THE SUGARS OF TRITICALE BRAN[1]

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ABSTRACT

The sugars in triticale bran have been examined quantitatively and qualitatively using paper, column, and thin-layer chromatography techniques. The brans were obtained from spring triticale crops grown in 1970, 1971, and 1973 in the same location. The triticale bran sugars were compared with the sugars in bran from 1973 crops of wheat and rye. The sugars found in triticale bran, from each year, in order of magnitude, were—sucrose, raffinose, kestose, nystose, and fructosylraffinose. In wheat bran, the sugars found, in order of magnitude, were—sucrose, raffinose, fructosylraffinose, nystose, and kestose; and in rye bran, in order of magnitude, were—sucrose, kestose, nystose, raffinose, and fructosylraffinose. Glucofructans were lowest in the triticale brans and highest in the rye bran. Fructose and glucose were not present in triticale or wheat brans and only traces occurred in the rye bran. None of the species contained maltose.

Triticale, a hybrid cereal grain, produced by the cross-breeding of wheat and rye, offers an important potential protein source for both human consumption and animal feed. A thorough review of the current research programs and the potential established so far for triticale has been published recently (1). With the exception of a brief comment in a report from this laboratory (2), to our knowledge, no information is available on triticale sugars, *i.e.*, monosaccharides and short-chain oligosaccharides. Klassen *et al.* (3) measured total reducing sugars related to α-amylase activity and kernel development in triticale, but did not describe the actual sugars. As part of our program for developing foods and feeds from cereal grain milling by-products, a detailed investigation of the sugars in triticale bran was undertaken and the results are reported here.

MATERIALS AND METHODS[4]

A spring triticale (6-TA-204) was grown on the same irrigated sites in Fort

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[4]Reference to a company or product name by the U.S. Department of Agriculture does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

Collins, Colo., during the years 1970, 1971, and 1973. During 1973, a wheat control, Chris, and a rye control, Tetra Petkus, were also grown at the same location. Bran samples were obtained by milling on a Quadrumat® Senior Mill.

Invertase was obtained from Difco Laboratories as Invertase Analytical. Galactose oxidase and glucose oxidase were obtained as Galactostat and Glucostat, respectively, from Worthington Biochemical Corporation.

Sugars were measured by the phenol-sulfuric acid method (4). Paper chromatography (descending) was carried out on Whatman No. 1 or 3MM paper in solvent 1-butanol-pyridine-water (6:4:3). Thin-layer chromatography (TLC) was performed on Brinkman precoated silica gel F254 plates, using acetone-water (78:22) as the developing solvent. Sugars were detected on paper with alkaline silver nitrate and on TLC plates with anisaldehyde-sulfuric acid.

A procedure for hydrolysis by invertase directly on paper chromatograms was employed (5). Mild acid hydrolysis refers to hydrolysis in 0.05*N* HCl for 30 min, at 100°C. Strong acid hydrolysis means 2*N* HCl at 100°C for 1 hr.

Enzymatic assays by glucose oxidase and galactose oxidase were performed according to procedures described by Worthington Biochemical Corporation (6).

In Table I, the values for sugars are absolute values. For mono-, di-, and trisaccharides, standard curves were available, whereas for tetrasaccharides, standard curves were computed. In hydrolysates containing mixtures of glucose and/or fructose and/or melibiose, after determining glucose and melibiose enzymatically, these values together with their respective computed absorbance values (phenol-sulfuric acid) were then used to compute fructose by difference in the total sugar determination with phenol-sulfuric acid reagent.

Isolation of Sugars from Bran

Five grams of bran were heated in 50 ml of 70% aqueous ethanol under reflux for 30 min. The mixture was cooled and filtered. The residue was washed with 20 ml of the same solvent. This extraction procedure extracts essentially the same quantity of sugars as does an earlier more laborious procedure described from

TABLE I
Identification and Distribution[a] of Sugars in Triticale Brans from Different Crop Years, and in Wheat and Rye Brans

