Characterization and Estimation of Barley Polysaccharides by Near-Infrared Spectroscopy. II. Estimation of Total β-D-Glucans

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ABSTRACT

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Total β-glucans were determined in 139 barleys, 84 of which were used for calibration and 55 for validation. They included covered two- and six-rowed regular (about 25% amylose in the starch) barleys, covered and hull-less waxy (up to 100% amylopectin) and high-amylose (up to 49% amylopectin) barleys, and chemically treated and gamma-irradiated barleys. Both commercial and varietal samples (from three locations) were used. The total β-glucan content ranged from 2.7 to 9.5% and was higher in waxy than in regular or high-amylose barleys. Equations for prediction of β-glucan content by the best fit of three wavelengths and the single-wavelength (2,264 or 2,348 nm) method (using the step-up program) were developed. The standard error of prediction for both was slightly above 0.6%. The three selected wavelengths were in the 2,260- to 2,380-nm region, previously found by us to be typical for β-glucans. In light of the highly heterogenous populations used in this study, the accuracy of predicting the β-glucan contents in the validation samples was affected by the identity of barleys used for developing the prediction equations. Preliminary studies indicated that even for more homogenous populations, the results may be affected by kernel size, hardness, and protein contents. The above parameters may have to be included in developing equations to predict the β-glucan contents of barleys.

Interest in assaying the contents of (1→3),(1→4)-β-D-glucan, referred to hereafter as β-glucan, in barley and malt has been increasing because of its nutritional and technological importance (Munck 1981). β-Glucan has been difficult to quantify because of its variable physical and chemical nature (Wood 1984). Despite the development in recent years of new and specific analytical techniques (Jorgensen and Aastrup 1988), there is no generally acceptable and simple screening procedure for β-glucan estimation aimed at the brewing and feed industries and plant breeding programs.

The few articles on the determination of barley β-glucan by near-infrared reflectance spectroscopy (NIRS) emphasized the difficulties involved in using this technique. Most articles dealt with soluble β-glucan or hot water extract by applying as many as six infrared wavelengths (Allison et al 1978, Morgan and Gothard 1979). The articles on determination of total β-glucan by the NIRS technique have been even more limited because of the presumption that near-infrared reflectance (NIR) spectra of different polysaccharides are similar. Henry (1985) used NIRS to distinguish barley β-glucans and unmodified wheat starch. Using a mixture of both components, he developed two equations for prediction of the total β-glucan content. One equation provided a multiple correlation coefficient of 0.69, which was based on three wavelengths (1,656, 1,676, and 1,752 nm). The second equation ($R = 0.93$) involved 12 wavelengths and was of no practical value because of overfitting of the data.

The main objective of this investigation was to evaluate the usefulness of NIRS analysis for rapid measurement of the total β-glucan content in highly diverse barley selections. The procedure is based on the findings described in Part I of this study (Czuchajowska et al 1992), in which NIR spectra were determined in barleys (covered and naked), isolated barley starches (large and small granules), and β-glucans (from barley and oats).

MATERIALS AND METHODS

Chemicals

β-Glucanase, C-0901, from *Penicillium funiculosum;* glucose oxidase, G-6766, from *Aspergillus niger;* peroxidase, P-6782, type VI-A, from horseradish; *o*-dianisidine, D-3252; amylose, A-0512, and amylopectin, A-8515, from potato, were purchased from Sigma Chemical Co., St. Louis, MO.

