sci. 42: 1-13 (2005)

Chillo S, Ranawana DV, Pratt M, Henry CJK. Glycemic response and glycemic index of semolina spaghetti enriched with barley β-glucan Nutrition 27: 653-658 (2011)

Cui W, Wood PJ, Blackwell B, Nikiforuk J. Physicochemical properties and structural characterization by two-dimensional NMR spectroscopy of wheat β-D-glucans - Comparison with other cereal β-D-glucans. Carbohydrate Polymers. 41: 249-258 (2000)

Foster-Powell K, Holt SHA, and Brand-Miller JC. International table of glycemic index and gylcemic load values: 2002. Am J. Cli Nutr 76: 5-56 (2002)

Goni I, Garcia - Alons A, Saura-Calixto F. A starch hydrolysis procedure to estimate glycemic index. Nutr. Res. (N.Y) 17: 427-437 (1997)

Guleria P, Kumari S, Dangi N. β-glucan: health benefits and role in food industry-a review. J. ERS. Tech&Eng 8: 255-263 (2015)

Izydorczyk MS, Biliaderist CG, Macri LJ, Macgregor AW. Fractionation of oat (1→3),(1→4)-β-D-glucans and characteristics of the fractions. J. Cereal Sci. 27: 321-325 (1998)

Izydorczyk MS, Dexter JE. Barley β-glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products-a Reiview. Food Research International 41: 850-868 (2008)

Izydorczyk MS, Jacobs M, Dexter JE. Distribution and structural variation of non-starch polysaccharides in milling fractions of hull-less barely. Cereal Chem 80: 645-653 (2003)

Izydorczyk MS, Lagasse SL, Hatcher DW, Dexter JE, Rossnagel BG. The enrichment of Asian noodles with fibre-rich fractions derived from roller

milling of hull-less barley. J. Sci&Food Agr. 85: 2094-2104 (2005)

Izydorczyk MS, Storsley J, Labossiere D, MacGregor AW, Rossnagel BG. Variationin total and soluvle β-glucan content in hulless barely: effects of thermal, physical, and enzymic treatments. J. Agric. Food Chem. 48: 982-989 (2000)

Jadhav SJ, Lutz SE, Ghorpade VM, Salunkhe DK. Barley: Chemistry and value-added processing. Crit. Rev. Food Sci. 2: 123-171 (1998)

Jeong HS, Kang TS, Park HJ, Jung IS, Lee HY Characteristics of viscosity and components of soluble extract in oats. Food Eng. Prog. 8: 40-46 (2004)

Jiang G and Vasanrhan T. MALDI-MS and HPLC quantification of oligosaccharides of lichenase-hydrolysed water-soluble β-glucans from ten barley varieties. J. Agri&Food Chem. 48: 3305-3310 (2000)

Kahlon TS and Smith GE. *In vitro* binding of bile acids by bananas, peaches, pineapple, grapes, pears, apricots and nectarines. Food Chem. 101: 1046-1051 (2007)

Kahlon TS and Woodruff CL. *In vitro* binding of bile acids by rice bran, oat bran, barleyand β-glucan enriched barley. Cereal Chem. 80: 260-263 (2003)

Kalra S and Jood S. Effect of dietary barley β-glucan on cholesterol and lipoprotein fractions in rats. J. Cereal Sci. 31: 141-145 (2000)

Kim HJ and White PJ, Impact of the molecular weight, viscosity, and solubility of β-glucan on *in vitro* oat starch digestibility. J. Agric Food Chem. 61: 3270-3277 (2013)

Kim HJ and White PJ. Interactional effects of β-glucan, starch, and protein in heated oat slurries on viscosity and *In vitro* bile acid binding. J. Agric. Food Chem. 60: 6217-6222 (2012)

Kim HJ and White PJ. *In vitro* bile-acid binding and fermentation of high, medium, and low molecular weight β-glucan. J. Agric. Food Chem. 58: 628-634 (2010)

Kim HJ, White PJ. *In vitro* digestion rate and estimated glycemic index of

oat flours from typical and high β-glucan oat lines. J. Agric, Food Chem. 60: 5237-5242 (2012)

Kim HJ and White PJ. Optimizing the molecular weight of oat β-glcuan for *in vitro* bile acid binding and fermentation. J. Agric. Food Chem. 59: 10322-10328 (2011)

Lee SH, Jang GY, Kim HY, Woo KS, Hwang IG, Kim KJ, Lee MJ, Kim TJ, Lee J.S, Jeong HS. Physicochemical properties of barley β-glucan with different heating temperatures. Korean J. Soc. Food Sci. Nutr. 41: 1764-1770 (2012)

Lee WJ. Changes in dietary fiber content of barley during pealing and cooking. Korean J. Food Sci. Technol. 24:180-182 (1992)

Li W, Cui SW, Wang W, Yada RY. Studies of aggregation behaviors of cereal β-glucan in dilute aqueous solutions by light scattering: part I. Structure effects. Food Hydrocolloids. 25: 189-195 (2010)

MacGregor AW, Fincher GB. Carbohydrates of the barley grain. pp. 73-130. In: Barely Chemistry and Technology. MacGregor AW, Bathy RS (eds). American Association of Cereal Chemists, St Paul, MN, USA (1993)

McCleary BV. Development od and integrated total dietary fiber method consistent with the Codex Alimentarius definition. Cereal Foods World. 55: 24-28 (2010)

