

Research Note

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

Hyo Jin Kim and Hyun Jung Kim*

Department of Food Bioengineering, Jeju National University

Abstract The effect of different temperatures (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, 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 optimally extracted at 65°C for 3 h and Jeju beer barley at 75°C. The extracted β -glucan resolubilized to 43.48–81.73% and the ratio of $\beta(1\rightarrow3)$ to $\beta(1\rightarrow4)$ linkage was in the range of 1:3.8–5.8. These results suggest that purification and properties of β -glucan depend not only on the water extraction temperature, but also on the barley variety.

Keywords: barley, β -glucan, resolubility, $\beta(1\rightarrow3)$: $\beta(1\rightarrow4)$ linkage, water extraction

Introduction

Barley has mainly been used for malting and feed purpose and offers a natural fiber in food products (1). Barley is a rich source for carbohydrates like starch, cellulose, and β -glucan (2). 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 (3). β -Glucans are composed of glucoses with $\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$ mixed linkage in ratio of about 2.3–3.0 to 1.0 (4). β -Glucans from different types of cereals showed the similar molecular structure but exhibited variation in the ratios of $\beta(1\rightarrow4)$ to $\beta(1\rightarrow3)$ linkages and molecular weights (5). With the structural characteristics, β -glucan can impart high in viscosity to aqueous solution (6) and nutritional effects (7). 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 (3,4).

About 75% of β -glucans are found in the barley endosperm cell wall and distributed uniformly throughout the endosperm (8). The β -glucans in the endosperm cell wall may be covalently bonded to protein forming large molecules (9). The extraction and purification of β -glucans in barley can be affected by flour particle size, temperature, pH, and ionic strength (10). 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 (11,12). As the steps for extraction were added, the purification of β -glucans were improved (10,11). Mostly, β -glucan purification was dependent on temperature of solvent and duration of extraction (12). 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.

Materials and Methods

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.

Chemical composition of barley flours

Chemical composition of barley flours were determined by AOAC method and AACC method (13,14). Moisture content of barley flours was measured with a moisture analyzer (MX-50, AND Co., Ltd., 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 76.13, and AOAC method 991.43 using

*Corresponding author: Hyun Jung Kim, Department of Food Bioengineering, Jeju National University, 102 Jejudaehakno, Jeju 63243, Korea
Tel: +82-64-754-3614
Fax: +82-64-755-3601
Email: hyunjkim@jejunu.ac.kr
Received April 8, 2016; revised May 10, 2016;
accepted May 11, 2016

assay kits (Megazyme International Co., Wicklow, Ireland).

Viscosity of barley suspension

The viscosity of barley suspension was determined by modification of the procedure of Jeong *et al.* (15). Barley flour suspension was prepared by 1:8, barley flour to water 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°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.

Extraction of β -glucan

β -Glucan was extracted from barley using the procedure of Kim and White (16) with modifications. Barley flour (15 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 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 a shaking water bath (JSSB-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 extraction yield.

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.* (17) with modifications. The β -glucan dispersion in water (1%, w/v) was agitated at 50°C for 12 h and centrifuged at 12,000×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)×100.

Ratio of $\beta(1\rightarrow3)$ to $\beta(1\rightarrow4)$ linkages in extracted β -glucans

Ratio of $\beta(1\rightarrow3)$ to $\beta(1\rightarrow4)$ linkages was determined to compare the structure of extracted β -glucan (18). Content of binding $\beta(1\rightarrow3)$ glucoses was determined as following: β -glucan (2 mg) was added to 1 mL sodium phosphate buffer (20 mM, pH 6.5) with 4U lichenase (Megazyme International Co.) which divided the $\beta(1\rightarrow4)$ 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×g for

10 min. After obtained 0.1 mL supernatant, content of total glucose was determined as follows: β -glucan (2 mg) was added to 1 mL sodium phosphate buffer (20 mM, pH 6.5) with 4U lichenase (Megazyme International Co.) and incubated at 40°C for 22 h. Reactant was treated with β -glucosidase (0.2U, Megazyme International Co.) at 50°C for 10 min. After the obtained 0.1 mL suspension, glucose content was determined. The $\beta(1\rightarrow4)$ linked glucoses was calculated by subtracting $\beta(1\rightarrow3)$ linked glucoses from total glucoses and ratio of $\beta(1\rightarrow3)$ to $\beta(1\rightarrow4)$ linkages was calculated as the amounts of $\beta(1\rightarrow4)$ linked glucose/ $\beta(1\rightarrow3)$ linked glucose.