	% Sugars in Bran				
Sugars	Triticale 1970	Triticale 1971	Triticale 1973	Wheat	Rye
Glucofructans	0.56	0.98	0.76	1.06	3.04
Fructosylraffinose	0.59	0.35	0.42	0.28	0.12
Nystose	0.34	0.28	0.25	0.16	0.40
Raffinose	1.56	1.22	1.48	2.16	0.28
Neokestose	0.65	0.69	0.75	trace	1.39
Sucrose	1.92	1.78	2.39	2.18	2.13
Glucose	0.00	0.00	0.00	0.00	0.04
Fructose	0.00	0.00	0.00	0.00	0.05
Total sugars	5.62	5.30	6.06	5.84	7.45

[a]Moisture-free basis.

our laboratory (7). The combined filtrate and washings were concentrated to approximately 10 ml and filtered through glass wool. One-half of the solution was chromatographed on a column of 200–400 mesh Dowex 50 (K^+) (8). The column was eluted with water at a rate of about 0.5 ml/min, and fractions of 5 ml were collected. Aliquots of 0.5 ml were removed from alternate tubes and assayed with phenol-sulfuric acid for total sugar content. Tubes representing peak elution of sugar peaks were pooled and evaporated to dryness. Those fractions of the peaks overlapping with preceding and following peaks were not included in the pooled material.

Each peak from the chromatography of each of the extracted brans was assayed for monosaccharides by paper and TLC after invertase hydrolysis, mild acid hydrolysis, and strong acid hydrolysis. In addition, each new peak species was investigated as follows.

Oligosaccharides. After mild acid hydrolysis and neutralization with 0.1*N* NaOH, the hydrolysate was assayed for glucose with glucose oxidase and for total sugar with phenol-sulfuric acid. This permitted the glucose:fructose ratio calculation.

Tetrasaccharides. The peak was chromatographed on 3MM paper for 72 hr.

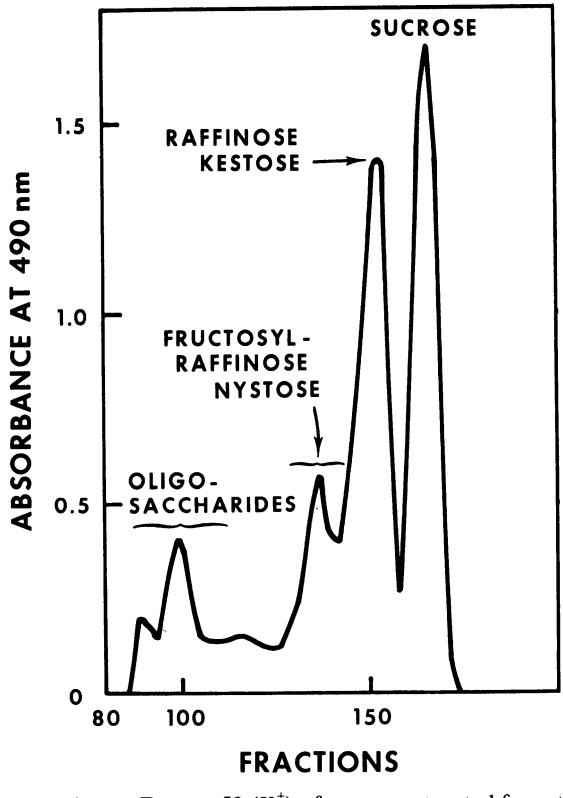

Fig. 1. Chromatography on Dowex 50 (K^+) of sugars extracted from triticale bran, 1970 crop.

The components present were then eluted with 10 ml of water. Each of the two components eluted was subjected to mild acid hydrolysis, neutralized, and assayed for total sugar, glucose, and melibiose, with phenol-sulfuric acid, glucose oxidase, and galactose oxidase, respectively. These data enabled the calculation to be performed for glucose:fructose, and melibiose:fructose ratios, and for quantitative assay of the tetrasaccharide components.

Trisaccharides. The peak was assayed for raffinose with galactose oxidase, then chromatographed on 3MM paper for 24 hr. The two components present were eluted, hydrolyzed with mild acid, and assayed for total sugar, glucose, and melibiose in the same manner as described for the tetrasaccharides in order to calculate ratios of sugars produced during hydrolysis, and to calculate the actual quantities of trisaccharide components.

Disaccharides and Monosaccharides. Before and after mild acid hydrolysis and neutralization, the extracts were assayed for total sugar and glucose with phenol-sulfuric acid reagent and glucose oxidase, respectively.

