Characterization of Cereal Sugars and Oligosaccharides

R. J. HENRY[1] and H. S. SAINI[2]

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ABSTRACT

Carbohydrates soluble in 80% ethanol from barley, oats, rye, rice, triticale, and wheat grains were fractionated and characterized by gel filtration and thin-layer chromatography. All cereal grains contained sucrose and small amounts of free glucose and fructose. Oligosaccharides were detected in all but the rice samples. Two series of oligosaccharides were found: the fructosyl derivatives of sucrose (fructan series) and the galactosyl derivatives of sucrose (raffinose series). Oats contained galactosyl derivatives of sucrose (raffinose and stachyose) but very little of the fructosyl derivatives. Both series of oligosaccharides were present in substantial amounts in barley, rye, and wheat. Rye contained greater concentrations of high molecular weight fructan (degrees of polymerization > 6) whereas wheat and barley had higher concentrations of fructan tri- and tetrasaccharides. Raffinose concentrations were highest in wheat and barley. Thin-layer chromatography indicated that 6^G fructosyl sucrose (neokestose) was the most abundant of the isomeric fructosyl sucroses in wheat and barley. Triticale cultivars contained less high molecular weight fructan than rye but more low molecular weight oligosaccharides (3–6 degrees of polymerization) than rye or wheat.

The importance of the soluble sugars of cereal grains in their utilization is well recognized. In breadmaking, the loaf volume can be directly related to the quantity of sugars in the flour, including those sugars produced from starch by diastatic action. Cereal grains contain variable amounts of monosaccharides, disaccharides, and oligosaccharides (Koch et al 1951). Glucose and fructose are the only two monosaccharides present in significant quantities in grain (Henry 1988). Sucrose is similarly the only disaccharide found in significant quantities in ungerminated grain. The identity of trisaccharides and higher polymers has not been fully established; two series of polymers are present: galactosyl derivatives of sucrose and fructosyl derivatives of sucrose (MacLeod and Preece 1954). Germinating grains may also contain saccharides derived from the breakdown of starch or cell wall polysaccharides (for example, maltose in malt). The relative quantitative importance of the two series of oligosaccharides (fructosyl and galactosyl derivatives of sucrose) has not been well established in cereals. In the trisaccharide fraction, all three primary fructosyl derivatives of sucrose and raffinose (a galactosyl derivative) have been reported from cereals but few studies have attempted their individual estimation. The exact structure of tetrasaccharides and higher polymers from cereals has not been determined.

This paper reports investigations aimed at establishing the identity and relative amounts of mono-, di-, and oligosaccharides in barley, oats, rice, rye, triticale, and wheat.

MATERIALS AND METHODS

Samples of barley, oats, rice, rye, triticale, and wheat were as described previously (Henry 1985) for characterization of the carbohydrates present. Ground grain was extracted with 80%, v/v, ethanol three times. Combined extracts were concentrated under vacuum and analyzed by gel filtration and thin-layer chromatography (TLC). More detailed quantitative analyses of wheat triticale and rye samples were undertaken on samples used in an earlier study of pentosan content (Saini and Henry 1988).

Gel Filtration Chromatography

Oligosaccharides were separated on an 84 × 1.9 cm column of Fractogel TSK HW-40 (S) (Merck) at room temperature. All fractions were analyzed for free or combined fructose and glucose. Oligosaccharides were hydrolyzed with acid, and then glucose, fructose, and galactose contents were determined. Wheat, rye, and triticale saccharides were separated by gel filtration on a 90 × 1.8 cm column of Bio Gel P-2 minus 400 mesh for quantitative analysis.

Isolation of Standards

A mixture of 1^F fructosylsucrose and 6^G fructosyl sucrose was isolated from bulbs of onion (*Allium cepa* L.) (Darbyshire and Henry 1978) by extraction with 80% ethanol and concentration of the trisaccharide fraction following gel filtration on Fractogel as described above.

