MALDI-MS and HPLC Quantification of Oligosaccharides of Lichenase-Hydrolyzed Water-Soluble β-Glucan from Ten Barley Varieties

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This study is the first to apply matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) to both qualitative and quantitative analyses of oligosaccharides of lichenase-hydrolyzed water-soluble β-glucan from barley. Compared to high-performance liquid chromatography (HPLC) with an evaporative light-scattering detector, MALDI-MS is a rapid technique with high accuracy and sensitivity and could be used to assess primary structural features of water-soluble β-glucan from different barley varieties.

Keywords: *Barley; β-glucan; MALDI-MS; oligosaccharides; quantification*

INTRODUCTION

Barley $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan ($\beta$-glucan) is the major component of barley endosperm cell walls, accounting for about 75% (w/w) of total cell-wall carbohydrates, the remainder (20−25%, w/w) being of protein and pentosans (arabinoxylan) (Fincher, 1975). Barley β-glucan could be used as a thickening agent in food applications due to its ability to develop high viscosity in aqueous solutions (McIntosh et al., 1991; Autio et al., 1992), but its excess could cause problems in brewing (Bamforth, 1985). Similar to other cereal β-glucans, barley β-glucan can also improve glucose regulation and reduce serum cholesterol levels in diabetic and hypercholesterolemic subjects, respectively (Bhatty, 1999). These physical and physiological properties of barley β-glucan depend largely on its molecular characteristics including its structural diversity.

β-Glucan from barley is a linear chain of glucose residues, containing about 70% $\beta(1\rightarrow4)$ and 30% $\beta(1\rightarrow3)$ glycosidic links (Igarashi and Sakurai, 1966; Woodward et al., 1983; Wood et al. 1994). Lichenase, a $(1\rightarrow3),(1\rightarrow4)$-$\beta$-D-glucan-4-glucanohydrolase, can cleave the $(1\rightarrow4)$ linkage of 3-O-substituted glucose units in β-glucan (Figure 1). The oligosaccharides released by lichenase hydrolysis are considered building blocks or fingerprints of the $\beta(1\rightarrow4)$-linked polysaccharide in the original chain. Analysis of the resulting oligosaccharides would allow the evaluation of primary structural features of β-glucans (Wood et al., 1994).

Several techniques have been used to analyze the oligosaccharides from lichenase-hydrolyzed barley β-glucan. Paper chromatography has been used, but this technique is time-consuming (approximately 47 h) and various enzymatic and colorimetric procedures have also been employed to identify the oligosaccharides from the hydrolyzed β-glucan (Clarke and Stone, 1966). Oligosaccharides released from β-glucans of oat products, barley, wheat, and rye have been analyzed by a high-perfor-
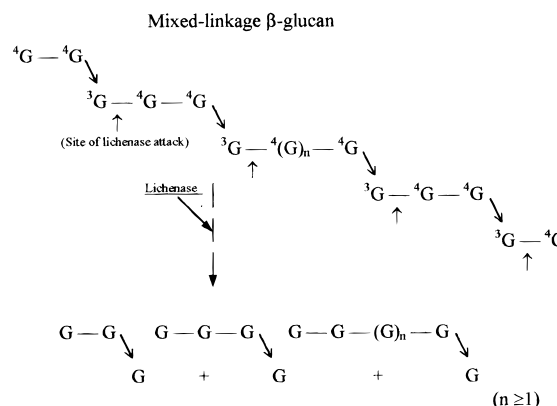

Figure 1. Hydrolytic action of lichenase on the mixed-linkage β-glucan.

mance liquid chromatography (HPLC) technique, which was developed by Wood and Weisz (1986) and later refined by Wood et al. (1991). However, this technique did not adequately resolve the oligosaccharides with degree of polymerization (DP) > 4 and, thus, provided inadequate information as to which oligosaccharides were present in the barley β-glucan chain. Derivatization of oligosaccharides released from barley β-glucan by lichenase hydrolysis, using potassium methylsulphinyl carbanion (Edney et al., 1991) and pyridylamination (Izawa et al., 1993), improved the HPLC resolution for oligosaccharides with higher DP, but derivatization with water sensitive reagents was required. By far the most used technique for analysis of homogeneous D-gluco-oligosaccharides and -polysaccharides (DP $\geq$ 50) is high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Koizumi et al., 1989). Wood et al. (1994) successfully applied it to analyze the oligosaccharides from lichenase-hydrolyzed barley β-glucan. However, the quantification of oligosaccharides by HPAEC-PAD was limited to the knowledge of weight response factors (Wood et al., 1994). In general, the sensitivity of PAD decreased rapidly from DP2 to DP6, while, for higher oligosaccharides (DP 7−17), the decrease in the sensitivity of PAD was

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minimal (Timmermans et al., 1994). The behavior of the mixed-linkage oligosaccharides in this study was similar to that of cellodextrins and other maltooligosaccharides reported by Koizumi et al. (1989) and van Riel and Olieman (1991). Initially, there was a rapid decline in weight response from glucose (weight response, 1.0) to cellobiose (0.65), but the rate of decline slowed after DP2 (0.4−0.5).

