# The Sugars of Wheat Bran[1]

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**ABSTRACT**

Sucrose, raffinose, neokestose, stachyose, and a fructosylraffinose have been shown to occur in wheat bran. Of these,the latter three have not been reported previously. Chromatographic evidence indicates the presence of glycerol, xylose, arabinose, glucose, and fructose.

Wheat bran, a by-product of flour milling, is composed of the pericarp and the outermost tissues of the seed including the aleurone layer (1). It constitutes almost 10% of the total weight of wheat milled for flour. On a moisture-free basis it contains about 70% carbohydrates, comprising, in approximate amounts, 43% hemicellulose, 35% cellulose, 14% starch, and 8% sugars (2). Considerable work has been reported on the hemicellulose carbohydrates of wheat bran (3), but observations on the sugar components are restricted to one report in which the presence of sucrose, raffinose, melibiose, glucose, galactose, fructose, and arabinose is recorded (4). As part of this Laboratory's program for investigating bran as a feed source, a more detailed investigation of the naturally occurring sugars in bran was undertaken and the results are reported here.

## MATERIALS AND METHODS

A sample of a Canadian hard red spring wheat bran was obtained from J. D. Summers of the University of Guelph, Ontario, Canada. A Gaines soft white wheat bran was obtained from Pullman, Washington. Invertase was purchased from Difco Laboratories as Invertase Analytical. Honey-bee invertase was prepared by a standard procedure (5). Nystose and 1-kestose were gifts from R. M. McCready (6). Neokestose and 6-kestose were gifts from D. Gross (7).

Eluted sugars were measured by the phenol-sulfuric acid method (8).

Paper chromatography was carried out on Whatman No. 1 or 3MM paper in the descending fashion in one of three solvents: A, 1-butanol-pyridine-water (6:4:3); B, 1-butanol-water (86:14); and C, ethyl acetate-pyridine-water saturated with boric acid (60:25:20). Thin-layer chromatography (TLC) was carried out on silica gel plates in solvent 1-propanol-ethyl acetate-water (7:2:1). Solvent compositions are given as volume ratios. In all cases where sugar ratios were performed, the sugars were separated by paper chromatography in solvent A. After separation, the spots or strips were eluted with water and the aqueous solution was analyzed. Sugars were detected on paper by one of three methods–alkaline silver nitrate, aniline phthalate, or periodate-benzidine–and on TLC plates with anisaldehyde-sulfuric acid. Gas-liquid chromatography (GLC) of methyl sugars

[1]Contribution from Western Regional Research Laboratory, Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710. Presented at the joint meeting, AACC-AOCS, Washington, D. C., March-April, 1968. Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

was carried out with an Aerograph gas chromatograph (A 350-B); 5% neopentyl-glycolsuccinate on 60- to 80-mesh Chromosorb W was used as the solid support (silanized) in aluminum columns (10 ft. by 0.125 in.). The flow of helium gas was about 23 ml. per min. and the temperature 160° C. in all analyses. Columns were resilanized at regular intervals.

Sugars were methylated by a modification of the procedure of Kuhn *et al* (9). About 10 mg. of sugar in 2.4 ml. of dried dimethylformamide and 0.6 ml. of methyl iodide containing 100 mg. of freshly prepared silver oxide was shaken at room temperature for 24 hr. in darkness. After this time a further 0.5 ml. of dimethylformamide, 0.2 ml. of methyl iodide, and 25 mg. of silver oxide were added, and shaking was continued 48 hr. longer. The methylated products were isolated in the usual manner (9). Methanolysis was carried out by refluxing for 30 min. in methanol containing 0.75% HCl. The HCl was removed by washing through Duolite A4 ($OH^-$) resin. Sucrose, maltose, melibiose, turanose, raffinose, 1-kestose, nystose, and stachyose were employed as methylation standards.

The powder diffraction technique (Cu radiation) was used for X-ray analyses.

A procedure (10) for hydrolyses by invertase and honey-bee invertase directly on paper chromatograms was used.

"Mild acid hydrolysis" refers to hydrolysis in 0.05*N* HCl at 100° C. for 30 min.; "strong acid hydrolysis" refers to hydrolysis in 2*N* HCl at 100° C. for 24 hr. (fructose is completely destroyed under these conditions).

#### Isolation of Sugars from Canadian Wheat Bran

Bran (25 g., 10% moisture) was heated under reflux for 1 hr. with 400 ml. of 70% aqueous ethanol. The mixture was cooled and filtered. The residue was again treated under the same conditions. The two filtrates were combined and concentrated to about 100 ml. The aqueous solution was twice extracted with 200 ml. of chloroform, then concentrated to about 20 ml. One-half of this solution was chromatographed on a column (120 by 4.5 cm.) of 200- to 400-mesh Dowex 50W x 4 ($K^+$ form) (6). The resin was conditioned and eluted with 0.2% potassium benzoate solution to prevent microbial growth. With an automatic fraction collector, fractions of 5 ml. were collected at a flow rate of 0.7 ml. per min. Aliquots of 0.1 ml. from alternate tubes were assayed for carbohydrate content. The peaks shown in Fig. 1 were pooled and investigated.