Acid Hydrolysis of Oligosaccharides

Optimum conditions for acid hydrolysis in hydrochloric acid were determined by studying the recovery of glucose, galactose, and fructose when solutions of glucose, galactose, fructose, sucrose, melibiose, raffinose, and stachyose (Sigma) were heated at 100°C for 1 hr in 0.0, 0.01, 0.1, and 1.0*M* acid. The 0.01*M* acid was sufficient to hydrolyze sucrose; however, the 1.0*M* acid was necessary to ensure complete hydrolysis of the linkage between the glucosyl and galactosyl linkage in melibiose, raffinose, and stachyose. Losses of glucose, galactose, and fructose in 1.0*M* acid were slight. This treatment was used to determine the monosaccharide composition of oligosaccharide fractions separated by gel filtration.

TLC

Oligosaccharides were characterized by their mobility on silica gel plates (Merck 5721). Some plates were equilibrated in 0.2*M* NaH_2PO_4 for 24 hr and then dried for 24 hr at 105°C before use. A solution of butanol, propan-2-ol, and water (3:12:4, v/v) and one of propan-2-ol, acetone, and water (2:2:1, v/v) were used as solvents, and urea-phosphoric acid was used to locate the separated oligosaccharides (Darbyshire and Henry 1981). Glucose, fructose, sucrose, raffinose, stachyose (Sigma), and onion trisaccharides were used as standards. Lanes were analyzed using a densitometer (Bio-Rad model 620 video densitometer).

Monosaccharide Determination

Galactose was determined using galactose dehydrogenase (Boehringer Mannheim), glucose using glucose oxidase, and fructose using resorcinol (Darbyshire and Henry 1981). Total sugars were determined using anthrone (Roe 1955). Monosaccharides were also determined by gas chromatography of alditol acetates (Harris et al 1988).

RESULTS AND DISCUSSION

Qualitative Analysis of Carbohydrates Soluble in 80% Ethanol by TLC

Components with a mobility similar to raffinose were detected in extracts from barley, wheat, rye, triticale, and oats. The large number of oligosaccharides from rye made interpretation difficult. No oligosaccharides were detected in rice.

[1]Queensland Wheat Research Institute, PO Box 2282, Toowoomba Q 4350, Australia.
[2]New South Wales Department of Agriculture, North Coast Agricultural Institute, Wollongbar NSW 2480, Australia.

The major fructosylsucrose in trisaccharide fractions from barley and wheat isolated by gel filtration had a mobility in TLC similar to 6^G fructosyl sucrose. Both 6^F and 6^G fructosylsucrose have been reported from wheat flour (Nilsson et al 1986), whereas 6^F and 1^F fructosylsucrose have been reported from barley leaves (Wagner and Wiemken 1987). Saunders and Walker (1969) found 6^G fructosyl sucrose but none of the other two isomers in wheat bran. At least one other isomer (probably 1^F fructosylsucrose) was present in lower concentration in the wheat analyzed in this study (Fig. 1). The presence of stachyose in oats was also indicated. Fructosyl derivatives of sucrose were not detected in oats but were present in barley, wheat, rye, and triticale.

Quantitative Analysis of Carbohydrates Soluble in 80% Ethanol by Gel Filtration

Separation of barley carbohydrates on a Fractogel TSK HW-40(5) column is shown in Figure 2; data were calculated from analysis of the monosaccharide composition of each fraction. Raffinose (galactosyl sucrose) eluted from the gel filtration column midway between fructosyl sucrose and $(\text{fructosyl})_2$ sucrose. Specific analysis for galactose in acid hydrolysates was necessary to distinguish these incompletely resolved oligosaccharides. Isomeric fructosyl sucroses were not separated on this column. Fructose behaved as a smaller molecule than glucose, as is usually found in these analyses. Members of the galactosyl sucrose series, raffinose, and stachyose eluted consistently earlier (at higher apparent molecular weight) than members of the corresponding fructosyl sucrose series. Acid hydrolysis of the oligosaccharides gave the expected monosaccharide compositions (Fig. 2). Gas chromatography confirmed the absence of other monosaccharides.

