Carbohydrate Research, 141 (1985) 13–19
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

NEAR-INFRARED REFLECTANCE ANALYSIS OF CARBOHYDRATES AND ITS APPLICATION TO THE DETERMINATION OF (1→3),(1→4)-β-D-GLUCAN IN BARLEY

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(Received September 17th, 1984; accepted for publication, November 3rd, 1984)

ABSTRACT

Near-infrared reflectance spectra of carbohydrates were shown to display significant differences that could be exploited to develop calibrations for the determination of specific carbohydrate fractions in mixtures. Wavelengths for determination of (1→3),(1→4)-β-D-glucans from barley were selected by examining the spectra of mixtures of barley β-D-glucan and starch. The wavelengths selected were then used to calibrate a near-infrared reflectance spectrophotometer for prediction of the β-D-glucan content of barley.

INTRODUCTION

Near-infrared reflectance analysis (NIRA) is a technique widely used for analysis of agricultural and food commodities[1]. The method relies upon statistical derivation of the relationship between the reflectance of near-infrared light (750–2500 nm) by solids or liquids and their chemical composition[2]. The technique has been most successfully applied to the measurement of the major constituents of the material being analysed (e.g., moisture, protein, and fat in foods). Determination of specific carbohydrates by NIRA has been limited because of the belief that the near-infrared spectra of carbohydrates were generally similar[3].

This paper reports the near-infrared reflectance spectra of a number of carbohydrates and a procedure for applying NIRA to the measurement of the composition of mixtures of carbohydrates. This procedure is illustrated by application to the analysis of (1→3),(1→4)-β-D-glucans in barley.

EXPERIMENTAL

Source of carbohydrates. — D-Arabinose, D-xylose, D-galactose, D-mannose, maltose, sucrose, cellulose, starch (unmodified, from wheat), and inulin (from dahlia) were from Sigma Chemical Co., St. Louis, MO, U.S.A. D-Glucose and D-fructose were from BDH Ltd., Poole, U.K. Barley β-D-glucan was from Biocon

0008-6215/85/$ 03.30

Biochemicals, Melbourne, Australia. All samples were dried over silica gel before spectroscopy.

Determination of barley β-D-glucan by near-infrared reflectance. — Barley was ground to pass a 0.8-mm sieve by using a laboratory mill 3100 (Falling Number AB) before determination of β-D-glucan by an enzymic method and NIRA. Two sets of barley samples were used. A set of 50 samples, including 31 different varieties grown over six different seasons and from 13 different sites, was used to produce calibrations for β-D-glucan. These samples contained from 2.90 to 5.16% of β-D-glucan, with a mean value of 4.05%. A second set of 25 barley samples, including 22 varieties grown over five seasons at seven different locations, was used to test the predictive capacity of the calibration equations.

Enzymic determination of β-D-glucan. — Total (1→3),(1→4)-β-D-glucan was determined by a simplified enzymic procedure[4]. The method involved heating 0.25 g of ground barley in 80% ethanol to inactivate enzymes, hydrolysis of the β-D-glucan with a high concentration of a purified β-D-glucanase, and estimation of the reducing sugars produced by reaction with *p*-hydroxybenzoic acid hydrazide.

Near-infrared reflectance spectroscopy. — Near-infrared reflectance spectra were recorded with a Technicon InfraAlyzer 500 C (InfraAlyzer 500 plus a Hewlett–Packard 1000 computer)[5]. Reflectance values were collected at 2-nm intervals from 100 to 2500 nm and stored as absorbance.

RESULTS

Near-infrared reflectance spectra of carbohydrates. — Near-infrared spectra of carbohydrates are shown in Figs. 1–3. The spectra of monosaccharides (Fig. 1) show more detail and differences than those of polysaccharides (Fig. 3). All of the spectra show a general increase in absorbance at longer wavelengths. Differences in the level of reflectance accross the entire spectrum may be due to difference in particle size.

The estimation of specific carbohydrates by NIRA was approached by first identifying the wavelengths providing maximum discrimination between the components in mixtures of the pure carbohydrates. The reflectance of actual samples at these wavelengths was then related to the content of the specific carbohydrate to be determined. The resulting equation could then be used to predict the carbohydrate content of unknowns.

Application to the determination of β-D-glucan in barley. — The (1→3),(1→4)-β-D-glucan content of barley is an important quality attribute[5]. The major component of the barley grain is starch, therefore determination of β-D-glucan in barley by NIRA is largely dependent upon distinction between the near-infrared spectra of the β-D-glucan and starch.

