THE CARBOHYDRATES OF THE GRAMINEAE

X. A Quantitative Study of the Carbohydrates of Wheat Germ[1]

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ABSTRACT

The embryonic plant and scutellum, obtained by hand-dissection of a soft white wheat, variety Holland, comprised 1.25 and 1.39% respectively of the dry weight of the kernel. The total sugar content of the embryonic plant was 21.9% and of the scutellum 18.4% on a defatted dry matter basis, or 20.1% of the total embryo. Paper chromatography revealed that the sugars consisted almost entirely of sucrose (embryo, 54.7%; scutellum, 62.0%) and raffinose (embryo, 45.3%; scutellum 38.0%) with only traces of glucose. The sugar content of the scutellum decreased when the kernels were exposed to moisture for 1 and 2 days, indicating that the sugar content of the germ may be a useful index of the storage history of wheat.

Commercial wheat germ milled from hard wheats which had been stored several months at 5°C. and then defatted, contained 16.8% total sugars (dry matter basis) consisting of 57.6% sucrose, 37.6% raffinose, 4.8% fructose, and small quantities of glucose.

A single wheat germ may be analyzed by the micro techniques which have been devised.

In 1886 it was shown (22) that wheat germ contained 15 to 18% of soluble carbohydrate material of which the major component was sucrose. A second unidentified, nonfermentable component, which had a high positive specific rotation, was thought to be raffinose, a trisaccharide isolated and crystallized in the same year from barley flour (18).

Several investigations on the composition of wheat germ followed thereafter (9,23,24), and in 1895 commercial wheat germ was shown (25) to contain sucrose and raffinose, both of which were obtained crystalline. In addition a small proportion of glucose was also reported (24) to be present in commercial wheat germ, since the extract exhibited a slight reducing action on Fehling's solution. A quantitative analysis of the carbohydrates in what appears to be the embryonic plant component of the wheat germ was carried out by determining the total extractable carbohydrates (raffinose plus sucrose) and subtracting from this value (24.3%) the percentage of raffinose (6.9%) (deduced from a galactaric (mucic) acid analysis) to give the sucrose content (17.4%).

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The presence of sucrose and raffinose in commercial wheat germ was placed beyond doubt in 1913 when both sucrose and raffinose were isolated and characterized by melting point and specific rotation (21). The same investigators reported that the embryonic plant component of wheat germ contained a relatively large proportion of glucose, since the aqueous extract of the germ readily yielded glucose phenylosazone. Bearing in mind that phenlyosazone formation is generally performed in an acidic medium, this assertion is open to question since both sucrose and raffinose would readily give rise to glucose phenylosazone.

Crystalline sucrose and raffinose were later isolated from wheat germ and characterized, and it was also reported (4,5) that the raffinose was present exclusively in the embryonic plant. The total carbohydrate content of the wheat germ was 9.4%, a lower value than that hitherto reported; it was said to consist of sucrose 5.2%, raffinose 4.0%, and reducing sugars 0.2%.

As part of a program initiated in these laboratories to study the carbohydrates of the Gramineae, it seemed desirable to reinvestigate the carbohydrates of wheat germ and to resolve, if possible, the difference between the results of Schulze and Frankfurt (25) and those of Colin and Belval (4,5) set forth above.

This paper, briefly reviewed elsewhere (15), is concerned with a study of the carbohydrates of commercial wheat germ and of (a) the embryonic plant (coleoptile-plumule-hypocotyl-coleorhize) portion and (b) the scutellum (scutellum and columnar epithelium) portion of hand-dissected wheat germ, using the modern techniques of column and partition chromatography. Some preliminary experiments have also been conducted on the effect of moisture on the carbohydrates of the wheat germ in the intact kernel.