#### Peaks

Peak I was concentrated to low volume and washed through Sephadex G25 (fine) to remove the potassium benzoate, and lyophilized.

The pooled peak II was concentrated to about 10 ml. and chromatographed on a column (75 by 2.5 cm.) of Sephadex G25 (fine) for further purification and desalting. The column was eluted with water at a rate of 0.5 ml. per min., and fractions of 5 ml. were collected. Aliquots of 0.2 ml. from alternate tubes were assayed for carbohydrate content. A single sharp peak was obtained, which was pooled and evaporated to dryness. The white residue was chromatographed on Whatman 3MM paper in system A over a

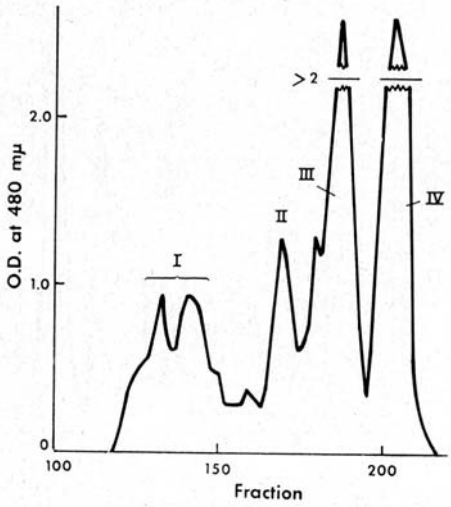


Fig. 1 (left). Chromatography on Dowex 50W ($K^+$) of sugars from Canadian wheat bran.

period of 72 hr. Two components were present and were eluted; both were nonreducing. The slower component ($R_{glucose}$ = 0.11) slowly crystallized from aqueous ethanol. It had m.p. 135°C. with a change in appearance at 102°-105°C. (lit. (11) for stachyose: m.p. 101°-105°C. and 150°C.).

The faster component ($R_{glucose}$ = 0.22) was obtained as an amorphous powder, $[\ ]_D^{23}$ +65.7. (*c* 0.6, water).

The pooled fractions (peak III) were concentrated to about 1 ml. and chromatographed on Whatman 3MM paper in solvent A over a period of 48 hr. Two nonreducing components with $R_{glucose}$ values of 0.29 and 0.52 were eluted and separately chromatographed on Sephadex G25 (fine) under conditions identical to those of peak II above. Each component gave a single sharp peak. The slower component crystallized from aqueous ethanol. It has m.p. 79°C. and $[\alpha]_D^{23}$ +104° (c 2.0, water) (lit. (12) for raffinose pentahydrate: m.p. 80°C. $[\alpha]_D$+105.2°).

The faster component was a very hygroscopic syrup. At one time it crystallized from ethanol but immediately became syrupy on exposure to the atmosphere. No crystallization was observed after seeding with 1- or 6-kestose. The material had $[\alpha]_D^{23}$ +22.7° (*c* 1.0, water) (lit. (7) for neokestose: $[\alpha]_D$+21 ±1°).

The pooled fractions (peak IV) were concentrated to small volume, part of which was washed through Sephadex G25 (fine).No attempt was made to crystallize the material.

#### Isolation of Sugars from the Gaines Wheat Bran

Gaines wheat bran (10 g.) was subjected to the same extraction procedure as the Canadian variety above, with appropriate reduction in quantity of reagents. Chromatography on the same Dowex column under the same experimental conditions gave the pattern illustrated in Fig. 2.

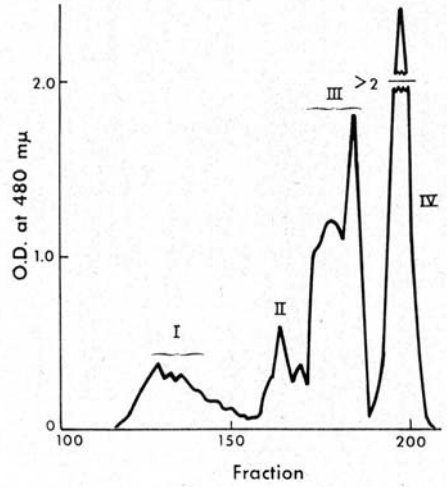


Fig. 2 (right). Chromatography on Dowex 50W ($K^+$) of sugars from Gaines wheat bran.

## RESULTS AND DISCUSSION

Paper-chromatographic examination of the water-soluble portion of the Canadian wheat bran extract showed the presence of mono-, di-, tri-, and tetrasaccharides and higher oligosaccharides. Chromatography on Dowex 50 ($K^+$) in the manner previously described for separation of short-chain sugars (6) afforded a separation of these sugars as shown in Fig. 1.