Measurement of β-D-glucan in barley by NIRA was approached by using mixtures of β-D-glucan and starch to select wavelengths for a calibration. Inspection of the spectra of barley β-D-glucan and starch (Fig. 3) showed that minor differences

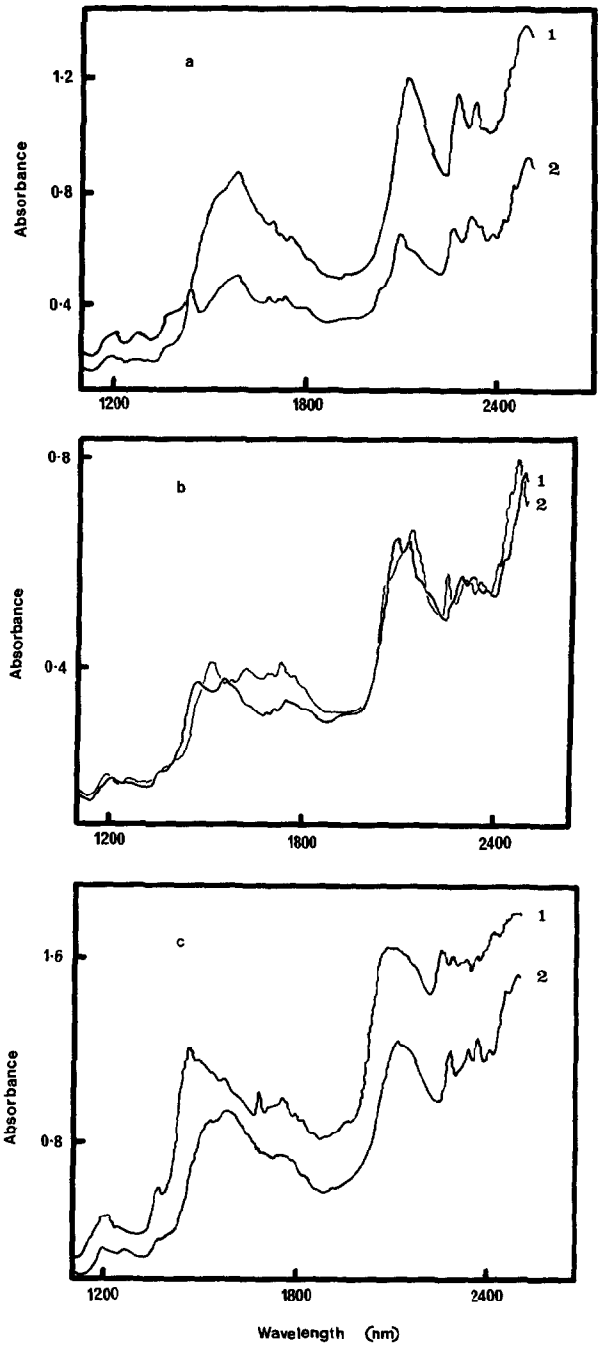

Fig. 1. Near-infrared spectra of monosaccharides: (*a*) 1, D-xylose; 2, D-arabinose; (*b*) 1, D-mannose; 2, D-galactose; (*c*) 1, D-fructose; 2, D-glucose.

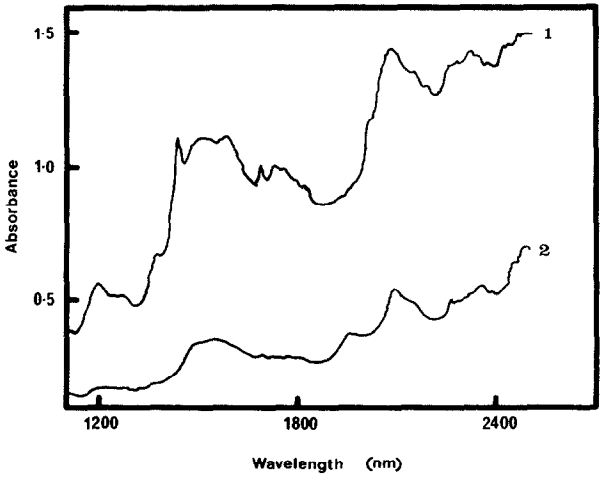

Fig. 2. Near-infrared spectra of disaccharides, 1, sucrose; 2, maltose.