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was originally used for determining the mass of large molecules such as proteins. MALDI-MS has also been used in molecular sizing and quantification of carbohydrates (Mock et al., 1991; Stahl et al. 1991; Wang et al., 1999a, 1999b). In our previous study (Wang et al., 1999a), it was found that a standard addition method [addition of a pure maltooligosaccharide internal standard (DP 7) to the debranched starch mixture] could be used to determine both the relative and absolute amounts of each maltooligo/polysaccharide using the weight concentration relationship, i.e., for individual maltooligo/polysaccharide, summation of all the MALDI-MS ion responses ($[M + Na]^+$ plus $[M + K]^+$ plus ($[M - H_2O + Na]^+$) giving an excellent linear correlation on a weight basis. This measurement is rapid and highly sensitive, and the technique often requires minimal sample purification. The objective of this study was to use MALDI-MS for both qualitative and quantitative analysis of the oligosaccharides, released from water-soluble barley β-glucan upon hydrolysis by lichenase. Also, an assessment of the variations in the primary structural features of $(1{\rightarrow}3),(1{\rightarrow}4)$-$\beta$-D-glucans from different barley varieties was carried out.

MATERIALS AND METHODS

General Methods. Lichenase (EC 3.2.1.73, *Bacillus subtilis*) and pullulanase (EC 3.2.1.41, *Bacillus acidopullulyticus*) were obtained from Megazyme International Ireland Ltd. (Wicklow, Ireland). Glucose, maltose, maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6), maltoheptaose (DP7), and α-amylase were purchased from Sigma Chem. Co. (St. Louis, MO). 2,5-Dihydroxybenzoic acid (DHB) was obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). Ten varieties of barley were provided by Dr. B. G. Rossnagel, from the University of Saskatchewan, which were ground using a UDY cyclone sample mill with 0.5 mm screen. The moisture of barley flour was determined according to the standard method of AACC (1982). Extraction of barley starch from flour and the determination of amylose content were carried out with the methods of Vasanthan and Bhatty (1995) and Chrastil (1987), respectively. The determination of total β-glucan in barley flour and the purity of water-soluble β-glucan extracted (room temperature) from barley flour followed the procedures for mixed-linkage β-glucan provided by Megazyme International Ireland Ltd. (Wicklow, Ireland) with their test kits.

Extraction and Purification of Water-Soluble β-Glucan from Barley. Barley flour was mixed with distilled water at a ratio of 1:10 (w/v) and stirred overnight at room temperature. The mixture was centrifuged at 7000 rpm for 15 min to obtain the first supernatant. The residue was dispersed in distilled water, stirred for 2 h, and then centrifuged to get the second supernatant. The combined supernatant was hydrolyzed with α-amylase and pullulanase at 40 °C for 16 h to convert starch fragments and oligosaccharides into glucose, heated in a boiling water bath for 5 min to kill the enzymes, and then filtered through a Whatman No. 1 filter paper (Jiang and Ramsden, 1999). Absolute ethanol was added to the filtrate so that the ethanol content in the aqueous mixture was 60% (v/v). The solution was allowed to stand for 2 h and then centrifuged at 5000 rpm for 10 min to obtain the precipitate

(crude water-soluble β-glucan). The crude water-soluble β-glucan was washed with absolute ethanol and centrifuged to remove the residual soluble glucose and obtain the "purified" water-soluble β-glucan. The "purified" water-soluble beta-glucan (still containing small amounts of protein and arabinoxylans) was further dispersed in distilled water, rotoevaporated at room temperature to remove the residual ethanol, and then freeze-dried.

Preparation of Barley β-Glucan Hydrolysates. To about 300 mg of purified water-soluble β-glucan, 1.0 mL of 50% (v/v) ethanol, and 12 mL of sodium phosphate buffer (20 mM, pH 6.5) were added, stirred, and incubated in a boiling water bath for 5 min. After being cooled to room temperature, the mixture was diluted with distilled water to a volume of 27 mL, followed by the addition of 3 mL of lichenase (150U) and incubation at 40 °C for 1.5 h. The β-glucan hydrolysate was heated in a boiling water bath for 5 min to kill the enzyme, cooled, and centrifuged at 10 000 rpm for 10 min. The supernatant (1 mL) was used for HPLC analysis to determine DP3 and DP4. The supernatant (about 25 mL) was rotoevaporated close to dryness and then diluted to 3 mL of a solution, which was then used for HPLC analysis to determine DP 5−7. The original supernatant (2 mL) was passed through a Sephadex G10 column (1.8×13 cm) for desalting. A flow rate of 0.7 mL/min was maintained as 1-mL fractions were collected. Fractions 10−17 (sugar peak separated from salt) were combined and made up to 8 mL. This sample fraction was suitable for MALDI-MS analysis without further purification.