RESULTS AND DISCUSSION

The chromatographic separation on Dowex 50 (K^+) of the sugars present in triticale bran from the 1970 crop is illustrated in Fig. 1. The patterns of all triticale brans investigated were similar to this. The separation of the sugars from the wheat and rye brans is shown in Figs. 2 and 3, respectively.

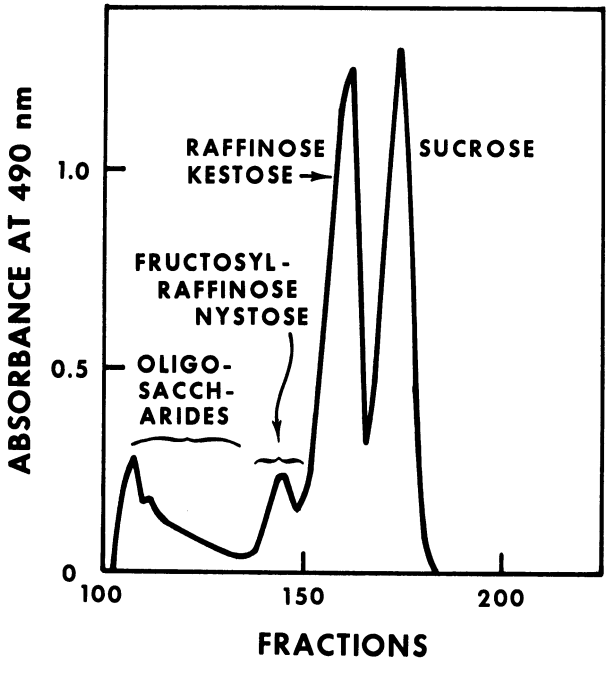

Fig. 2. Chromatography on Dowex 50 (K^+) of sugars extracted from wheat bran.

Oligosaccharides. In all cases this was a complicated mixture of oligosaccharides containing glucose and fructose. The ratio of glucose to fructose was: triticale 1970, 1:7.0, 1971, 1:8.2, 1973, 1:8.7; wheat 1:8.0; rye 1:12.7. It is likely that these components were glucofructans of the type previously encountered in wheat bran (7) and generally found in cereals (9, 10). Only traces of uronic acid residues were observed; these have not been included in the analyses.

Tetrasaccharides. Separation of the two tetrasaccharides was successfully achieved by quantitative chromatography on 3MM paper. On paper and TLC these nonreducing components migrated like fructosylraffinose and nystose. Their distribution coefficient (K_D) (8) in the column chromatographic system used was identical to standards for these two compounds. Invertase hydrolysis of the faster moving component yielded glucose and fructose; mild acid hydrolysis yielded glucose and fructose in the ratio, respectively, triticale 1970, 1:3.36, 1971, 1:3.00, 1973, 1:3.23; wheat, 1:2.89; rye, 1:3.27. All evidence points to this tetrasaccharide being glucose-(fructose)$_3$. This component has been identified as nystose (11), although the authors have made no attempt to establish the absolute configuration of this tetrasaccharide.

Invertase hydrolysis of the slower moving component yielded melibiose and fructose. Mild acid hydrolysis yielded a mixture of melibiose and fructose in the respective ratio, triticale 1970, 1:2.01, 1971, 1:2.20, 1973, 1:2.50; wheat 1:1.76, 1:1.76, and rye 1:1.84. Complete identification of a compound which exhibited identical chromatographic behavior and which yielded the same sugars on acid or invertase hydrolysis from another wheat bran was made by our laboratory (12), and based on data before and after hydrolyses, it appears that this component in all species was fructosylraffinose.

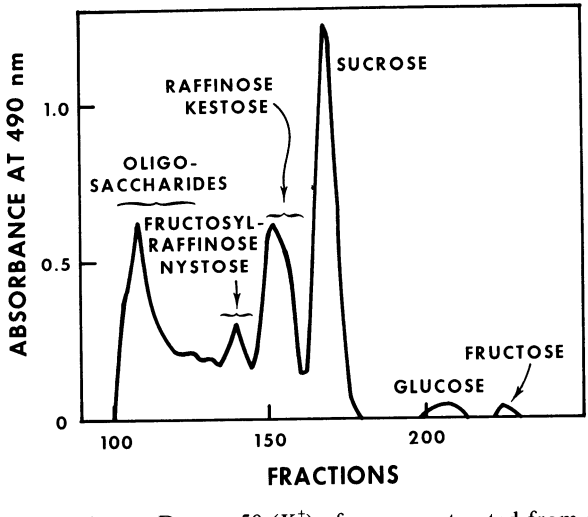

Fig. 3. Chromatography on Dowex 50 (K^+) of sugars extracted from rye bran.