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).

Results and Discussion

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% (9). 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 (19), 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 (20). MacGregor and Fincher (21) reported a 4.0–7.0% of β -glucan in barley. And Batty (22) 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 (23). 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

Table 1. Chemical composition¹⁾ 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 \pm 0.09 ^{a 2), 3)}	2.43 \pm 0.16 ^a	1.14 \pm 0.09 ^{ab}	77.44 \pm 1.62 ^a	10.55 \pm 1.21 ^a	6.85 \pm 0.40 ^a	39.70 \pm 0.71 ^a	433.00 \pm 12.73 ^b
Jeju blue barley	7.70 \pm 0.07 ^d	2.45 \pm 0.17 ^a	1.29 \pm 0.21 ^{ab}	72.86 \pm 3.18 ^a	7.53 \pm 2.09 ^b	5.13 \pm 0.37 ^b	33.00 \pm 0.28 ^b	1116.00 \pm 8.49 ^a
Jeju beer barley	9.10 \pm 0.08 ^c	2.46 \pm 0.06 ^a	1.05 \pm 0.12 ^b	73.73 \pm 0.96 ^a	5.69 \pm 0.59 ^b	3.58 \pm 0.38 ^c	22.62 \pm 0.28 ^c	85.50 \pm 1.84 ^c
Black barley	12.00 \pm 0.09 ^b	2.11 \pm 0.21 ^b	1.34 \pm 0.09 ^a	73.53 \pm 2.76 ^a	7.41 \pm 0.84 ^b	4.16 \pm 0.25 ^c	21.10 \pm 0.42 ^d	75.00 \pm 0.28 ^c

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

²⁾Each value is mean \pm standard deviation.

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

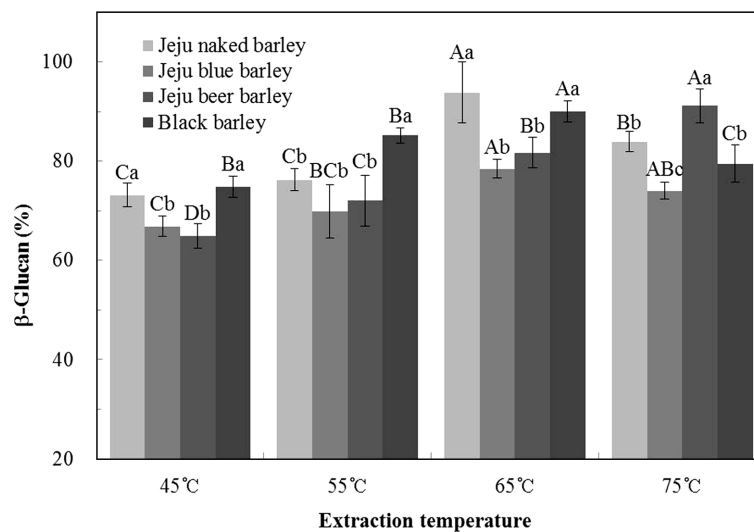


Fig. 1. β -Glucan concentration after extraction of different barley varieties at 45, 55, 65, and 75°C. Data are expressed as mean \pm standard deviation. Means with different capital letters on the bars indicate significant difference ($p < 0.05$) within the same barley variety and means with small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

room temperature, respectively. The viscosity of Jeju naked barley, which β -glucan was the highest as 6.85%, was the highest. Aastrup (24) 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 (25). According to Izydorczyk *et al.* (23) 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.

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 -glucan 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.* (25) 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 (26) 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, Saulnier *et al.* (27) used a hot water extraction procedure in the presence of thermostable α -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.