HPLC. An HPLC system, equipped with a Shimadzu Ezchrom chromatography data system, a Varian 9010 solvent delivery system (Sunnyvale, CA), an HP Series 1050 autosampler, a Jordi Gel DV 13 polyamine (250 mm length $\times$ 4.6 mm i.d.) column (Bellingham, MA), and an evaporative light-scattering detector (Alltech. 500 ELSD, Mandel Scientific Co. Ltd., Guelph, ON, Canada), was used for oligosaccharide determination. Eluents A and B were distilled water and acetonitrile, respectively. The solvent gradient used was as follows: A 10% (v/v) and B 90% (v/v) at the beginning; A 40% (v/v) and B 60% (v/v) at 25 min; A 0% (v/v) and B 100% (v/v) at 26 min; A 10% (v/v) and B 90% (v/v) at 30 min. The detector temperature was set at 125 °C, and a flow rate of 1.0 mL/min was maintained. Standard glucose, maltose, DP3, DP4, DP5, DP6, and DP7 were used. The percentage of individual peak was calculated as the ratio between the area of a given peak and that of total peak area.

MALDI-MS. MALDI-MS was performed using the Bruker Proflex III linear mode, Bruker Analytical Systems Inc. (Billerica, MA). Oligosaccharides, which cocrystallized with matrix on the probe using the solvent evaporation, were desorbed and ionized by a nitrogen laser pulse (337 nm) and then accelerated under 20 kV with time-delayed extraction before entering the time-of-flight mass spectrometer. DHB (2,5-dihydroxybenzoic acid) was used as matrix. The preparation of matrix followed Wang et al. (1999b). Laser strength was selected on the basis of the degree of polymerization of oligosaccharides to obtain good signal-to-noise ratios. The number of laser pulses was received as 3×60 or 180 laser pulses for a final MALDI-MS spectrum.

Quantification of oligosaccharides (DP3−DP12) using MALDI-MS was achieved using the standard addition method (Wang et al., 1999a; Wang et al., 1999b). Maltoheptaose (DP7, 32.096 μg/mL) was used as an added standard. The above desalted oligosaccharide samples from lichenase-hydrolyzed barley β-glucan were mixed with distilled water or the above standard DP 7 (2:1, v/v). Samples (without DP 7) and standard addition samples were then mixed with the matrix (1:1, v/v). The following detail procedures were carried out with the methods of Wang et al. (1999b). For individual maltooligo/polysaccharide, summation of all ion responses occurred ($[M + Na]^+$ plus $[M + K]^+$ plus ($[M - H_2O + Na]^+$), giving an excellent linear correlation on a weight basis (Wang et al., 1999a). Concentration of individual maltooligosaccharides in the standard mixture was as follows: DP3 = 0.82 mg/mL; DP4 = 0.93 mg/mL; DP5 = 1.22 mg/mL; DP6 = 1.63 mg/mL; DP7 = 1.69 mg/mL.

Analysis of Lichenase-Hydrolyzed Barley β-Glucan

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Table 1. Extractable Percentage and Purity of Water-Soluble (WS) β-Glucan from 10 Varieties of Barley[a]

	amylose (%)[b]	β-glucan (%)[c]	WS β-glucan (%)[d]	purity of β-glucan
		Waxy		
SB94794	0.25(0.16)	7.27(0.49)	57.90(1.21)	83.28(1.29)
Candle	4.82(0.63)	6.38(0.33)	63.90(0.95)	82.84(1.57)
SB94912	5.62(0.64)	7.14(0.44)	53.16(0.87)	76.42(0.10)
SB94917	4.51(0.76)	7.41(0.03)	47.70(1.03)	77.28(0.71)
		Regular		
CDC Dawn	22.26(1.33)	3.65(0.52)	39.23(0.41)	72.06(0.20)
Phoenix	23.72(1.45)	4.44(0.17)	38.42(0.37)	70.78(1.12)
SB94860	23.80(1.37)	6.25(0.02)	40.69(0.56)	71.17(0.75)
SR93102	29.54(1.68)	5.94(0.58)	33.57(0.39)	71.26(0.15)
		High Amylose		
SB94893	48.30(2.79)	7.04(0.49)	43.38(0.33)	65.41(0.31)
SB94897	41.38(2.52)	7.67(0.01)	47.23(0.68)	66.09(0.73)

[a] All the results were obtained in triplicate on dry weight basis (w/w). Standard deviations are in parentheses. [b] Amylose content of purified barley starch. [c] β-Glucan content of barley flour. [d] Extractable water-soluble β-glucan was expressed as % of total β-glucan.