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Barleys

We used in this study a total of 139 barleys, 84 for calibration and 55 for validation. The calibration set included three subsets described in Table I. A subset of 22 covered, commercial samples, from the 1989 crop, regular in amylose (22–28%) and grown at 15 sites (Pacific Northwest area), was obtained from the Washington Department of Agriculture, Inspection Service, Colfax. Those samples contained 3.0–5.8% β-glucan, with a mean value of 4.1%. A second subset of 36 samples, from the 1990 harvest, included 12 two- and six-rowed cultivars (all covered and regular in amylose content) grown at three locations (Pullman, Lind, and Royal Slope, WA) and was provided by S. E. Ullrich, Agronomy Department, Washington State University. The sites differ in microclimate (rainfall and temperature) during the growing season; the Lind location represents dryland compared with the higher rainfall in Pullman and Royal Slope. The β-glucan content in those samples ranged from 2.7 to 5.6% (mean value 4.2%). A third subset of 26 barley trials, from the 1990 crop, included 14 selections (all waxy genotypes containing up to 100% amylopectin) grown in Pullman, WA, and was supplied by S. E. Ullrich. Those samples contained 4.5–9.5% β-glucan (mean value 7.2%) and included covered, hull-less, chemically treated (by sodium azide and ethyl methane sulfonate), and gamma-irradiated barleys (as examples of mutants from a plant breeding program).

Additionally, two independent series of barleys were used to verify the predictive capacity of the calibration equations. Those

TABLE I
Description of Barleys

Barley Types	Number of Samples	Total β-Glucan, % Range	Total β-Glucan, % Mean
Calibration set (84)			
Covered, commercial, two-rowed, 22–28% amylose	22	3.0–5.8	4.1
Covered, 12 each from three locations, two-rowed and six-rowed, 23–26% amylose	36	2.7–5.6	4.2
Covered and hull-less, chemically treated, gamma-irradiated, waxy (up to 100% amylopectin)	26	4.5–9.5	7.2
Validation set (55)			
Regular, waxy, chemically treated, gamma-irradiated	34	3.7–9.3	5.2
Naked and covered, various amylose-amylopectin ratios, modified	21	4.8–9.1	6.9

samples, including 10 cultivars from the 1988–89 crops, were provided by S. E. Ullrich. In the first set of 34 samples, the content of β-glucan ranged from 3.7 to 9.3% (mean value 5.2%), and the samples included regular, waxy, chemically treated, and gamma-irradiated barleys. A second series of 21 samples included barleys that were naked and covered and varied in amylose-amylopectin ratios (regular, high-amylose, and waxy), that were modified by mutagenic agents and gamma-irradiation, and that contained 4.8–9.1% of β-glucan (mean value, 6.9%). All grain samples used for analyses were ground in a grinder (Udy Corp., Fort Collins, CO) fitted with round, 0.5-mm sieve openings.

Analytical Methods

Moisture was determined by oven drying for 1 hr at 130°C (AACC 1983). The content of total β-glucan was measured enzymatically as described by Ahluwalia and Ellis (1984). β-Glucan was extracted from barley flour with 50 m*M* perchloric acid at 96°C for 3 min. The extract was incubated with *P. funiculosum* β-glucanase preliminarily heated at 70°C for 1 hr (pH 4.0) to inactivate the starch-degrading enzymes (Bamforth 1983). The glucose released by hydrolysis of β-glucan was analyzed with a glucose oxidase-peroxidase reagent (Lloyd and Whelan 1969), and results are expressed on a polysaccharide basis (glucose × 0.9). Appropriate substrate and enzyme blanks were included to correct for any free glucose not emanating from β-glucan. The mean standard error of the β-glucan estimate was ±0.25 for 139 assayed barley samples; two replicate assays differed between 0.00 and 0.63%. Quantification of amylose (as percentage of starch) was made according to Hovenkamp-Hermelink et al (1988). All analyses were performed at least in duplicate; mean results of all analyses are reported on a moisture-free basis.

NIRS

All samples were scanned by NIRS on a Technicon 500 InfraAlyzer (Technicon, Tarrytown, NY). The readings were taken with reference to a ceramic standard throughout the range 1,100–2,500 nm, unless otherwise stated. Statistical analyses were made with the software furnished with the Technicon instrument.

The samples were scanned from 1,100 to 2,500 nm at 4-nm intervals, collecting 350 data points per sample. The Technicon best-fit combination of three wavelengths search program, selecting from all possible combinations, was used to choose the wavelengths. In addition, the step-up search program was used for 22 commercial samples to calculate the prediction equation for β-glucan at a single wavelength. This program was used to select the single wavelength that best differentiated among whole meal, starch, and β-glucans in a specific spectral region.