### Peak I

This was a complicated mixture of oligosaccharides containing mainly fructose but also a uronic-type residue. A morethorough investigation of this mixture will be reported at a later date. At this point it appears that the components are probably fructans of the type previously encountered in cereals (3,13).

### Peak II

Initially, paper chromatography indicated two nonreducing components which migrated at the rate expected for tetrasaccharides. Separation of the components was achieved by preparative scale paper chromatography on Whatman 3MM paper. The crystalline slower- moving component had a $R_{glucose}$ value identical to that of stachyose and, on mild acid hydrolysis or invertase treatment, behaved in a manner identical with that of stachyose, yielding fructose and manninotriose (1:1). Strong acid hydrolysis destroyed the fructose but yielded glucose and galactose (1:2). Final evidence for stachyose was provided when the X-ray pattern of the crystalline material and that of standard stachyose proved to be identical. Stachyose is quite widespread in nature (11,14) but has not previously been reported in wheat.

The second noncrystalline component, on mild acid hydrolysis or invertase treatment, yielded fructose and melibiose (2:1). Strong acid hydrolysis destroyed fructose and yielded galactose and glucose (1:1). These ratios and

GLC of the methanolized methylated material indicated the possibility of the "fructosylraffinose" structure Gal-(1→6)-Glu-(1→2)-Fru-(?2)-Fru. Permethylfructose, permethylgalactose, and methyl-2,3,4-trimethylglucoside as well as an unidentified mono-OH component were found in approximately equal amounts, suggesting that the second fructose unit is linked to the terminal fructose of the raffinose skeleton. Only one report of a fructosylraffinose has been previously recorded in which paper chromatography provided evidence for its occurrence in wheat (15). No structural work was attempted, but its possible presence substantiates the above observations.

**Peak III**

The crystalline material had a $R_{glucose}$ value identical to that of raffinose, and like raffinose yielded fructose and melibiose (1:1) on mild acid hydrolysis or invertase treatment. GLC of the methanolized methylated component gave a pattern identical with that of raffinose treated in the same manner. The X-ray analysis provided conclusive evidence that the component was raffinose. This sugar has long been established as a component of all wheat fractions (3) and is usually second to sucrose in concentration.

The second component, on mild acid hydrolysis or invertase treatment, yielded only glucose and fructose (1:2). Action of honey-bee invertase was negative. The Raybin test (16) was positive. The material did not crystallize after seeding with either 1-kestose or 6-kestose. GLC of the methanolized methylated material showed tetramethylfructose and methyl-2,3,4-trimethylglucoside (about 2:1) indicative of neokestose [Fru-(2→6)-Glu-(1→2)-Fru]. When the borate complex (R. M. Saunders, unpublished work) of this material was paper-chromatographed along with the borate complexes of authentic 1-kestose [Fru-(2→1)-Fru-(2→1)-Glu], 6-kestose [Fru-(2→6)-Fru-(2→1)-Glu], and neokestose, the product from wheat bran migrated the same as that of neokestose. Optical rotation values support the neokestose identification. Also, the compound was a very hygroscopic syrup, as reported previously for neokestose (7). The positive Raybin test actually indicates a sucrose molecule skeleton in which the fructose moiety is not further substituted, a factor which is compatible with the neokestose structure. To support this observation, it was compared in this test with nystose [Glu-(1→2)-Fru-(1→2)-Fru-(1→2)-Fru] and 1-kestose (both negative) and sucrose and raffinose (both positive). Honey-bee invertase, which is actually an alpha-glucosyltransferase, did not hydrolyze the molecule, because in neokestose the glucose moiety is substituted at C-6. Under the same conditions, 1-kestose was hydrolyzed. Neokestose has not previously been established as a component of the wheat kernel, although it does occur in wheat straw (17). Other workers have realized the presence of a "glucodifructose" in wheat flour (3), but structural identities have not been established. All three known nonreducing kestoses, 1-, 6-, and neokestose, can be produced by transfer of a beta-D-fructofuranosyl radical to sucrose by the action of invertases (7) as well as occurring naturally (14). The bran under investigation does actually contain a fructofuranosidase (R. M. Saunders, unpublished work), but it is unlikely that this has reacted with residual sucrose during storage to produce neokestose since neither 1- nor

6-kestose is present and glucose is not present in large amounts. In the normal transfructosylation reaction all three kestoses are usually produced.

**Peak IV**

Paper chromatography indicated a single component behaving in all solvents like sucrose. Mild acid hydrolysis and action of invertase and honey-bee invertase yielded glucose and fructose (1:1). GLC of the methanolized methylated sugar and comparison with authentic sucrose treated under identical conditions indicated that this peak was sucrose. Sucrose was established as a wheat component as long ago as 1886 (18), and numerous investigators have established its presence in all fractions of wheat.