RESULTS AND DISCUSSION

Extraction of Water-Soluble β-Glucan from Barley. Solubility and viscosity of β-glucan in aqueous solutions depend mainly upon the concentration and molecular characteristics of water-soluble β-glucan (Gómez et al., 1997). The data given in Table 1 show that the waxy barley has a higher content of both total β-glucan and extractable, water-soluble β-glucan than regular barley. For waxy varieties, the water-soluble β-glucan [% (w/w) of total β-glucan] ranged from 47% to 64%, whereas, for regular and high amylose varieties, the range was from 33% to 47%. These results were in agreement with the previously reported data (Bhatty, 1987; Bhatty et al., 1991; Knuckles et al., 1992; Lee et al., 1997). In addition, the purity of water-soluble β-glucan, extracted from waxy barley, was higher than that of regular and high amylose varieties. This may be due to the differences among varieties in the contents of nonstarch polysaccharide (i.e. hemicellulose) and protein.

Qualitative Analysis of Oligosaccharides by HPLC. The content of soluble oligosaccharides, produced by the action of lichenase on purified barley β-glucan, did not change after 0.5 h of hydrolysis. Therefore, it may not be necessary to extend the treatment longer than 0.5 h. However, routine analysis often uses 1.5 h for hydrolysis (Wood et al., 1994). The relative percentage of each oligosaccharide is given in Table 2. Glucose and cellobiose were not found in any of lichenase-hydrolyzed water-soluble barley β-glucan samples. Compared to previously published data by Edney et al. (1991) and Wood et al. (1994), the results (the relative percentages of DP 3–DP 7 in Table 2) were more comparable to those of the former. The relative percentages of DP4–DP7 (Table 2) were somewhat higher than those of the latter. HPAEC-PAD has a higher sensitivity than an HPLC connected to an evaporative light-scattering detector and thus can detect oligosaccharides of higher DP (DP > 7). On the other hand, due to unavailability of proper standards, the PAD response factors for oligosaccharides with higher DP are not known. Therefore, it is likely that the slightly low relative percentages of DP4–DP7 reported by Wood et al. (1994) were due to the variations in the sensitivity of techniques employed.

Quantification of Oligosaccharides by MALDI-MS and HPLC. For successful MALDI-MS quantification of oligosaccharides, selection of an appropriate matrix and the conditions of sample preparation are very important. The matrix should provide a good spot-to-spot repeatability and a linear response for a series of oligosaccharides of interest. If possible, fragmentation of the oligosaccharides or their reaction with the matrix should be avoided, and there should be no discrimination against high and low mass ions at a single run/scan. A protocol has been successfully developed in our laboratory to quantify molecular characterization of maltooligo/polysaccharides from debranched starch amylopectin of corn and barley using MALDI-MS (Wang et al. 1999a). In our experiments, 2,5-dihydroxybenzoic acid (DHB) was used as the selected matrix since it has been proven to be a suitable matrix for quantification of maltooligosaccharides using MALDI-MS (Wang et al., 1999a). Using DHB as the matrix and an excess of sodium chloride (0.01 M concentration), the responses (Figure 2) for the predominant ionic form (molecular mass of positive ion plus one sodium atom, $[M + Na]^+$) for each standard (DP 3–DP 7) were linear with almost the same slope but different intercept as Wang et al. (1999a), indicating that the MALDI-MS machine was operated properly in this study. The supernatant of lichenase-hydolyzed water-soluble barley β-glucan was passed through a desalting column (Sephadex G10), and the fractions were pooled before analysis by MALDI-MS. The MALDI-MS spectrum of oligosaccharides released from purified water-soluble barley β-glucan is shown in Figures 3. It was found that the oligosaccharide with the same DP released by lichenase hydrolysis, from water-soluble barley β-glucan, formed the same molecular adduct ions, e.g. sodium and potassium adduct ions (Figure 3) as maltooligosaccharides (Wang et al., 1999a).

There is a structural difference between maltooligosaccharides used as standards and the mixed-linkage oligosaccharides released by lichenase hydrolysis, from water-soluble barley β-glucan. Maltooligosaccharides are a linear chain of glucose residues connected by α(1→4) glycosidic linkages. Oligosaccharides released by lichenase hydrolysis, from water-soluble barley β-glucan, are a linear chain of β-glucose residues but have a kink due to β(1→3) linkage at the terminal end (Figure 1). However, in view of the very similar behavior (weight responses in PAD) between maltooligosaccharides and the mixed-linkage oligosaccharides from barley β-glucan and the lack of authentic standards of mixed-linkage oligosaccharides, a series of concentrations for each of the authentic maltooligosaccharides (DP3–DP7) was used to construct standard curves and their HPLC responses, which were then used to quantify the oligosaccharides (DP3–DP7) in the water-soluble barley β-glucan hydrolysate. A standard addition method was employed in MALDI-MS to determine both the relative and absolute amounts of each oligosaccharide using the weight concentration relationship, i.e., for individual maltooligo/polysaccharide, summation of all ion responses ($[M + Na]^+$ plus $[M + K]^+$ plus ($[M–H_2O + Na]^+$) giving an excellent linear correlation on a weight basis (Wang et al., 1999a). The data obtained from both analyses are given in Table 3.