Trisaccharides. Paper and TLC showed only two components which could be separated on 3MM paper. The compounds migrated like raffinose and kestoses, and had K_D values in the column chromatographic system identical to raffinose and kestoses. Mild acid or invertase hydrolysis of the slower component yielded only melibiose and fructose, whereas the faster component yielded only glucose and fructose. No attempt was made to crystallize these components, although it is clear from these observations and from previous analogous data obtained for the same components from wheat bran (7, 13), that these components are raffinose and a kestose. In the earlier reports (7, 13), the kestose component of wheat bran was established to be neokestose. This has not been carried out here, although from the analogous behavior, we have seen no reason to doubt that the kestose in the brans investigated here is neokestose.

Disaccharides. Paper and TLC indicated a single component behaving like sucrose, and which had a K_D value in the Dowex system identical to that of sucrose. Mild acid or invertase hydrolysis yielded only glucose and fructose (1:1), indicative of sucrose.

Monosaccharides. Trace amounts of glucose and fructose were found only in the rye bran. Glucose was identified by being assayed as 100% glucose with glucose oxidase. Fructose was identified by its K_D value in the column system and by its migration rate on paper and TLC. In addition, its uv spectrum in HCl (14) was identical to the uv spectrum of a standard in HCl.

DISCUSSION

The identification of the sugars and their calculated percentage composition of the original brans are shown in Table I. The percentage composition was calculated by knowing the total area under the peaks (Figs. 1, 2, 3), the ratio of components to each other within each peak, and their respective absorbance values with the phenol-sulfuric acid reagent.

Only small differences are apparent in the distribution of sugars in triticale brans from different years. Sucrose was the predominant sugar, followed by raffinose, kestose, fructosylraffinose, and nystose. Upon comparison on the distribution patterns of the triticale bran sugars with the patterns of the wheat and rye sugars, several differences were apparent. The total sugar content of triticale brans averaged 5.66% and was thus closer to the value for wheat than for rye bran. The glucofructan content was considerably lower in triticale than in wheat or rye. In the triticales, fructosylraffinose was at a higher level than it was in either wheat or rye, whereas nystose, raffinose, and kestose were intermediate between the equivalent values for wheat or rye. Sucrose content varied in the triticales and was thus higher or lower than wheat or rye although the average sucrose value was lower in the triticales. In wheat, those sugars containing galactose accounted for 41.8% of total sugars, whereas those sugars containing only glucose and fructose accounted for 58.2% of total sugars. Sugars containing galactose comprised 5.4% of rye bran total sugars and sugars containing only glucose and fructose made up the remainder—94.6%. In the triticales, sugars containing galactose comprised 33.1% (average) of total sugars, whereas sugars containing only glucose and fructose accounted for 66.9% (average). From these data on sugars of brans, triticale is somewhat between its parents, but more like wheat than rye.

In the wheat bran, almost no kestose was present although it was present at high levels in three other wheat brans previously investigated (7, 13). It was not reported, however, in two other wheat brans recently investigated (15). No stachyose was found in any of the brans reported here. It has been found in wheat bran by some workers (7, 13) but not by others (15, 16). These discrepancies are presumably due to varietal differences.

No maltose was found in any of the species examined. Klassen *et al.* (3) found that reducing sugar increased in the maturing triticale kernel and suggested this was due to α-amylase activity during development and was associated with kernel shriveling. The results of this study do not dispute this, though they indicate that if α-amylase activity does occur during development, hydrolysis does not proceed as far as the maltose stage.

Caution should be exercised in drawing a general conclusion from the small number of samples analyzed. It is emphasized that the results reported here pertain only to the varieties analyzed, and not necessarily to all varieties of triticale, wheat, and rye.

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