RESULTS AND DISCUSSION

Prediction Equations of β-Glucan by NIRS

The combined 84 samples and four subsets were used to form the prediction equations for β-glucan. The number of samples used for calibration, the range of β-glucan values, and the selected wavelengths for the linear regression equations are listed in Table II. Also, the respective correlation coefficients and standard errors of estimate (SEE) are given. The 84 samples, with β-glucan in the range of 2.68 to 9.51%, showed a good linear relation between the three wavelengths of best fit and total β-glucan. A multiple correlation coefficient of $R = 0.871$ and an SEE of 0.869 were obtained (Fig. 1). With a similar range of β-glucan contents but after exclusion of six outliers from calculations (in which the difference between actual and predicted value was larger than 1.5% β-glucan), the correlation coefficient increased to $R = 0.924$ and SEE decreased to 0.677.

The correlation coefficients and selected wavelengths by the best-fit combination of three wavelengths method were of interest to us for two reasons. First, we wanted to observe the quality of the relation between total β-glucan and NIRS wavelengths of barley characterized by a broad range of chemical composition and physical properties of barley genotypes. The second reason concerned the wavelengths selected by the best-fit method. The linear response between β-glucan and the combination of three wavelengths was good, especially if we consider the very high diversity of the material used. The three selected NIRS wavelengths (Tables II and III) belong to the upper region of

TABLE II
β-Glucan, the Best-Fit Three Wavelengths, Multiple Correlation Coefficients, and Standard Errors of Estimate (SEE) for the Calibration and Validation Sets

Barley Samples Included	Number of Samples	Range of β-Glucan (%)	Wavelengths (nm)	Correlation Coefficient	SEE
Calibration sets					
Regular,[a] waxy, three locations	84	2.68–9.51	2,234 2,374 2,500	0.871	0.869
Regular, waxy, three locations less outliers	78	2.99–9.51	2,234 2,374 2,500	0.924	0.677
Regular, three locations (varieties) only	36	2.68–5.58	1,590 1,604 2,136	0.586	0.575
Regular only (commercial)	22	2.99–5.78	2,094 2,122 2,374	0.919	0.317
Waxy only	26	4.52–9.51	1,912 2,108 2,360	0.936	0.567
Regular and waxy pooled	48	2.99–9.51	2,234 2,360 2,500	0.939	0.703
Validation sets					
Regular, waxy, chemically treated, gamma-irradiated	34	3.67–9.34	2,094 2,472 2,500	0.878	0.741
Naked and covered, various amylose-amylopectin ratios, modified	21	4.80–9.07	1,464 1,534 1,772	0.893	0.698

[a]See text.

absorbance. This particular area was previously described by us (Czuchajowska et al 1992) as a region in which major differences among barley β-glucan and barley starch and whole meal occurred. The two other regions of absorbance in which differences were found (Czuchajowska et al 1992) were not selected by the software for the equations. Selected wavelengths and equations are presented for several subsets of the 84 samples used for calibration in Tables II and III. The reason for distinguishing among these subsets was to obtain genotypically better-defined material. These subsets are discussed below.

A high multiple correlation coefficient (0.919) and a low SEE (0.317) for β-glucan was obtained for the subset of 22 commercial samples that represented regular barley (Table II). These commercial samples were grown in the Pacific Northwest region. The content of β-glucan ranged from 2.99 to 5.78%. The size of SEE indicated small differences between laboratory values of β-glucan and NIRS values for the corresponding samples. The selected wavelengths for these 22 samples (Table II) represented two areas of major differences between barley β-glucan and starch and barley whole meal (Czuchajowska et al 1992). The wavelengths 2,094 and 2,122 nm are associated with interaction of starch and protein. Areas of absorbance including the discussed wavelengths were also considered by Henry (1985) for the determination of barley β-glucan content of β-glucan and starch mixtures. The third wavelength, 2,374 nm, was found by us (Czuchajowska et al 1992) to be in the major region of differences between barley β-glucan and barley whole meal. It was used as a primary NIRS wavelength for determination of cellulose, hemicellulose, and dietary fiber (Osborne and Fearn 1986, Williams and Norris 1987). In our present study this particular wavelength was selected regularly by the software in several subsets.