Materials and Methods

Commercial Germ. Wheat germ milled from hard red spring wheat supplied by General Mills, Inc., Minneapolis, and containing approximately 7.4% moisture was stored in a sealed container at about 5°C. until the various analyses were made. It was relatively free from scutellum material and consisted largely of the embryonic plant contaminated with some flour and appreciable amounts of bran. Before analysis, the germ was ground in a Wiley laboratory mill to pass sieve openings 0.5 mm. in diameter. When the grinding was stopped after only about two-thirds of the material had passed through the sieve, the contamination with bran flecks was greatly reduced.

Hand-Dissected Germ. Since large-scale isolation of pure wheat

germ is impossible, recourse was had to hand-dissection. In this way data were also secured on the percentage of germ present in the kernel. A sample of sound, plump, soft white wheat, variety Holland, containing 9.2% moisture (determined by drying for 1 hour at 130°C.), was employed. Subsamples of the same wheat containing 12.3 and 12.9% moisture, obtained by exposing them in a desiccator containing water at room temperature (24° – 25°C.) for about 18 and 24 hours respectively were also dissected with specially made micro tools. With the assistance of a binocular microscope (30×), the embryonic plant could be separated from the germ by first removing the pericarp covering the germ and applying a slight pressure with a scalpel at point B (Fig. 1). When the kernels contained about 9% moisture, it was a relatively simple matter to obtain the embryonic plant free from the scutellum, but at the higher moistures, it was often contaminated with scutellum. On the other hand, it was difficult to dissect pure scutellum from the wheat kernels at 9% moisture, since the scutellum adhered by its columnar epithelial layer to the endosperm. However, the scutellum was detached from dry kernels by passing the very thin blade of a specially-made scalpel between the scutellum and the endosperm; any endosperm remaining attached to its convex side was removed by careful scraping under the microscope. It was quite difficult to remove all the bran particles from the edge of the shield of the scutellum. The weights of the original kernels, and of the embryonic plant and scutellum, were recorded.

Extraction of Carbohydrates. The commercial wheat germ was dried *in vacuo* at 75°C. over phosphorus pentoxide to constant weight and the loss in weight computed as moisture. The dried sample was

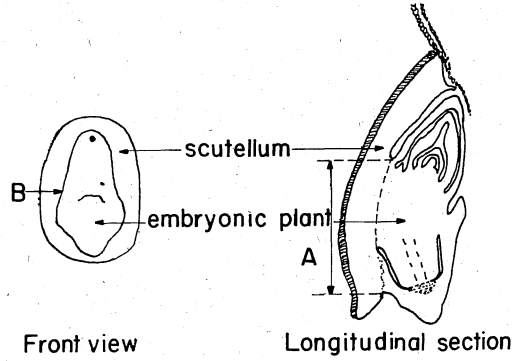

Fig. 1. Diagram illustrating the embryonic plant, A, and the scutellum parts of wheat germ. To remove the embryonic plant, pressure was exerted at B. The scutellum was detached by inserting a specially made scalpel between the scutellum and the endosperm.

extracted with anhydrous diethyl ether in a Soxhlet apparatus for 6 to 7 hours and the percentage of ether-extractable material determined.

(a) Large-scale extraction. For the large-scale isolation of the carbohydrates, 9.665 g. of germ were employed. In order to render the carbohydrate material more accessible to extraction with water, the defatted material was boiled with 95% aqueous ethanol for 1 hour and, without separation, the mixture was freed from solvent by evaporation *in vacuo*. To the residue, 150 ml. of water were added and the mixture boiled for 1.5 hours. The mixture was cooled and centrifuged and the clear yellow liquid evaporated (bath temperature 50° to 60°C.) *in vacuo* almost to a syrup. Absolute ethanol was added to precipitate protein, which was removed by centrifugation. The clear solution was reconcentrated as before and the treatment with ethanol repeated. The process of concentration and treatment with ethanol was repeated until no more precipitate was produced. Each precipitate was redissolved in water and examined for the presence of sugars by paper chromatography. If any were detected, the solution was treated with ethanol to precipitate impurities; the sugars in the mother liquor were recovered by evaporation and combined with the main bulk of the material. During all the manipulations the pH was checked and the solutions maintained neutral or slightly alkaline by the addition of a little dilute ammonium hydroxide to prevent any hydrolysis of the sucrose and raffinose during the concentrations.