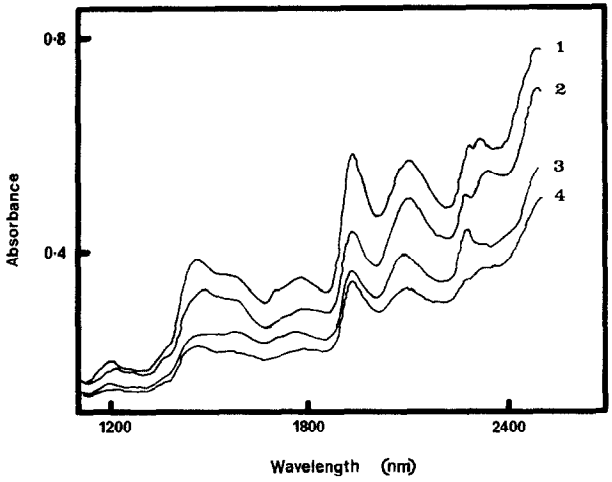

Fig. 3. Near-infrared spectra of polysaccharides, 1, starch; 2, α-cellulose; 3, inulin; 4, β-D-glucan (barley).

were present between 1600 and 1800 nm. This region of the spectrum probably includes the C–H first overtones[7]. A search was made for the best wavelengths for measuring β-D-glucan in a series of 11 mixtures containing 0–10% by weight of barley β-D-glucan in starch. The combination of three wavelengths giving the best correlation with β-D-glucan content was determined (Table I). A search considering points at 14-nm intervals gave 1702, 1744, and 2192 as the best combiation of three wavelengths. The spectrum between 1600 and 2400 nm was then divided into 200-nm regions. Each region was searched, considering points at 2-nm intervals. The best combination of three wavelengths in each of the four regions is given in Table I. The best combination of any three wavelengths from these 12 wavelengths

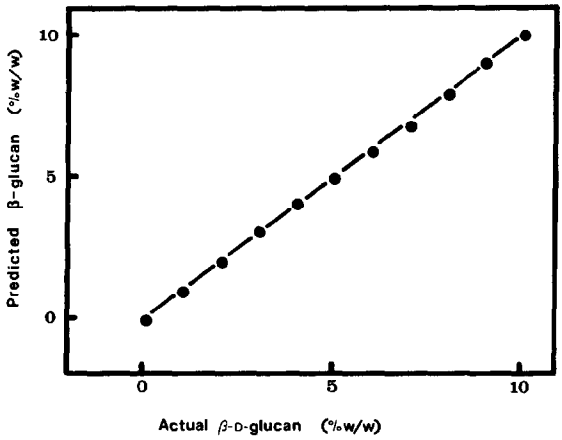

Fig. 4. Prediction of β-D-glucan content of mixtures of β-D-glucan and starch by NIRA. Equation: β-D-glucan (%) = 73.27 + 9742.9 (absorbance 1656 nm) − 6225.9 (absorbance 1676 nm) − 3365.5 (absorbance 1752 nm). Multiple correlation-coefficient, 0.999; root-mean-square difference, 0.116%.

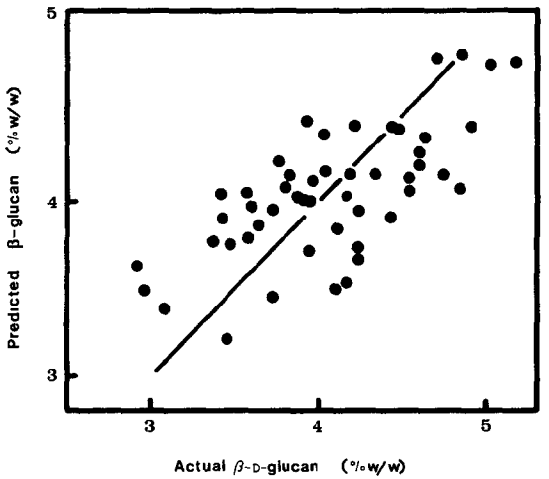

Fig. 5. Prediction of β-D-glucan content of barley by NIRA. Equation: β-D-glucan (%) = 9.87 − 498.3 (absorbance 1656 nm) + 307.5 (absorbance 1676 nm) + 150.5 (absorbance 1752 nm). Multiple correlation-coefficient, 0.691. Actual β-D-glucan was estimated by an enzymic procedure.