The above discussed subset of commercial regular barley samples was selected to find the correlation of β-glucan content with only one wavelength, 2,264 or 2,348 nm. The step-up program was applied and that wavelength selected. The linear correlation coefficients and SEE values were 0.843 and 0.821, respectively, for 2,264 nm and 0.606 and 0.643, respectively, for 2,348 nm. The high correlation confirmed the findings presented in Part I of this study (Czuchajowska et al 1992) concerning the distinction between NIR spectra of barley β-glucan and spectra of barley starch and whole meal.

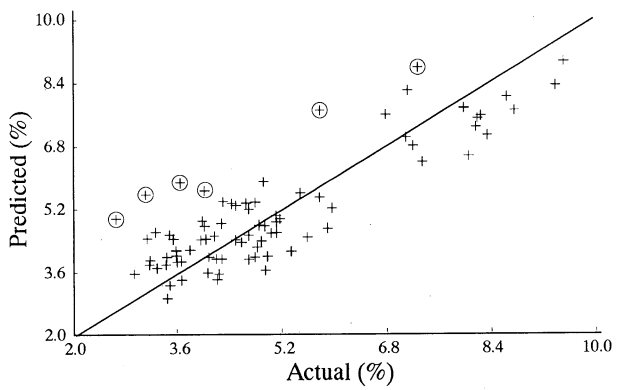

Fig. 1. Plot of linear correlation between enzymatically determined and near-infrared-reflectance-spectroscopically determined β-glucan for 84 calibration prediction samples. Circled values are outliers.

Waxy genotypes of barley contained much higher percentages of β-glucan than regular barleys. In this study the 26 waxy barley samples covered a range of β-glucan from 4.52 to 9.51%. The correlation coefficients for β-glucan ($R = 0.936$) indicated a good linear relation between the laboratory values of this component and the best-fit combination of three wavelengths (Table II).

The next subset used for calibration contained two series of samples discussed above. It contained 22 samples of commercial regular barley and 26 samples of waxy barley (Table II). The β-glucan in the combined subsets ranged from 2.99 to 9.51%; the multiple correlation coefficient was $R = 0.939$ and the SEE was 0.703.

The above results for calibration, on the basis of a total of 84 samples (22, 26, and 36), concerned 1) 22 regular commercial barley samples, 2) 26 waxy commercial barley samples or 22 regular commercial plus 26 waxy barley samples (48), and 3) 36 (12 × 3) samples. The 22, 26, and 22 + 26 samples gave satisfactory linear responses between total β-glucan and the combination of three wavelengths. The 36 samples of regular barley (12 cultivars, each from three locations; Table I) included in the total of 84 samples, however, gave a linear correlation coefficient of only 0.586. The instrument-selected wavelengths for this subset did not include the most typical region of distinction between β-glucan and barley whole meal, as determined in Part I of this study (Czuchajowska et al 1992). Those samples were therefore examined more closely. Pullman and Royal Slope are similar in climate; however, Lind represents the dryland. Growing conditions seemed to have little affect on the β-glucan content of barley, but growing conditions significantly affected protein content and 1,000-kernel weight (not reported here). The latter parameters may affect particle size distribution and, indirectly, NIR spectra (Williams 1975, Watson et al 1977, Williams and Thompson 1978, Norris and Williams 1984). When only one location was selected, the correlation coefficients were as follows: $R = 0.92$ for Royal Slope with 0.306 SEE, $R = 0.96$ for Lind with 0.178 SEE, and $R = 0.97$ for Pullman with 0.229 SEE. Also, the selected wavelengths differ for each location, which might be due to differences in chemical composition and in physical properties. The slopes of the linear regressions of β-glucan were different for each location. This resulted in poor correlation coefficients when all samples were pooled.

The variation in particle size was most pronounced for all barleys in this study and may have had the largest effect on the 36 samples from the three locations. Such effects, theoretically, may be compensated in part, at least, by derivatization of the spectra (Williams and Norris 1987). There is, however, an optimum for the derivative size (gap) and wavelength segment for best results. If those parameters are not optimized, the calibration could be affected adversely and actually be inferior to that based on log $(1/R)$. Such an optimization would require a very large number of samples, varying widely in particle size and density, and use of an instrument with a very high resolution. Such an approach was deemed impractical in this study.