**Hexose, Pentose, Triose**

Prior to chromatography on Dowex 50W ($K^+$) of the water-soluble alcoholic extract of bran, paper-chromatographic investigation in systems A and B and TLC indicated the presence of glucose, fructose, xylose, arabinose, and glycerol. Continuation of the elution shown in Fig. 1 eventually elutes the monosaccharides, but owing to their low concentration, this was not carried out here. Glucose and fructose have previously been noticed in wheat bran (4). Xylose and arabinose are components of hemicellulose of wheat bran (19), and thus it is not surprising that traces of the two pentoses were found. The small amount of glycerol may arise by residual lipase action since bran is rich in esterases (20); the appearance of glycerol has been noted by other workers (21).

Table I summarizes the identification of the peak components and the indicated monosaccharides, lists their $R_{glucose}$ value in solvent A, and shows their calculated percentage composition of the total sugars and of the original Canadian wheat bran.

TABLE I. IDENTIFICATION OF PEAK COMPONENTS IN FIGURE I AND INDICATED MONOSACCHARIDES IN CANADIAN WHEAT BRAN: THEIR DISTRIBUTION AND $R_{glucose}$ VALUE

| Peak | Sugars Present | Percentage Distribution of Sugars | Percentage of Sugars in Bran (Moisture-Free) | $R_{glucose}$ in Solvent A |
|---|---|---|---|---|
| I | Fructans<br>Uronic sugar | 11.6 | 0.75 | 0 0.10 |
| II | Stachyose | 2.6 | 0.17 | 0.11 |
| | Fructosylraffinose | 5.1 | 0.33 | 0.22 |
| III | Raffinose | 22.7 | 1.47 | 0.29 |
| | Neokestose | 19.3 | 1.25 | 0.52 |
| IV | Sucrose | 35.3 | 2.29 | 0.76 |
| | Xylose | 3.4 | 0.22 | 1.24 |
| | Arabinose | | | 1.12 |
| | Glucose | | | 1.00 |
| | Fructose | | | 1.12 |
| | Glycerol | | | 1.54 |
| | Total sugars | 100.0 | 6.48 | |

Paper-chromatographic examination of the water-soluble portion of the Gaines wheat bran extract gave results similar to those observed with the Canadian variety. The presence of glucose, fructose, xylose, arabinose, and higher oligosaccharides was indicated. The oligosaccharides isolated by column chromatography were tentatively identified by their analogous behavior on paper chromatograms compared to the sugars isolated from Canadian wheat bran. The sugar components of the Gaines wheat bran are listed in Table II, with their tentative identification and their respective precentage

TABLE II. IDENTIFICATION OF PEAK COMPONENTS IN FIGURE 2 AND INDICATED MONOSACCHARIDES IN GAINES WHEAT BRAN, AND THEIR DISTRIBUTION

| Peak | Sugars Present[a] | Percentage Distribution of Sugars | Percentage of Sugars in Bran (Moisture-Free) |
|---|---|---|---|
| I | Fructans | 11.0 | 0.70 |
| II | Stachyose<br>Fructosylraffinose | 11.2 | 0.71 |
| III | Raffinose<br>Neokestose | 40.3 | 2.58 |
| IV | Sucrose | 35.1 | 2.25 |
| | Xylose<br>Arabinose<br>Glucose<br>Fructose | 2.4 | 0.15 |
| | Total sugars | 100.0 | 6.39 |

[a] Tentative identification only.

composition of the total sugars and of the bran. Very close agreement is observed in the distribution of the sugars in the two brans from differing wheat types. That these brans were obtained and processed in differing environmental conditions emphasizes that the sugars found here have not been influenced by an external factor such as microbial contamination. Sucrose is the predominant sugar, followed by raffinose, in agreement with the previous report (4). Neokestose is next in concentration, then smaller amounts of the other sugars. It is somewhat surprising that neokestose was not recorded by the previous workers, considering the amount actually present. Unlike the previous report, melibiose and galactose were not observed here, although it is conceivable that these could arise by degradation of raffinose during isolation. The over-all percentage of sugars, 6.48 and 6.39, is higher than the previously reported 5.31 (2). This could be due to more efficient extraction and the more efficient assay technique of phenol-sulfuric acid, and to the difference in bran samples.

**Acknowledgments**

We wish to thank R. M. McCready of this Laboratory for specimens of nystose, 1-kestose, and honey-bee invertase, and for valuable discussion; and D. Gross of Tate and

Lyle Laboratories, England, for specimens of 6-kestose and neokestose. Our thanks also to K. J. Palmer of this Laboratory for the X-ray analyses.

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[Received November 6, 1967. Accepted May 29, 1968]