was 1656, 1676, and 1752, exactly the same as the best combinations between 1600 and 1800 nm. This result supports the visual assessment of this region as being important for distinction between β-D-glucan and starch. The prediction of the β-D-glucan content of the β-D-glucan–starch mixtures from the reflectance at these wavelengths was excellent (root-mean-square difference, 0.12%, Fig. 4). The equation relating the reflectance of standard mixtures to their β-D-glucan content did not allow the prediction of β-D-glucan in barley samples. However, these

TABLE I

SELECTION OF WAVELENGTHS FOR THE DETERMINATION OF BARLEY β-D-GLUCAN IN THE PRESENCE OF STARCH

Range considered (nm)	*Interval (nm)*	*Best 3 wavelengths (nm)*		
1100–2500	14	1702	1744	2192
1600–1800	2	1656	1676	1752
1800–2000	2	1860	1972	2000
2000–2200	2	2068	2088	2148
2200–2400	2	2224	2248	2290

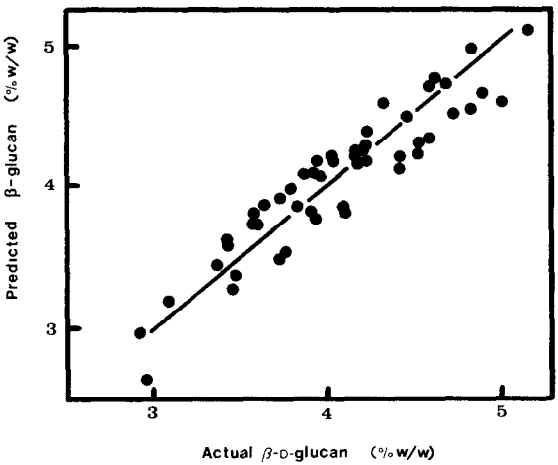

Fig. 6. Prediction of β-D-glucan content of barley by NIRA. Equation: β-D-glucan (%) = −7.79 + 692.3 (absorbance 1656 nm) − 1341.0 (absorbance 1676 nm) + 8.80 (absorbance 1752 nm) + 8.094 (absorbance 1860 nm) + 860.9 (absorbance 1972 nm) − 1310.9 (absorbance 2000 nm) + 407.6 (absorbance 2068 nm) − 803.2 (absorbance 2088 nm) + 521.9 (absorbance 2148 nm) +155.7 (absorbance 2224 nm) − 15.9 (absorbance 2248 nm) + 7.48 (absorbance 2290 nm). Multiple correlation-coefficient, 0.931. Actual β-D-glucan was estimated by an enzymic procedure.

wavelengths could be used to develop calibrations for β-D-glucan in ground barley. The relationship between predicted and actual β-D-glucan for a calibration set of 50 barley samples using the best three wavelengths for β-D-glucan–starch mixtures is given in Fig. 5 (root-mean-square difference, 0.397%). The relationship could be improved by including all 12 wavelengths selected from standard mixtures (Fig. 6, root-mean-square difference, 0.224%).

These equations were able to predict the β-D-glucan content of other samples, not included in the calibration set. The root-mean-square difference for the prediction set (25 samples) was 0.403% for the three-wavelength equation. However, the 12-wavelength equation was inferior at prediction (root-mean-square difference, 0.557%).

DISCUSSION

Few assignments of bands in the near-infrared spectra of carbohydrates have been made[7,8], although considerable work has been done at longer wavelengths[9,10].

The near-infrared spectra of simple carbohydrate such as monosaccharides and oligosaccharides show substantial differences, suggesting that their specific determination in mixtures by NIRA should be possible. Giangiacomo *et al.*[6] reported successful prediction of D-glucose, D-fructose, and sucrose in pure mixtures by NIRA between 950 and 1850 nm. NIRA has also been used to measure sucrose in cake mixes[7].

The spectra of polysaccharides did not show such large differences, but their distinction has been achieved in the present study. The use of mixtures of the components to be analysed allows selection of the wavelengths most useful for their distinction. This approach to wavelength selection may produce calibrations having wide application than those based only upon an analysis of actual samples. Similar procedures could be applied to the determination of other polysaccharides, such as pentoglycans in wheat and amylose in rice.

The advantage of NIRA over other methods for carbohydrate determination is the speed of the analysis. More than one sample per min may be analysed.

ACKNOWLEDGMENT

This work was supported by funds from the Australian Wheat and Barley Industry Research Councils.

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