CHANGES IN THE SOLUBLE CARBOHYDRATES DURING BROWNING OF WHEAT EMBRYOS[1]

PEKKA LINKO, YU-YEN CHENG, AND MAX MILNER[2]

ABSTRACT

Changes in the soluble carbohydrates of wheat embryos as affected by storage at temperature and moisture values pertinent to conditions which produce germ-damaged ("sick") wheat in commercial grain were investigated chromatographically. From the total sugar content of 28.6% in commercial wheat germ, raffinose (38.1%) and sucrose (55.9%) are the major components, with fructose (2.8%), glucose (2.1%), and melibiose (1.1%) present in smaller quantities. Storage for 8 days at various moisture contents from 8.9 to 25%, and temperatures from 29° to 50°C., produced characteristic increases of reducing sugars, at the expense of the nonreducing.

Browning, as indicated by fluorescence, increased with moisture content and temperature, but only after discoloration was visually apparent, and particularly at moisture values beyond 15%. A decrease in nonreducing sugars, as well as an increase in reducing carbohydrates which apparently form browning intermediates with available free amino acids, preceded marked increases in fluorescence.

At moisture levels related to practical storage conditions, several unknown sugarlike compounds appeared quickly in stored commercial germ. These compounds disappeared later as fluorescence values rose. However, only one of these unknowns appears in intact viable wheat grains when sufficient moisture permits germination.

It has been shown recently that only moderate wetting of wheat embryos, either excised or in the intact kernel, immediately activates several enzyme systems that cause rapid changes in the amounts of free amino acids (12,13). This led to the suggestion that amino acids liberated from proteins might, under practical storage conditions, react nonenzymatically with reducing sugars in the embryo to yield the browning reaction products shown to be characteristic of "sick" wheat (4). For these reasons, the behavior of soluble carbohydrates during storage of wheat at different moistures and temperatures becomes important in formation of "sick" wheat.

The discovery by Richardson and Crampton (18) of sucrose as the predominant soluble carbohydrate in wheat germ was soon followed by the crystallization and identification of raffinose (19). Liebig (11) published evidence for the presence of glucose in wheat meal. Kluyver (9) determined, both by chemical methods and by fermentation tech-

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[2] Respectively, Assistant Professor, Graduate Research Assistant, and Professor, Kansas State University.

niques, the presence of fructose, glucose, raffinose, and sucrose in ungerminated wheat. Dubois (5) crystallized raffinose and sucrose from wheat germ and also obtained chromatographic evidence for the presence of trace amounts of fructose and glucose.[3] The evidence (10,20) for the presence of fructose, glucose, maltose, and sucrose in wheat flour was confirmed by Montgomery and Smith (15) through isolation of the crystalline compounds.

Ramstad and Geddes (17) found a marked increase in reducing sugars in soybeans stored at more than 15% moisture content. An equally marked decrease in nonreducing sugars followed. According to Milner *et al.* (14), total sugars in wheat decrease with storage at moisture levels above 15.4%; the reducing sugars showed little or no increase below 20.8% moisture. Montgomery and Smith (16) pointed out that the quantity of soluble carbohydrates in wheat is likely to depend upon the highest moisture level to which the grain has been exposed. Houston *et al.* (8) have reported similar changes in rice, where a decrease in nonreducing sugars is followed by some increase in reducing sugars at moisture values above 14%. They found that at 32°C. and 16.5% moisture, nearly all nonreducing sugars were lost. Bottomley *et al.* (2) have shown that decrease in nonreducing sugars in corn parallels mold count more closely than does increase in fat acidity.

The present work was undertaken to study the changes in individual soluble carbohydrates in commercial wheat germ during short storage periods under various conditions of moisture and temperature.

Materials and Methods

Materials. Fresh granular wheat germ containing 8.9% moisture, supplied by International Milling Co., Minneapolis, Minn., was used. The germ was stored in a moisture-proof container at +4°C.

For germination experiments, an Ohio-grown soft red winter wheat, variety Seneca, with moisture content of 11.6% was used.

Moisture. Moisture content (wet-weight basis) was determined by drying the samples for 1 hour at 130°C. Samples of different moisture contents were prepared by adding the calculated amount of distilled water, followed by vigorous mixing. Samples were stored in moisture-proof containers at various temperatures, and remained mold-free during the experimental periods used.

Determination of Sugars. Soluble carbohydrates were extracted from wheat germ with 70% (w/v) ethanol, after which the extract

[3] These studies are presented in detail in the paper by Dubois, Geddes, and Smith entitled "The Carbohydrates of the Gramineae. X. A Quantitative Study of the Carbohydrates of Wheat Germ" which appears in this issue (pp. 557–568). W. F. G., *Editor.*

was passed through Amberlite IR-120 (H^+) resin. The eluate containing the sugars was distilled in vacuum to a small volume.