Resolubility and ratio of $\beta(1 \rightarrow 3)$ to $\beta(1 \rightarrow 4)$ 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

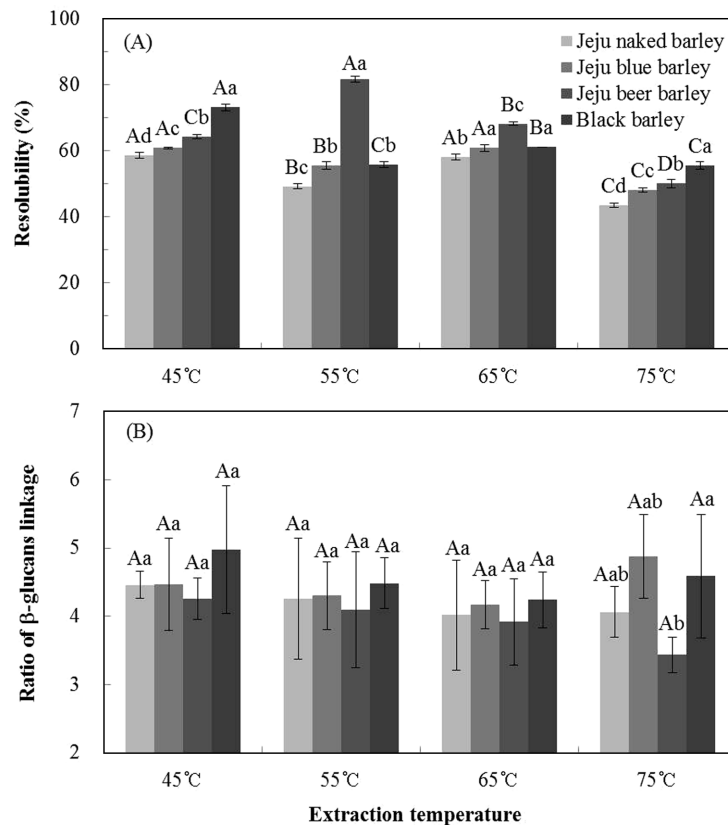


Fig. 2. Resolubility (A) and ratio of β -(1 \rightarrow 3) to β -(1 \rightarrow 4) linkages (B) of extracted β -glucan from different barley varieties at 45, 55, 65, and 75°C. Data are expressed as mean \pm standard deviation. Means with different capital letters on the bars indicate significant difference ($p < 0.05$) 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.

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 (28) so the β -glucan with high resolubility possibly contained low molecular weight β -glucan. Resolubility was also influenced by the ratio of β (1 \rightarrow 3): β (1 \rightarrow 4) linkages (5). Since the β (1 \rightarrow 3) linkage broke up the regularity of the β (1 \rightarrow 4) linkage in β -glucan molecules, the molecule could be more soluble and flexible to be high resolubility.

The ratio of β (1 \rightarrow 3) and β (1 \rightarrow 4) linkages in β -glucan is an important factor in determining the physical properties, such as solubility, viscosity and gel formation (8). The ratio of β (1 \rightarrow 3) to β (1 \rightarrow 4) linkages in β -glucan extracts is shown in Fig. 2(B) and ranged from 3.44 to 4.98. Izydorczyk *et al.* (6) reported that the ratio of β (1 \rightarrow 3) to β (1 \rightarrow 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 (29) indicated that the more β -glucan purified, the more ratio of β (1 \rightarrow 3): β (1 \rightarrow 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.* (6). Thus, the extraction procedure in this study was optimized to purify β -glucan highly.

Conclusions

Chemical compositions including β -glucan concentration were dependent on barley varieties 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 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 Jeju beer barley was 75°C. Resolubility of extracted β -glucan was dependent on barley varieties and extraction temperature. Ratio of β (1 \rightarrow 3) to β (1 \rightarrow 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 extraction temperature of water. It is possible to produce highly purified and uniform β -glucan which can impact nutritional and textural properties of food developed with barley.

Acknowledgment

This research was supported by the 2015 scientific promotion program funded by Jeju National University.