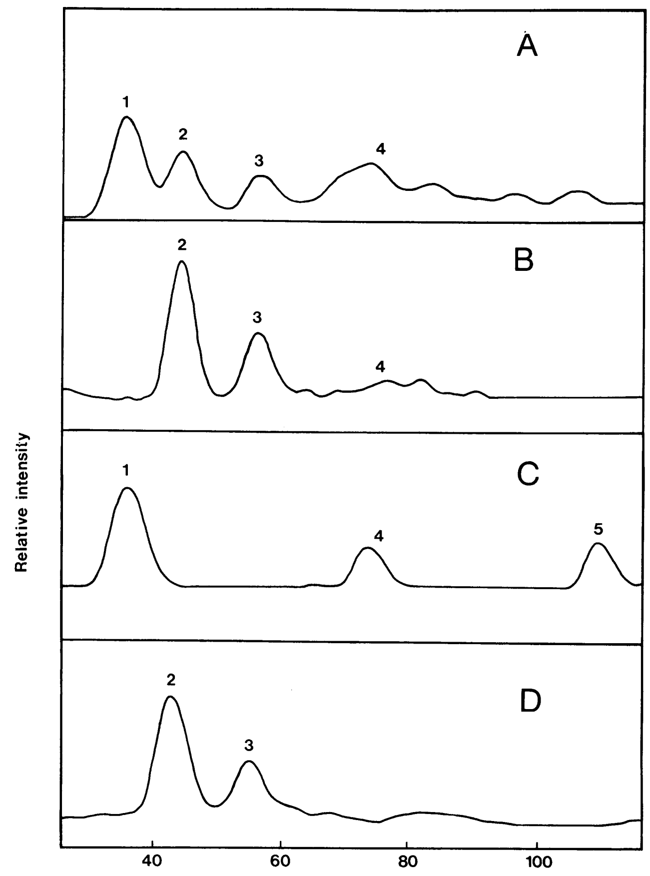

Fig. 1. Thin-layer chromatography of trisaccharides from wheat (analyzed using a densitometer): **A,** 80% ethanol-soluble sugars from wheat grains; **B,** trisaccharides from wheat (fructan trisaccharide peak) isolated by gel filtration; **C,** standard sugars (Sigma); **D,** trisaccharides from onion (isolated by gel filtration). Peak identities: sucrose (**1**), 6^G fructosylsucrose (nenkestose) (**2**), 1^F fructosylsucrose (isokestose) (**3**), raffinose (**4**), and stachyose (5).

Wheat and barley contained both fructosyl and galactosyl derivatives of sucrose: rye contained much more fructan, and oats almost no fructan but more stachyose (Table I). MacArthur and D'Appolonia (1979) reported the presence of higher concentrations of stachyose in oats than in wheat. No evidence was found for the presence of "fructosylraffinose" (White and Secor 1953, Saunders and Walker 1969) in any of the samples analyzed.

Comparison with Analysis by Other Methods

The glucose content of these grains determined after separation by gel filtration was much lower than that determined by direct analysis of extracts (Henry 1985). The glucose oxidase method was found to give a weak response with sucrose, presumably

TABLE I
Carbohydrates Extracted from Cereal Grains in 80% (v/v) Ethanol

Carbohydrate	Wheat (mg/g)	Barley (mg/g)	Rye (mg/g)	Oats (mg/g)
Glucose	...[a]	0.72	0.10	0.01
Fructose	1.45	0.78	0.07	...[a]
Sucrose	7.91	13.6	7.07	4.34
Raffinose	4.72	2.34	0.79	0.52
Stachyose	0.06	0.02	0.02	0.31
Fructosyl sucrose, DP[c] 3	2.86	2.59	0.93	...[b]
$(\text{Fructosyl})_2$ sucrose, DP 4	1.52	2.00	1.63	0.03
$(\text{Fructosyl})_3$ sucrose, DP 5	0.45	0.31	1.15	...[b]
$(\text{Fructosyl})_{\geq 4}$ sucrose, DP > 5	3.06	2.33	12.9	0.18
Total	20.7	24.4	21.4	5.23

[a] Levels too low for reliable estimation.
[b] Not detected.
[c] DP = Degrees of polymerization.