Evaporation of the aqueous ethanol solution *in vacuo* (bath temperature 50° to 60°C.) gave the soluble carbohydrates as a white amorphous powder which was dried *in vacuo* to constant weight.

(b) Micro-extraction. For the micro-extractions, about 100 mg. of commercial wheat germ, 15 to 20 mg. of hand-dissected embryonic plant material, and 10 to 15 mg. of the scutellum were employed. After drying *in* vacuo at 75°C. over phosphorus pentoxide and removal of most of the lipid by extraction with absolute diethyl ether, the residue was boiled with 95% aqueous ethanol for 1 hour and the solvent evaporated without separation. The extraction of the sugars was carried out with about 1 ml. of boiling water. The extract was filtered and the residues washed with a small amount of water. Losses were largely eliminated by carrying out the successive extractions with diethyl ether, ethanol, and water in the same vessel (Fig. 2). The combined extract and washings were passed first through a weak cation exchange resin (Duolite C3) and then through an anion resin (Duolite A4). The eluate was concentrated *in vacuo* to dryness and weighed.

To determine whether the resins used for the removal of protein and inorganic material absorbed any of the sugars from the aqueous extract of the wheat germ, the following experiments were conducted:

(a) Aqueous solutions containing known amounts of D-glucose, sucrose, and raffinose were passed successively through the resins and, after adjustment of the solution to a known volume, the sugar in the final eluate was determined by the phenol sulfuric acid method (see below). Repeated experiments showed that recovery was quantitative.

(b) An aqueous solution containing a mixture of 500 γ of sucrose and 500 γ of raffinose was added to a 10-ml. aliquot from 25 ml. of aqueous solution containing the carbohydrates from 0.0228 g. of embryonic plant. The 10 ml. of aqueous extract of the germ with and without the added sugars were treated with the two resins and the solutions analyzed after concentration for sucrose and raffinose by paper chromatography using the phenol-sulfuric acid reagent as described in the next section. The difference between the two results indicated that the recovery of sucrose and raffinose was 99%.

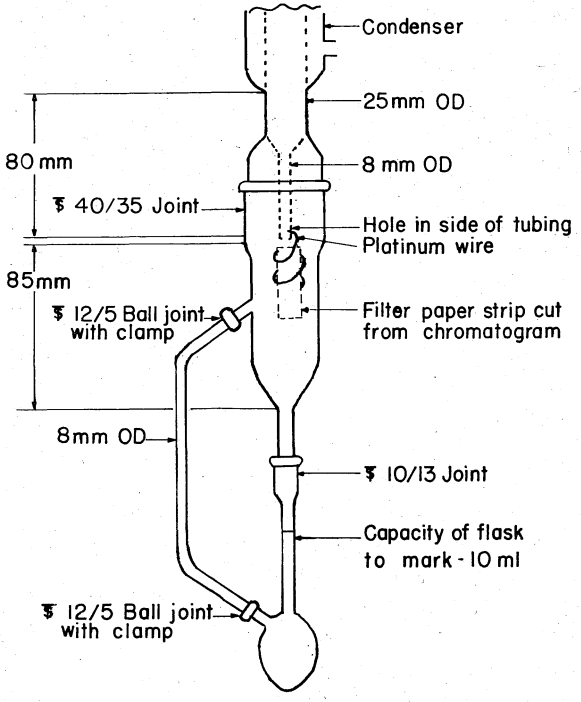

Fig. 2. Diagram of apparatus employed for the microextraction of lipids and sugars from wheat germ tissues.