In summary, the following statements can be made. Good correlation coefficients for β-glucan were obtained for commercially blended samples representing regular barley, waxy barley, and both genotypes. Small numbers of barley cultivars representing different growing conditions gave low correlations and differed in the selection of best-fit wavelengths. The best correlation with lowest SEE was obtained for the wavelengths

TABLE III
Prediction Equations for Barley β-Glucans

Barley Samples Included	Equation Coefficients and Wavelengths[a]
Regular, waxy, three locations (84)	$67.182 - 1{,}037.526\ (A_{2,234}) + 1{,}315.645\ (A_{2,374}) - 454.373\ (A_{2,500})$
Regular, waxy, three locations less outliers (78)	$71.919 - 1{,}173.441\ (A_{2,234}) + 1{,}504.98\ (A_{2,374}) - 521.753\ (A_{2,500})$
Regular (commercial) only (22)	$10.346 + 1{,}427.206\ (A_{2,094}) - 1{,}779.502\ (A_{2,122}) + 309.009\ (A_{2,374})$
Waxy only (26)	$24.835 - 266.868\ (A_{1,912}) - 212.620\ (A_{2,108}) + 362.053\ (A_{2,360})$
Regular and waxy pooled (48)	$66.444 - 686.237\ (A_{2,234}) + 870.566\ (A_{2,360}) - 332.606\ (A_{2,500})$

[a] A indicates absorbance at the given wavelength.

TABLE IV
Barleys Used for Calibration and Prediction, Laboratory Range of β-Glucan in Validation, Bias, and Standard Errors of Prediction (SEP)

Barley Samples Included in Calibration	Numbers of Samples in Validation	Range of β-Glucan in Validation	Bias	SEP
Regular, waxy, three locations less outliers (78)	34	3.67–9.34	2.06	1.13
	21	4.8–9.07	1.18	0.79
Regular (commercial) only (22)	12 Pullman	3.28–5.33	−0.27	0.76
	12 Royal Slope	3.83–5.58	−0.59	0.53
	12 Lind	2.68–4.43	0.77	0.74
	36 Pooled	2.68–5.58	0.00	0.90
Waxy only (26)	21	4.8–9.07	2.61	1.27
Regular and waxy pooled (48)	34	3.67–9.10	1.92	0.79
	21	4.8–9.07	1.61	1.08

representing at least two regions in which differences between barley β-glucan and whole meal of barley appeared.

Validation of Equations of β-Glucan by NIRS

Equations for prediction of β-glucan for subsets of samples are summarized in Table III. These equations were applied to separate sets of barley samples with known β-glucan values. The selected prediction equations and validation sets, laboratory ranges of β-glucan, and standard errors of prediction (SEP) are listed in Table IV. The results for the 36 samples (12 cultivars from three locations) were not validated because of the low (0.586) correlation coefficient (Table II).

The equation, based on 78 samples covering the wide range of β-glucan (Table II), was used to predict two sets of samples: the set of 34 samples in which β-glucan ranged from 3.67 to 9.34% and the set of 21 samples with β-glucan content between 4.80 and 9.07%. Both prediction sets required correction for bias (Table IV). The correction for bias, which is the systematic error between laboratory values and NIRS values, reduced SEP by over 50% for both prediction sets (Table IV). The 21 barley samples represented three barley genotypes (waxy, regular, and high-amylose) of the commonly grown cultivars in the Pacific Northwest area. These samples showed good agreement between laboratory values of β-glucan and those predicted by NIRS (Fig. 2A). Again, both sets of samples of 34 and 21 were used for validation for β-glucan of the equation obtained by 48 barleys (22 regular plus 26 waxy) (Table II). In both cases, a correction for systematic error was required. When corrected for the bias, the 21 samples of barley, which covered a wide range of β-glucan, had an SEP of about 1.08 and showed good agreement between laboratory and predicted values (Fig. 2B). The prediction equation for waxy samples only (Table II) covered a range of β-glucan from 4.52 to 9.51; that equation was validated only by 21 commonly grown barley cultivars, which ranged from 4.80 to 9.07 in β-glucan content and was covered by the calibration set (Table IV and Fig. 2C).