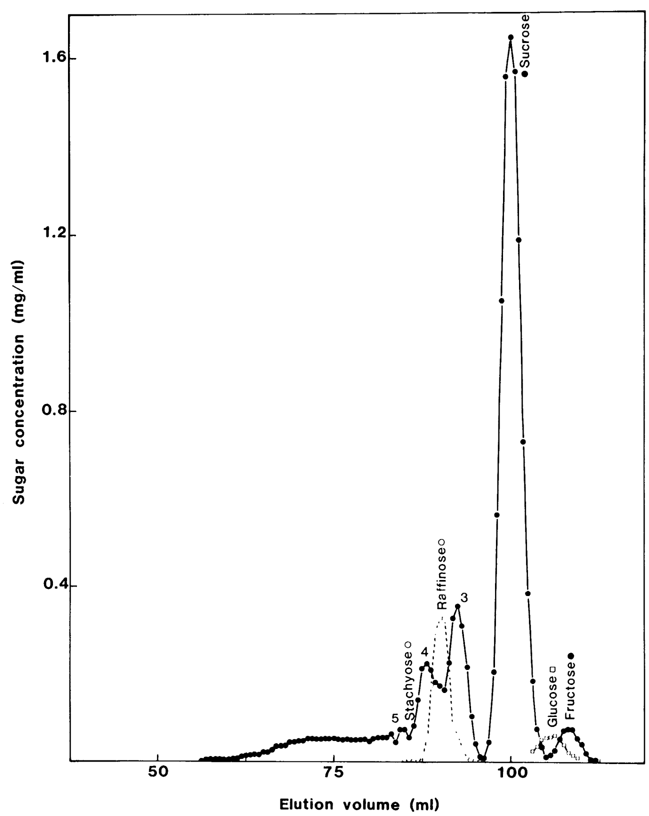

Fig. 2. Separation of 80% ethanol-soluble carbohydrates from barley grain on Fractogel TSK HW-40(S). Glucose was determined using glucose oxidase. Sucrose and oligosaccharides were determined by acid hydrolysis and separate estimation of glucose, fructose, and galactose. Trisaccharide glucose/fructose 1:2, experimental 1:1.96; raffinose glucose/fructose/galactose 1:1:1, experimental 1:0.92:1.05; tetrasaccharide glucose/fructose 1:3; experimental 1:3.05. Numbers on peaks refer to the degrees of polymerization of the (fructosyl) sucrose oligosaccharides.

TABLE II
Comparison of Total Soluble Sugars and Component Saccharides of Triticale with Wheat and Rye

Grain	Total Soluble Sugars[a]	Component Saccharides[a] (g/100 g, dry weight) Degrees of Polymerization						
		1	2	3	4	5	6	>6
Wheat								
Mean (2 varieties)	1.67 a	tr.	0.72 a	0.51 ab	0.13 a	0.03	0.03	0.26 a
Standard error	0.31	···	0.11	0.11	0.06	0.05	0.06	0.09
Rye								
Mean (2 varieties)	3.06 b	tr.	0.97 ab	0.43 a	0.10 a	0.10	0.00	1.45 b
Standard error	0.31	···	0.11	0.11	0.06	0.05	···	0.09
Triticale								
Mean (6 varieties)	2.98 b	0.06	1.20 b	0.76 b	0.31 b	0.17	0.08	0.36 a
Standard error	0.13	0.14	0.05	0.05	0.02	0.02	0.02	0.04
Level of significance among species means[b]	**	ns	**	*	**	ns	ns	**

[a]In columns significantly different amounts of total soluble sugars and individual components are followed by different letters.
[b]*, Significance level $P < 0.05$; **, $P < 0.01$; ns, not significant.