It was evident that the sensitivity of MALDI-MS to oligosaccharides from lichenase-hydrolyzed water-soluble barley β-glucan was higher than that of HPLC with an

Table 2. Relative Percentages of Oligosaccharides of Lichenase-Hydrolyzed Water-Soluble β-Glucan from 10 Varieties of Barley As Determined by HPLC[a]

	DP3	DP4	DP5	DP6	DP7
			Waxy		
SB94794	60.08(0.89)	30.51(0.18)	5.35(0.54)	2.55(0.18)	1.50(0.01)
Candle	60.66(0.69)	30.44(0.13)	5.18(0.76)	2.27(0.44)	1.44(0.37)
SB94912	63.17(0.09)	30.18(0.01)	4.56(0.32)	2.07(0.30)	nd
SB94917	60.95(0.35)	31.25(0.04)	5.43(0.10)	2.37(0.12)	nd
average	61.22(1.35)	30.60(0.46)	5.13(0.39)	2.32(0.20)	0.74(0.85)
			Regular		
CDC Dawn	58.81(0.16)	32.77(0.01)	5.55(0.21)	2.86(0.05)	nd
Phoenix	56.74(0.91)	34.95(1.31)	5.84(0.48)	2.47(0.08)	nd
SB94860	60.02(0.37)	32.06(0.10)	5.36(0.69)	2.56(0.65)	nd
SR93102	60.44(0.68)	31.88(1.15)	5.14(0.21)	2.53(0.25)	nd
average	59.00(1.66)	32.92(1.41)	5.47(0.30)	2.61(0.17)	nd
			High Amylose		
SB94893	57.71(0.40)	31.17(0.88)	5.85(1.09)	3.38(0.31)	1.88(0.05)
SB94897	58.62(0.67)	32.07(0.17)	6.18(0.19)	3.13(0.33)	nd
average	58.17(0.64)	31.62(0.64)	6.02(0.23)	3.26(0.18)	0.94(1.33)

[a] All the results were done in triplicate on weight (%). Standard deviations are in parentheses. nd means not detected.

Figure 2. MALDI-MS spectrum of standard maltooligosaccharides. Individual maltooligosaccharides were dissolved in 0.01 M NaCl solution at a concentration of DP3 = 0.82 mg/mL, DP4 = 0.93 mg/mL, DP5 = 1.22 mg/mL, DP6 = 1.63 mg/mL and DP7 = 1.69 mg/ml. DHB was used as the matrix. "a.i." means arbitrary intensity and "*m/z*" means the mass-to-charge ratio.

Figure 3. MALDI-MS spectrum of oligosaccharides from lichenase-hydrolyzed water-soluble barley β-glucan passed through desalting procedure. DHB was used as the matrix. 1. [DP 3 + Na]+; 2. [DP 3 + K]+; 3. [DP 6 + Na]+; 4. [DP 7 + Na]+; 5. [DP 8 + Na]+; 6. [DP 9 + Na]+; 7. [DP 10 + Na]+; 8. [DP 11 + Na]+; 9. [DP 12 + Na]+.

evaporative light-scattering detector. In the present study, the original supernatant of the barley β-glucan hydrolysate was analyzed by HPLC and only DP3 and

DP4 could be detected and quantified. For HPLC quantification of DP5−DP7, the supernatant had to be concentrated. However, the sample concentration required for MALDI-MS was minimal (diluted to $^1/_{12}$ concentration of the original supernatant); moreover, higher polymers (up to DP12) could be detected and quantified using a single sample.

The sum of the absolute percentage of DP3 as well as DP4 for 10 varieties of barley analyzed by MALDI-MS varied from 85% to 93% (average 89.42%), indicating that DP3 and DP4 chain units were main building blocks of water-soluble barley β-glucan. This result is in agreement with the findings by Woodward et al. (1983), Wood et al. (1991, 1994), and Edney et al. (1991). The absolute percentages of oligosaccharides with DP5−DP9 as determined by MALDI-MS were very similar to those analyzed by HPLC or HPAEC-PAD (Yin and MacGregor, 1989; Wood et al., 1994). For all 10 varieties in this study, following a decline through DP8, there was an increase in the DP9 oligosaccharide content. The content of oligosaccharide with DP > 9 was very low.