Paper Chromatography. (a) Qualitative. Whatman No. 1 filter paper was used throughout, some papers being freed from soluble carbohydrate impurities by washing (13). The following solvents were employed: A, phenol saturated with water (irrigation time, 15 to 18 hours) (19); B, 1-butanol:propionic acid:water (4:1:5) (irrigation time 70 hours) (3); C, 1-butanol:ethanol:water (4:1:5) (irrigation time 70 hours) (19). The sugars were detected on the chromatograms by spraying either with ammoniacal silver nitrate and heating for 1 to 2 minutes at 100°C. (19) or with a solution of N,N'-dimethyl-*p*-aminoaniline (0.2 g.) in 50% aqueous ethanol (50 ml.) containing trichloroacetic acid (1 g.) and conc. hydrochloric acid (1 ml.) and heating for 5 to 10 minutes at 130°C. (3).

(b) Quantitative. The mixture of sugars was separated by solvent A and the components were determined by the phenol-sulfuric acid reagent (7,8), reference being made in the usual way to standard curves for fructose, sucrose, and raffinose (7). The absorbance of the colored solution formed by the reaction of the sugars with the phenol-sulfuric acid reagent was determined in a Beckman DU spectrophotometer at 490 mμ.

In one experiment, the sugars from commercial wheat germ were separated by paper chromatography and determined by means of Dreywood's anthrone reagent (6,16).

Microanalysis of Sugars Separated by Paper Chromatography. This procedure was adopted for the separation of as little as 200 γ of sugars in order to permit the analysis of the sugars in a single, hand-dissected wheat germ.

The section of the paper chromatogram containing the sugar component to be analyzed was excised, rolled up, and suspended from the end of a reflux condenser with a platinum loop (see Fig. 2). Water (5 ml.) was placed in the flask and the apparatus assembled. The water was boiled under the reflux and drops of liquid fell from the condenser onto the paper held in the loops and the sugars were extracted.[2]

After 30 minutes of refluxing the extract was allowed to cool and the volume adjusted to 10 ml. The solution was filtered through sintered glass and a suitable aliquot (2 ml.) of this solution was treated with 80% phenol (0.1 ml.) and sulfuric acid (5 ml.), and the absorbance of the colored solution determined as already described (8).

Results

Commercial Wheat Germ. The commercial wheat germ which

[2] The present technique being followed in these laboratories for extracting the sugars from paper chromatograms is described in the paper by Dubois *et al.* (7).

had been stored at 7.3% moisture (determined by drying *in vacuo* at 75°C. over phosphorus pentoxide) for several months at about 5°C. contained 14.9% lipid (dry matter basis, determined by extraction with diethyl ether).

Chromatograms irrigated with the three solvent systems employed and developed with ammoniacal silver nitrate or with the N,N′-dimethyl-*p*-aminoaniline reagent revealed the presence of sucrose and raffinose (major components) and glucose and fructose (minor components). The quantitative data obtained by the various methods are summarized in Table I.

The mean quantity of total sugars extracted was 16.8% and the results (apart from one fructose analysis) obtained by the micro and semimicro extraction techniques are in good agreement. The mean composition of the sugars was sucrose 57.6%, raffinose 37.6%, and fructose 4.8%. However, some glucose was present in all samples of sucrose which were separated by paper chromatography using Solvent A (Table I). These results confirm reports in the literature that the major carbohydrate components of commercial wheat germ are sucrose and raffinose but small quantities of reducing sugar are present (24,25).

Isolation of Crystalline Sucrose. Evaporation of the aqueous solution of the component of commercial wheat germ corresponding to sucrose which was obtained by chromatography on a cellulose column

TABLE I

THE CARBOHYDRATES OF COMMERCIAL WHEAT GERM

					MEAN
Weight of germ extracted (dry matter basis) g.		9.665		0.100	
Total sugars extracted, % d.m. basis		16.9		16.7	16.8
Component sugar, %					
Fructose	5.6	6.2	4.7	2.7	4.8
Sucrose	56.6	55.3[a]	57.4	61.1	37.6
Raffinose	37.8	38.5	38.0	36.2	57.6
Chromatographic analysis					
Method	Column (2)[b]	Paper (7)	Paper (7)	Paper (7)	
Solvent[c]	A	B	A	A	
Sugar reagent	Direct weighing	Anthrone-sulfuric acid (6)	Phenol-sulfuric acid (7)	Phenol-sulfuric acid (7)	

[a] Paper chromatographic analysis using solvent B showed that some glucose was present in all samples of sucrose separated. In this case 7.5% of the total sugars consisted of glucose.