References

- Jadhav SJ, Lutz SE, Ghorpade VM, Salunkhe DK. Barley: Chemistry and value-added processing. *Crit. Rev. Food Sci.* 38: 123-171 (1998)
- McCleary BV, Holmes MG. Enzymatic quantification of (1-3),(1-4) β -D-glucan in barley and malt. *J. I. Brewing* 91: 285-295 (1985)
- Wood PJ. Cereal β -glucans in diet and health. *J. Cereal Sci.* 46: 230-238 (2007)
- Stone BA, Clarke AE. (1 \rightarrow 3, 1 \rightarrow 4)- β -Glucan in higher plants. pp. 431-489. In: *Chemistry and Biology of (1 \rightarrow 3)- β -Glucans*. Stone BA, Clarke AE (eds). La Trobe University Press, Victoria, Australia (1992)
- 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)
- Izydorczyk MS, Biliaderist CG, Macri LJ, Macgregor AW. Fractionation of oat (1 \rightarrow 3),(1 \rightarrow 4)- β -D-glucans and characteristics of the fractions. *J. Cereal Sci.* 27: 321-325 (1998)
- 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)
- Miller SS, Fulcher RG. Distribution of (1 \rightarrow 3)(1 \rightarrow 4)- β -D-glucan in kernels of oat and barley using microspectrofluorometry. *Cereal Chem.* 71: 64-68 (1994)
- Baik BK, Steven EU. Barley for food: Characteristics, improvement, and renewed interest. *J. Cereal Sci.* 45: 233-242 (2008)
- Wood PJ, Siddiqui IR, Paton D. Extraction of high viscosity gums from oat. *Cereal Chem.* 55: 1038-1049 (1978)
- Charles SB, Louise JC. The potential use of cereal (1 \rightarrow 3,1 \rightarrow 4)- β -D-glucans as functional food ingredient. *J. Cereal Sci.* 42: 1-13 (2005)
- Izydorczyk MS, Storsley J, Labossiere D, MacGregor AW, Rossnagel BG. Variation in total and soluble β -glucan content in hull-less barley: Effects of thermal, physical, and enzymatic treatment. *J. Agr. Food Chem.* 48: 982-989 (2000)
- AOAC. Official methods of analysis of AOAC Intl. 16th ed. Method 991.43. Association of Official Analytical Chemists, Arlington, VA, USA (1995)
- AACC International. Approved Method of the AACC, 10th ed. Method 21-23.01, 76.13. Association of Cereal Chemists, St. Paul, MN, USA (2000)
- 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)
- Kim HJ, White PJ. In vitro bile-acid binding and fermentation of high, medium, and low molecular weight β -glucan. *J. Agr. Food Chem.* 58: 628-634 (2010)
- Lee SH, Jang GY, Kim HY, Woo KS, Hwang IG, Kim KJ, Lee MJ, Kim TJ, Lee JS, Jeong HS. Physicochemical properties of barley β -glucan with different heating temperatures. *Korean J. Soc. Food Sci. Nutr.* 41: 1764-1770 (2012)
- Woodward JR, Fincher GB, Stone BA. Water-soluble (1 \rightarrow 3), (1 \rightarrow 4)- β -D-glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure. *Carbohydr. Polym.* 3: 207-225 (1980)
- Lee WJ. Changes in dietary fiber content of barley during peeling and cooking. *Korean J. Food Sci. Technol.* 24:180-182 (1992)
- Bamforth CW. Barley β -glucans. Their role in malting and brewing. *Brew. Dig.* 57: 22-27 (1982)
- MacGregor AW, Fincher GB. Carbohydrates of the barley grain. pp. 73-130. In: *Barley Chemistry and Technology*. MacGregor AW, Bathy RS (eds). American Association of Cereal Chemists, St Paul, MN, USA (1993)
- Batty RS. Physicochemical properties of roller-milled barley bran and flour. *Cereal Chem.* 70: 397-402 (1993)
- Izydorczyk MS, Storsley J, Labossiere D, MacGregor AW, Rossnagel BG. Variation in total and soluble β -glucan content in hull-less barley: effects of thermal, physical, and enzymatic treatment. *J. Agr. Chem.* 48: 982-989 (2000)
- Aastrup S. The relationship between the viscosity of an acid flour extract of barley and its β -glucan content. *Carlsberg Res. Commun.* 44: 289-304 (1979)
- Wood PJ, Siddiqui IR, Paton D. Extraction of high-viscosity gums from oat. *Cereal Chem.* 55: 1038-1049 (1978)
- McCleary BV. Purification of (1 \rightarrow 3)(1 \rightarrow 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)
- 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)
- Buliga GS, Brant DA, Fincher GB. The sequence statistics and solution conformation of a barley (1 \rightarrow 3, 1 \rightarrow 4)- β -glucan. *Carbohydr. Res.* 157: 139-156 (1986)
- Phillip DR, Stone BA. Water-soluble (1 \rightarrow 3),(1 \rightarrow 4) β -glucans from barley (*Hordeum vulgare*) endosperm. IV. Comparison of 40 and 65°C soluble fractions. *Carbohydr. Polym.* 8: 85-97 (1988)