However, the molar ratio of tri- to tetrasaccharides found in this study (ranging from 2.3 to 2.8) was lower than that reported by Wood et al. (1994) (ranging from 2.8 to 3.1). This may be due to the variations in the techniques and barley varieties used in their studies. Wood et al. (1991, 1994) reported that the inclusion of lichenase-treated oat β-glucan and glucose as external standard in each analysis may help to detect the changes in the tri- to tetrasaccharide ratio and total oligosaccharide response relative to glucose (response factor, ≃0.4). The results from MALDI-MS analyses have higher standard deviations than those obtained from HPLC or HPAEC-PAD, but the direct use of standard maltooligosaccharides for quantification of oligosaccharides released from purified barley water-soluble β-glucan seemed rapid and convenient. Wood et al. (1994) reported that the accuracy of molar ratio of tri- to tetrasaccharide of the released oligosaccharides as analyzed by HPAEC-PAD was ±10%, due to the different weight response factors of tri- and tetrasaccharides to PAD. The average molar ratio, calculated from Table 3, of tri- to tetrasaccharide in the lichenase-hydrolyzed water-soluble barley β-glucan analyzed by MALDI-MS for waxy, regular, and high-amylose barley varieties was very similar to that determined by HPLC analysis.

Analysis of Lichenase-Hydrolyzed Barley β-Glucan

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Table 3. Absolute Contents of Oligosaccharides in Lichenase-Hydrolyzed Water-Soluble β-Glucan from 10 Varieties of Barley As Determined by MALDI-MS and HPLC[a]

	DP3	DP4	DP5	DP6	DP7	DP8	DP9	DP10	DP11	DP12
					Waxy					
SB94794										
(H)[b]	57.73	29.31	5.14	2.45	1.45	nd	nd	nd	nd	nd
stdev	0.89	0.18	0.54	0.18	0.01					
(M)[c]	61.93	30.98	4.24	1.67	0.65	0.60	1.45	0.28	0.19	0.15
stdev	1.78	0.69	0.50	0.32	0.17	0.16	0.27	0.09	0.08	0.08
Candle										
(H)	58.74	29.48	5.02	2.20	1.39	nd	nd	nd	nd	nd
stdev	0.69	0.13	0.76	0.44	0.37					
(M)	61.54	31.29	3.98	1.44	0.59	0.54	1.37	0.39	0.31	0.27
stdev	1.85	1.29	0.91	0.39	0.01	0.11	0.35	0.12	0.13	0.10
SB94912										
(H)	59.83	28.58	4.32	1.98	nd	nd	nd	nd	nd	nd
stdev	0.09	0.01	0.32	0.30						
(M)	61.65	30.79	3.89	1.35	0.47	0.43	1.28	0.31	0.26	0.22
stdev	1.10	1.28	0.41	0.17	0.19	0.22	0.11	0.09	0.10	
SB94917										
(H)	54.84	28.12	4.89	2.13	nd	nd	nd	nd	nd	nd
stdev	0.35	0.04	0.10	0.12						
(M)	56.47	29.02	4.15	1.73	0.69	0.63	1.47	nd	nd	nd
stdev	1.18	0.26	0.14	0.25	0.06	0.12	0.38			
				DP3:DP4(waxy):[d] (H) = 2.64(0.08); (M) = 2.61(0.04)						
					Regular					
CDC Dawn										
(H)	55.29	30.81	5.22	2.69	nd	nd	nd	nd	nd	nd
stdev	0.16	0.01	0.21	0.05						
(M)	58.31	31.87	5.06	1.96	0.42	0.4	1.21	nd	nd	nd
stdev	2.19	1.94	0.56	0.14	0.15	0.12	0.23			
Phoenix										
(H)	51.76	31.88	5.33	2.25	nd	nd	nd	nd	nd	nd
stdev	0.75	0.48	0.42	0.08						
(M)	52.71	32.08	4.81	1.80	0.67	0.58	1.48	0.35	0.31	0.22
stdev	0.91	1.31	0.48	0.18	0.27	0.12	0.13	0.06	0.06	0.04
SB94860										
(H)	57.92	30.94	5.17	2.47	nd	nd	nd	nd	nd	nd
(M)	58.81	31.88	4.76	2.32	0.74	0.70	1.85	0.35	0.32	nd
stdev	1.05	0.62	0.53	0.51	0.24	0.20	0.27	0.08	0.09	
SR93102										
(H)	57.99	30.59	4.93	2.43	nd	nd	nd	nd	nd	nd
stdev	0.68	0.62	0.21	0.25						
(M)	60.37	32.06	4.75	2.10	0.45	0.39	1.24	nd	nd	nd
stdev	0.97	0.62	0.88	0.56	0.29	0.17	0.32			
				DP3:DP4(regular): (H) = 2.37(0.16); (M) = 2.38(0.14)						
					High Amylose					
SB94893										
(H)	53.35	28.82	5.41	3.13	1.74	nd	nd	nd	nd	nd
stdev	0.40	0.88	0.55	0.31	0.05					
(M)	55.48	30.44	5.09	2.94	0.84	0.76	1.79	0.41	0.38	0.29
stdev	1.22	1.10	0.59	0.45	0.27	0.21	0.39	0.08	0.13	0.09
SB94897										
(H)	54.10	29.60	5.70	2.89	nd	nd	nd	nd	nd nd	
stdev	0.67	0.17	0.19	0.33						
(M)	55.70	30.77	5.08	2.77	0.85	0.71	1.70	0.38	0.33	nd
stdev	1.96	0.50	0.91	0.62	0.16	0.08	0.46	0.05	0.07	
				DP3:DP4(high Amylose): (H) = 2.43(0.01); (M) = 2.39(0.01)						
Total Average										
(H)	56.16	29.91	5.11	2.46	1.53	nd	nd	nd	nd	nd
stdev	2.65	1.15	0.37	0.35	0.19					
(M)	58.30	31.12	4.58	2.01	0.64	0.59	1.48	0.35	0.30	0.23
stdev	3.14	0.95	0.47	0.53	0.15	0.14	0.23	0.04	0.06	0.05