And finally, the prediction equation for commercial regular barleys with the lowest SEE of 0.317 was validated by the regular barley samples grown under three different conditions. As stated, the 12 cultivars from each location showed similar β-glucan contents but differed strongly in protein content and 1,000-kernel weight. These properties may have affected the prediction of the combined samples from all three locations. However, each location considered separately gave satisfactory results after correction of a small bias. The SEP was 0.76 for Pullman, 0.53 for Royal Slope, and 0.74 for Lind. However, overfitting of data resulting from the small number of degrees of freedom (12 − 4 = 8) for those samples cannot be excluded. As mentioned throughout the text, the estimation of β-glucan content in barley by NIRS may be affected by the kernel weight and/or protein content through their effects on particle size distribution of a milled sample. Those factors, as well as the β-glucan content, may be affected by cultural practices and growth conditions. The effects of those factors on determination of β-glucan by NIRS will be the subject of Part III in this series.

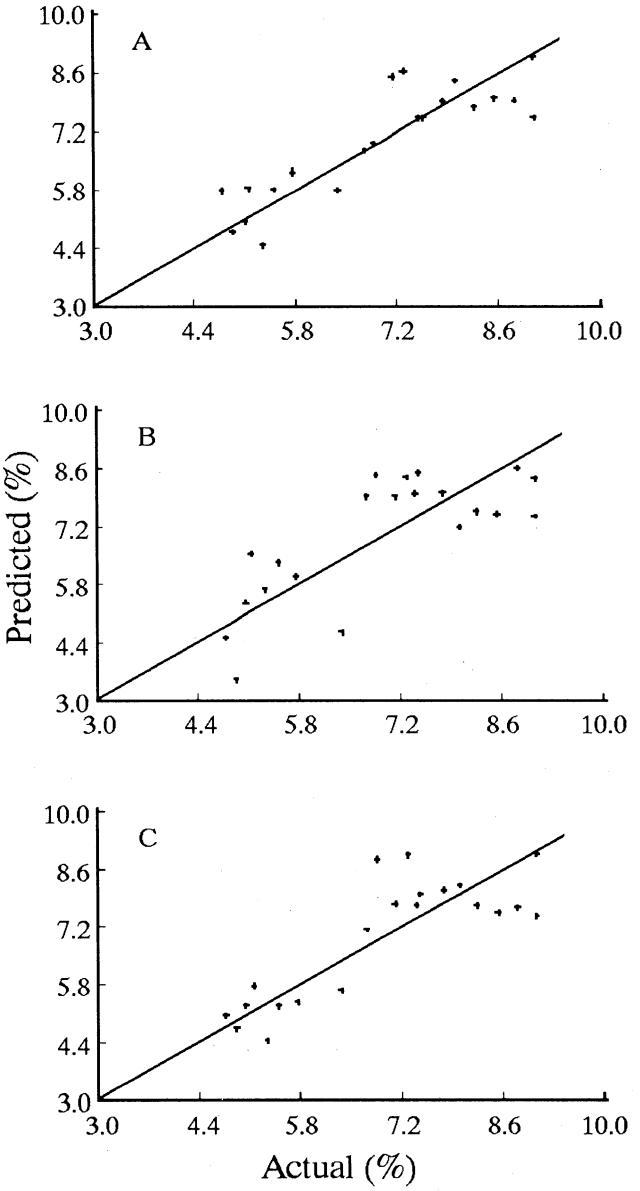

Fig. 2. Relationship between measured and predicted β-glucan content for the validation set of 21 samples representing regular, waxy, and high-amylose barleys, on the basis of equations developed for 78 samples (total less outliers) (**A**), 48 samples (regular and waxy) (**B**), and 26 samples (waxy only) (**C**). For identification of samples, see Table I and text.

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