[b] Numerals in parentheses refer to references in "Literature Cited."

[c] Solvent A = phenol saturated with water, B = 1-butanol:ethanol:water (4:1:5).

(2,12) gave a syrupy product which crystallized spontaneously. The residue was dissolved in the minimum amount of water and ethanol was added until a faint turbidity was produced. After 1 day in an open container, slow evaporation gave crystalline sucrose. After the mother liquor was removed, crystallization from aqueous ethanol gave pure sucrose m.p. and mixed m.p. 184°C. $[\alpha]_D^{22}$ +64.4° in water (c, 1.0).

Isolation of Crystalline Raffinose. The fraction of the carbohydrate material extracted from commercial wheat germ whose R_F value corresponded to raffinose crystallized spontaneously. Crystallization first from aqueous ethanol as described above for sucrose and then from water yield pure raffinose m.p. and mixed m.p. 125°C., $[\alpha]_D^{23}$ +99.5° in water (c, 0.5).

Hand-Dissected Wheat Germ. The results obtained by dissection and analysis of soft white wheat, variety Holland, are recorded in Table II. The total embryo comprised 2.64% by weight of the kernel (dry matter basis). The percentage of germ or embryo reported in the literature varies rather widely. Thus, Girard (10) reported values from 1.16 to 1.50% for four French wheats; Osborne and Mendel (17) in 1919 reported 1.5%, presumably for American wheat; Percival (20) recorded 2.8 to 3.5%, and Grischenko (11) found values ranging between 2.56 and 3.25% for six samples representing two varieties of Russian wheat. Bailey (1) determined the germ content of several samples of hard red spring, hard red winter, durum, and soft wheats grown in Canada or the United States. The average germ content for the soft wheats was 2.66%, and the average for all wheats was 2.61%. The germ content obtained in the present study is in good agreement with these values. The embryo component comprised 47.5% and the scutellum 52.5% of the total weight of the germ.

Sucrose and raffinose were the only sugars detected by paper chromatography when the chromatograms of the extracts were sprayed with N,N′-dimethyl-*p*-aminoaniline trichloroacetate; when sprayed with ammoniacal silver nitrate, trace amounts of glucose were detected in addition to sucrose and raffinose. No fructose could be detected even when the carbohydrate mixture was put on the chromatogram as a syrup.

The analyses for total sugars in Table II agree quite well in view of the fact that only 10 to 23 mg. of material were extracted. The low value for the total sugars in the embryonic plant obtained in experiment 1 was probably due to losses during manipulation. In subsequent experiments the losses were largely eliminated by carrying out the successive extractions with diethyl ether, ethanol, and water in the same vessel.

The sugar content of the total embryo of 20.1%, expressed on a defatted moisture-free basis for the hand-dissected material, agrees much more closely with the value of 24.3% reported for wheat germ by Schulze and Frankfurt (25) than the value, 9.2%, recorded by Colin and Belval (4,5); however, the proportions of sucrose, 56.5%, and raffinose (43.5%) deduced from their analyses are in quite good agreement with those in Table II. Schulze and Frankfurt (25) found that raffinose comprised 28.3% of the sugars of the germ.