[a] All the results (absolute percentages of DP 3−DP 12 in b-glucan) were obtained in triplicate and on a dry weight basis. Standard deviations (stdev) are in parentheses. nd means not detected. [b] (H) is the abbrviation of HPLC. [c] (M) is the abbreviation of MALDI-MS. [d] The average molar ratio of DP3:DP4.

In general, there is higher content of water-soluble β-glucan in waxy varieties of barley than in nonwaxy varieties of barley (Lee et al., 1997; Bhatty, 1999), which may partly result in higher viscosity of β-glucan in waxy varieties of barley than nonwaxy varieties of barley. In this study, the molar ratio of tri- to tetrasaccharide in the β-glucan hydrolysate for waxy varieties (varying from 2.6 to 2.8) was somewhat higher than that for regular and high amylose varieties (varying from 2.3 to 2.6). This suggested that there would be a higher content of (1→3)-β-linkage in the structure of water-soluble β-glucan from waxy varieties of barley. Since the (1→3)-β-linkages occur at irregular intervals (Buliga et al., 1986; Woodward et al., 1988; Gómez et al., 1997), the β-glucan has an irregular shape overall, which reduces its tendency to pack into stable, regular molec-

ular aggregates. Therefore, β-glucans in the waxy varieties of barley are relatively more soluble in water.

In conclusion, MALDI-MS provides a rapid and sensitive means for quantification of oligosaccharides released from water-soluble barley β-glucan by lichenase hydrolysis. MALDI-MS takes about 20 min for determining peak heights for 10 probe positions rather than 1 h for each analysis by HPAEC-PAD. The sample concentration used by MALDI-MS was $^1/_{12}$ of that used by HPLC or HPAEC-PAD. The differences of the molar ratio of tri- to tetrasaccharide in the lichenase-hydrolyzed water-soluble β-glucan between waxy and nonwaxy barley varieties, analyzed by MALDI-MS and HPLC in this study, might partly explain the differences in water solubility.

LITERATURE CITED

American Association of Cereal Chemists. Approved Methods of the AACC. Method 44−15A; The Association: St. Paul, MN, 1982.

Autio, K.; Myllymaki, O.; Suortti, T.; Saastamoinen, M.; Poutanen, K. Physical properties of (1→3), (1→4)- β-D-glucan preparates isolated from Finnish oat varieties. *Food Hydrocolloids* **1992**, *5*, 513−522.

Bhatty, R. S. The potential of hull-less barley. *Cereal Chem.* **1999**, *76* (5), 589−599.

Bhatty, R. S. Relationship between acid-extract viscosity and total soluble and insoluble β-glucan contents of hulled and hulless barley. *Can. J. Plant Sci.* **1987**, *67*, 997−1008.

Bhatty, R. S.; MacGregor, A. W.; Rossnagel, B. G. Total and acid-soluble β-glucan content of hull-less barley and its relationship to acid-extract viscosity. *Cereal Chem.* **1991**, *68*, 221−227.

Bamforth, C. W. Biochemical approaches to beer quality. *J. Inst. Brew.* **1985**, *91*, 154−160.

Buliga, G. S.; Brant, D. A.; Fincher, G. B. The sequence statistics and solution configuration of barley (1→3), (1→4)- β-D-glucan. *Carbohydr. Res.* **1986**, *157*, 139−156.

Chrastil, J. Improved colorimetric determination of amylose in starches or flours. *Carbohydr. Res.* **1987**, *159*, 154−158.

Clarke, A. E.; Stone, B. A. Enzymatic hydrolysis of barley and other β-glucan by a β-(1→4)-glucan hydrolase. *Biochem. J.* **1966**, *99*, 582−588.

Edney, M.J.; Marchylo, B.A.; MacGregor, A.W. Structure of total barley β-glucan. *J. Inst. Brew.* **1991**, *97* (1), 39−44.

Fincher, G.B. Morphology and chemical composition of barley endosperm cell walls. *J. Inst. Brew.* **1975**, *81*, 116−122.