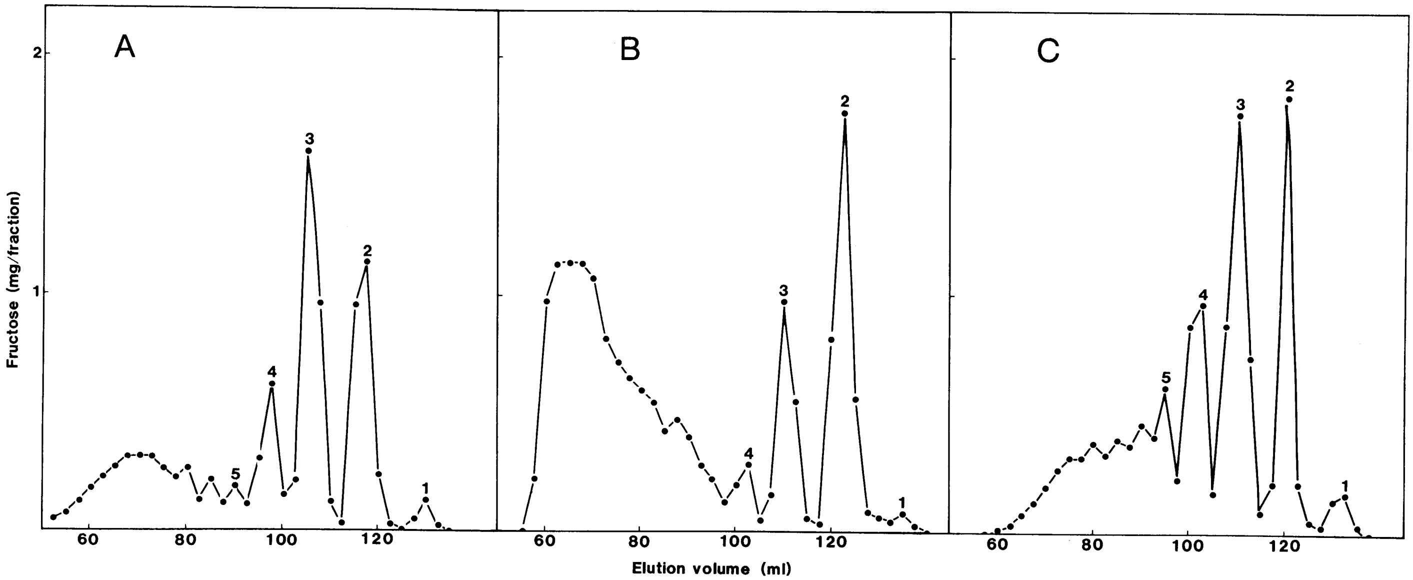

Fig. 3. Comparison of 80% ethanol-soluble carbohydrates from: **A,** wheat; **B,** rye; and **C,** triticale separated by gel filtration on Bio-Gel P2. Fructose content of fractions was determined by reaction with resorcinol. Hydrolysis of some fractions with α-galactosidase (coffee bean, Sigma) released galactose detected with galactose dehydrogenase. Numbers on peaks refer to the degrees of polymerization.

because of limited hydrolysis of the sucrose under the assay conditions. Although the color produced from glucose was more than 10 times that from an equal weight of sucrose, the vast excess of sucrose in cereal grains resulted in an apparently higher glucose content when crude extracts were analyzed. The results obtained after separation by gel filtration are much closer to the lower glucose results obtained by gas chromatography of trimethylsilyl ethers (Henry 1985). Gel filtration indicated fructan levels in agreement with earlier colorimetric values for total fructose (Henry 1985).

Comparison of Triticale Varieties with Wheat and Rye

Results for analysis by gel filtration of six varieties of triticale are compared with two wheat and two rye samples in Table II with examples in Figure 3. Rye contained the most high molecular weight oligosaccharide (degrees of polymerization [DP] > 6). However smaller oligosaccharides were present in highest concentrations in triticale (Table II). The distribution of carbon between polymers of different size may be due to the need to increase the average degree of polymerization as the total carbohydrate level increases to maintain osmotic balance. Thus, triticale, with mean sugar levels higher than wheat, has more tri- and tetrasaccharides. Rye, however, with still higher sugar levels, has less tri- and tetrasaccharide because the carbohydrate has been polymerized further, resulting in more fructan with a DP above 6. Similar effects of total sugar content on DP have been reported in other plants (Darbyshire and Henry 1979).

Differences between the six triticale varieties analyzed were not significant.

Biosynthesis of Oligosaccharides

The product of sucrose/sucrose fructosyltransferase in the leaves of barley (Wagner and Wiemken 1987), wheat (Henry and Darbyshire, *unpublished*), and in other higher plants (Henry and Darbyshire 1980, Pollock 1986) is 1^F fructosylsucrose (isokestose). The major fructan trisaccharide in cereal grains identified in this study is 6^G fructosylsucrose (neokestose), which may be produced by the action of a fructan/fructan fructosyltransferase. However the fructans of grasses are (2→6) linked rather than (2→1) linked. The first member of the (2→6) linked fructan series 6^F fructosylsucrose (kestose) is not present in large amounts in the grain but is found in leaves (Wagner and Wiemken 1981). These results suggest the need for more work to establish the structure and biosynthesis of fructans in cereal grains.

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