McClear BV, Holmes MG. Enzymatic quantification of (1-3),(1-4) β-D-glucan in barley and malt. J. I. Brewing 91: 285-295 (1985)

McCleary BV. Purification of (1→3)(1→4)-β-D-glucan from barley flour. pp. 511-514. In: Methods in Enzymology. Wood WA, Kellogg ST (eds). Academic Press, San Diego, CA, USA (1988)

Newman CW and Newman RK. A brief history of barley foods. Cereal Foods World. 51: 4-7 (2006)

Miller SS, Fulcher RG. Distribution of (1→3)(1→4)-β-D-glucan in kernels of oat and barley using microspectrofluorometry. Cereal Chem. 71: 64-68 (1994)

Papageorgiou M, Lakhdara N, Lazaridou A, Biliaderis CG, Izydorczyk MS. Water extractable (1→3, 1→4)-β-D-glucans from barley and oats: An intervarietal study on their structural features and rheological behavior. J. Cereal Chem. 42: 213-224 (2005)

Phillip DR, Stone BA. Water-soluble (1→3),(1→4)β-glucans from barley (Hordeum vulgare) endosperm. IV. Comparison of 40°C and 65°C soluble fractions. Carbohydr. Polym. 8: 85-97 (1988).

Quinde Z, Ulllrich S, Baik BK. Genotypic variation in colour and discolouration potential of barley-based food products. Cereal Chem. 81: 752-758 (2004)

Sayar A, Jannink JL, White PJ. *In vitro* bile acid binding of flours from oat lines varying in percentage and molecular weight distribution of β-glucan. J. Agric. Food Chem. 53: 8797-8803 (2005)

Saulnier L, Gevaudan S, Thibault JF. Extraction and partial characterization of β-glucan from the endosperms of two barley cultivars. J. Cereal Sci. 19: 171-178 (1994)

Siebenhandl S, Grausgruber H, Pellegrini N, Rio DD, Fogliano V, Pernice R, and Berghofer E. Phytochemical profile of main antioxidant in different fraction of purple and blue wheat, and black barley. J. Agric. Food Chem. 55: 8541-8547 (2007)

Singh J, Dartois A, Kaur L. Starch digestibility in food matrix: a review. Trends in Food Sci & Tech. 21: 168-180 (2010)

Skendi A, Billiaderis CG, Lazaridou A, Izydorczyk MS. Structure and rheological properties of water soluble β-glucans from oat cultivars of Avena sativa and Avena bysantina. J. Cereal Sci. 38: 15-31 (2003)

Stone BA, Clarke AE. (1→3, 1→4)-β-Glucan in higher plants. pp. 431-489. In: Chemistry and Biology of (1→3)-β-Glucans. Stone BA, Clarke AE (eds). La Trobe University Press, Victoria, Australia (1992)

Susanne S, Heinrich G, Nicoletta P, Daniele DR, Vincenzo F, Rira P,

Emeerich B. Phytochemical profile of main antioxidants in different fractions of puple and blue wheat, and black barley. J. Agr. Food Chem. 55: 8541-8547 (2007)

Thondre PS and Henry CJK. High-molecular-weight barley β-glucan in chapatis (unleavened Indian flatbread) lowers glycemic index. Nutrition Research. 29: 480-486 (2009)

Tosh SM, Wood PJ, Wang Q. Gelation characteristics of acid-hydrolyzed oat beta-glucan solutions solubilized at a range of temperature. Food Hydrocolloids. 17: 523-527 (2003)

Tudorica CM, Kuri V, Brennan CS. Nutritional and physicochemical characteristics of dietary fiber enriched pasta. J. Agri. Food Chem 50: 347-356 (2002)

Wood PJ. Cereal β-glucans in diet and health. J. Cereal Sci. 46: 230-238 (2007)

Wood PJ. Relationship between solution properties of cereal β-glcuans and physiological effect - A review. Trend in Food Sci&Tech 15: 646-320 (2004)

Wood PJ, Siddiqui IR, Paton D. Extraction of high viscosity gums from oat. Cereal Chem. 55: 1038-1049 (1978)

Wood PJ, Weisz J, Blackwell BA. Molecular characterization of cereal β-D-glucans. Structural analysis of oat β-D-glucan and rapid structural evaluation of β-D-glucans from different sources by high performance liquid chromatography of oligosaccharides released by lichenase. Cereal Chem. 68: 31 - 39 (1991)

Woodward JR, Fincher GB, Stone BA. Water-soluble (1→3), (1→4)-β-D-glucans from barley (Hordeum vulgare) endosperm. II. Fine structure. Carbohydr. Polym. 3: 207-225 (1980)

Yao N, White PJ, Jannink JL, Alavi S. Impact of drt solids and bile acid concentrations on bile acid binding capacity of extruded oat cereals. J.

Agric. Food Chem. 56: 8672-8679 (2008)

Zhang Q, Abe T, Takahachi T, Sarahara T. Variations in *in vitro* starch digestion of glutinous rice flour. J. Agric. Food Chem. 44:2672-2674(1996)

Zheng GH and Sosulski FW. Determination of water separation from cooked starch and flour pastes after refrigeration and freeze-thaw. J. Food Sci. 63: 134-139 (1998)

Zhou M, Robards K, Glennie-Holmes M, Helliwell S. Structure and pasting properties of oat starch. Cereal Chem. 75: 273-281 (1998)

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