Gómez, C.; Navarro, A.; Garnier, C.; Horta, A.; Carbonell, J. V. Physical and structural properties of barley (1→3), (1→4)- β-D-glucan. III. Formation of aggregation analyzed through its viscoelastic and flow behavior. *Carbohydr. Polym.* **1997**, *34*, 141−148.

Igarashi, O.; Sakurai, Y. Studies on the nonstarchy polysaccharides of the endosperm of naked barley. *Agric. Biol. Chem.* **1966**, *30*, 642−645.

Izawa, M.; Kano, Y.; Koshino, S. Relationship between structure and solubility of (1→3), (1→4)-β-D-glucan from Barley. *J. Am. Soc. Brew. Chem.* **1993**, *51* (3), 123−127.

Jiang, G. S.; Ramsden, L. Characterization and yield of the arabinogalactan-protein mucilage of taro corms. *J. Sci. Food Agric.* **1999**, *79*, 671−674.

Koizumi, K.; Kubota, Y.; Tanimoto, T.; Okada, Y. High-performance anion-exchange chromatography of homogeneous D-gluco-oligosaccharides and -polysaccharides (po-lymerization degree ≥50) with pulsed amperometric detection. *J. Chromatogr.* **1989**, *464*, 365−373.

Knuckles, B. E.; Chiu, M. M.; Betschart, A. A. β-glucan-enriched fractions from laboratory-scale dry milling and sieving of barley and oats. *Cereal Chem.* **1992**, *69*, 198−202.

Lee, C. J.; Horsley, R. D.; Manthey, F. A.; Schwarz, P. B. Comparison of β-glucan content of barley and oat. *Cereal Chem.* **1997**, *74*, 571−575.

McIntosh, G. H.; Whyte, J.; McArthur, R.; Nestel, P. J. Barley and wheat foods influence on plasma cholesterol concentrations in hypercholesterolemic men. *Am. J. Clin. Nutr.* **1991**, *53*, 1205−1209.

Mock, K. K.; Davey, M.; Cottrell, J. S. The analysis of underivertised oligosaccharides by matrix-assisted laser desorption mass spectrometry. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 644−651.

Stahl, B.; Steup, M.; Karas, M.; Hillenkamp, F. Analysis of neutral oligosaccharides by matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* **1991**, *63*, 1463−1466.

Timmermans, J. W.; van Leeuwen, M. B.; Tournois, H.; de Wit, D.; Vliegenthart, J. F. G. Quantification analysis of the molecular weight distribution of inulin by means of anion exchange HPLC with pulsed amperometric detection. *J. Carbohydr. Chem.* **1994**, *13*, 881−888.

van Riel, J.; Olieman, C. Selectivity control in the anion-exchange chromatographic determination of saccharides in dairy products using pulsed amperometric detection. *Carbohydr. Res.* **1991**, *215*, 39−46.

Vasanthan, T.; Bhatty, R. S. Starch purification after pin-milling and air-classification of waxy, normal and high amylose barley. *Cereal Chem.* **1995**, *72*, 379−384.

Wang, J.; Jiang, G. S.; Vasanthan, T.; Sporns, P. MALDI-MS characterization of maltooligo/polysaccharides from de-branched starch amylopectin of corn and barley. *Starch/ Staerke* **1999a**, *51* (7), 243−248.

Wang, J.; Sporns, P.; Low, N. H. Analysis of food oligosaccharides using MALDI-MS: quantification of fructooligosaccharides. *J. Agric. Food Chem.* **1999b**, *47* (4), 1549−1557.

Wood, P. J.; Weisz, J. HPLC analysis of cereal β-glucans (Abstr.). *Cereal Foods World* **1986**, *31*, 595.

Wood, P. J.; Weisz, J.; Blackwell, B. A. Structural studies of (1→3), (1→4)- β-D-glucans by ^{13}C-nuclear magnetic response spectroscopy and by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharides released by lichenase. *Cereal Chem.* **1994**, *71*, 301−307.

Wood, P. J.; Weisz, J.; Blackwell, B. A. 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.* **1991**, *69* (1), 31−39.

Woodward, J. R.; Fincher, G. B.; Stone, B. A. Water-soluble (1→3), (1→4)- β-D-glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure. *Carbohydr. Polym.* **1983**, *3*, 207−225.

Woodward, J. R.; Phillip, D. R.; Fincher, G.B. Water-soluble (1→3), (1→4)-β-D-glucans from barley (*Hordeum vulgare*) Endosperm. IV. Comparison of 40 °C and 65 °C soluble fractions. *Carbohydr. Polym.* **1988**, *8*, 85−97.

Yin, X. S. and MacGregor, A. W. Substrate specificity and nature of action of barley β-glucan solubilase. *J. Inst. Brew.* **1989**, *95*, 105−109.