Effect of Varying the Moisture Content of Wheat on the Sugars in the Germ. The analyses in Table III show that when wheat kernels are moistened the content of total sugars in the embryonic plant and particularly in the scutellum decrease, probably because of translocation or increased respiration. The decrease is very striking since

TABLE II

CARBOHYDRATES OF THE EMBRYONIC PLANT AND SCUTELLUM FRACTIONS OBTAINED BY HAND-DISSECTION[a]

	Proportion of Kernel	Lipid Content[b]	Total Sugars[a]	Component Sugars (Percent of Total)	
				Sucrose	Raffinose
	%	%	%	%	%
Embryonic plant[c]					
1			19.3	54.0	46.0
2			22.1	52.8	47.2
3	1.25	15.3	23.3	55.7	44.3
4			23.0	56.4	43.6
Mean			21.9	54.7	45.3
Scutellum					
1	1.39	12.6	19.1	62.2	37.8
2			17.6	61.7	38.3
Mean			18.4	62.0	38.0
Total embryo[d]	2.64		20.1	58.5	41.5

[a] Expressed as percent of defatted germ, dry matter basis. Replicate No. 4 was carried out with only 1.44 mg. of sample.
[b] Diethyl ether.
[c] The average weight of the embryonic plant component of a single wheat kernel was 0.48 mg.
[d] Calculated.

TABLE III

EFFECT OF VARYING THE MOISTURE CONTENT OF WHEAT ON THE SUGARS IN WHEAT GERM[a]

	Moisture, 9.2%		Moisture, 12.3%		Moisture, 12.9%
	Scutellum	Embryonic Plant	Scutellum	Embryonic Plant	Scutellum
Total sugars, %	18.4	22.0	11.8	17.9	13.6
Sucrose, % of total	62.0	54.7	57	56	51
Raffinose, % of total	38.0	45.3	43	44	49

[a] Sugar content is expressed on a dry-matter, defatted-tissue basis.

the kernels at 12.3 and 12.9% moisture were only stored over water for 18 and 24 hours respectively. These limited observations indicate that the ratio of sucrose to raffinose remains constant in the embryo but decreases in the scutellum upon short-time storage of the wheat at 12.3 to 12.9% moisture content.

Discussion

Commercial wheat germ is contaminated with some bran and flour and the lower total sugar content (16.8%, d.m. basis) of the sample used in these studies in comparison with that (20.1% d.m. basis) of the total embryo of hand-dissected germ would be expected. The sugars of hand-dissected germ consisted almost entirely of sucrose (58.5%) and raffinose (41.5%), although trace amounts of glucose were also detected when the chromatograms were sprayed with ammoniacal silver nitrate. Although the sugars of the commercial wheat germ also consisted mainly of sucrose (57.6%) and raffinose (37.6%), fructose (4.8%) along with some glucose was present. It thus appears that the carbohydrates of sound wheat germ are sucrose and raffinose and that the glucose and fructose in commercial wheat germ probably result from the partial hydrolysis of sucrose and raffinose. It is significant that upon storage of the wheat over water for 18 to 24 hours the sucrose content of the scutellum decreased. Recently, Linko *et al.* (14) have reported that fresh granular wheat germ milled in the United States contained 28.6% total sugars (dry matter basis) consisting principally of sucrose (55.9%), raffinose (38.1%), fructose (2.8%), glucose (2.1%), and meliboise (1.1%). The total sugar content of this sample was not expressed on a defatted basis and the results are therefore appreciably higher than those obtained in the present study. The relative percentages of sucrose and raffinose are, however, in close agreement. It appears that the carbohydrates of sound pure wheat germ are sucrose and raffinose and the glucose, fructose, and melibiose reported in commercial wheat germ probably result from the partial hydrolysis of sucrose and raffinose. This observation is in agreement with the results of many studies in these and other laboratories which have shown that storage of wheat at moisture contents above about 14.5% (wet basis) results in a progressive decrease in nonreducing sugar content. It is possible that the sugar content and nature of the sugars in wheat germ may be used as an index of the storage history of wheat.

The above investigations have also revealed that micro amounts of wheat germ in the order of 10 to 20 mg. can be analyzed with satisfactory accuracy and micro extraction and analytical procedures

may be employed to study the sugar contents of germs from individual kernels.

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