Whatman No. 4 filter paper was used without prewashing for chromatography. The first solvent for two-dimensional chromatography was water-saturated phenol (Merck, reagent grade); the second, 1-butanol-acetic acid-water (63:10:27, by volume). The latter also was used as a solvent for one-dimensional chromatography. The chromatograms were normally developed at 27°C. for about 36 hours in 1-butanol-acetic acid-water, at which time fructose migrated to the bottom edge of the paper. However, for the detection of possible presence of pentoses and tetroses, shorter developing times were used. Eight to ten hours' development in phenol-water was found satisfactory.

Two principal reagents were used to detect sugars on paper chromatograms:

1. *Silver nitrate.* This reagent was used for general detection of sugars. The chromatogram was dipped into silver nitrate solution (0.1 ml. of saturated aqueous silver nitrate diluted to 20 ml. with acetone, after which silver nitrate was brought back to solution by addition of water droplets), dried at room temperature, and then dipped into 0.5*N* sodium hydroxide in aqueous methanol.[4] Black spots developed immediately. Chromatograms were again dried at room temperature for 5 to 10 minutes, after which they were slowly pulled through Kodak X-ray fixing solution (1). Chromatograms were finally thoroughly washed with tapwater to remove excess fixing solution. Paper tearing could be avoided by careful handling. The method yielded relatively stable chromatograms showing black spots against white background. The individual sugars were identified by co-chromatography with known carbohydrates.

2. *2,3,5-Triphenyltetrazolium chloride.* Chromatograms were dipped into a freshly prepared mixture of equal volumes of 4% methanolic 2,3,5-triphenyltetrazolium chloride and 1*N* sodium hydroxide in aqueous methanol (6). The papers were then heated 10 minutes at 75°C. in a water-saturated atmosphere. Red spots developed on a slightly pink background. This reagent is much less sensitive than silver nitrate, and it was used only to detect reducing sugars. For quantitative determinations, the size of the largest spot was used as a guide in cutting out all the other sugar spots, as well as a blank. The color was extracted with 5 to 10 ml. of methanol-acetic acid mixture (10:1, v/v), and the extinction was read at 482 mμ.

[4] Sodium hydroxide was dissolved in a minimum amount of distilled water, and diluted to desired volume with anhydrous methanol.

One-dimensional runs in 1-butanol-acetic acid-water were satisfactory for the determination of fructose, glucose, maltose, and melibiose. Raffinose and sucrose were determined according to the method of Williams and Bevenue (21) by hydrolyzing the solution to be analyzed on the origin of a chromatogram by means of invertase. Two applications of 10-μl. spots each of invertase (National Biochemicals Corp., 200 mg. ml.) per 2-μl. spot of sugar solution were made, after which fructose, glucose, and melibiose were determined as usual.

Results and Discussion

As indicated by Table I, raffinose and sucrose are the major soluble carbohydrates in commercial wheat germ; fructose, glucose, and melibiose are present in smaller quantities. Other unidentified sugars are

TABLE I
SOLUBLE CARBOHYDRATES IN COMMERCIAL WHEAT GERM
(Moisture-free basis)

SUGAR	QUANTITY	PERCENT OF TOTAL SUGAR
	mg/g	%
Fructose	8	2.8
Glucose	6	2.1
Melibiose	3	1.1
Raffinose	109	38.1
Sucrose	160	55.9
Other	Traces	
Total	286	100.0

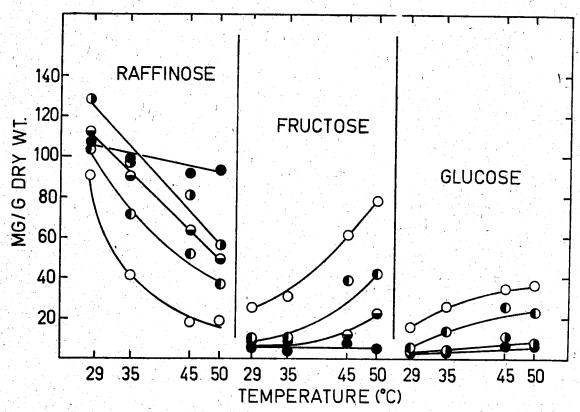

Fig. 1. Effect of temperature on the change in concentration (moisture-free basis) of raffinose, fructose, and glucose in commercial wheat germ stored 8 days at various moisture levels. Percent moisture: ● = 8.9; ◑ = 13; ◒ = 15; ◐ = 18; ○ = 25.

present only in traces. The total amount of soluble sugars found in the present work is close to 29% (moisture-free basis), a somewhat higher value than Dubois (5,16) reported (see footnote 3).

Figure 1 shows the effect of temperature on the concentration of fructose, glucose, and raffinose after small samples of commercial wheat germ were stored at different moisture contents and at various temperatures for 8 days. If germ is kept at the original 8.9% moisture level, temperature increase up to 50°C. causes almost no changes in sugar concentrations. Changes become more pronounced, however, with increasing moisture content. Raffinose decreases, and reducing sugars increase with increasing temperature. Figure 2 shows in greater

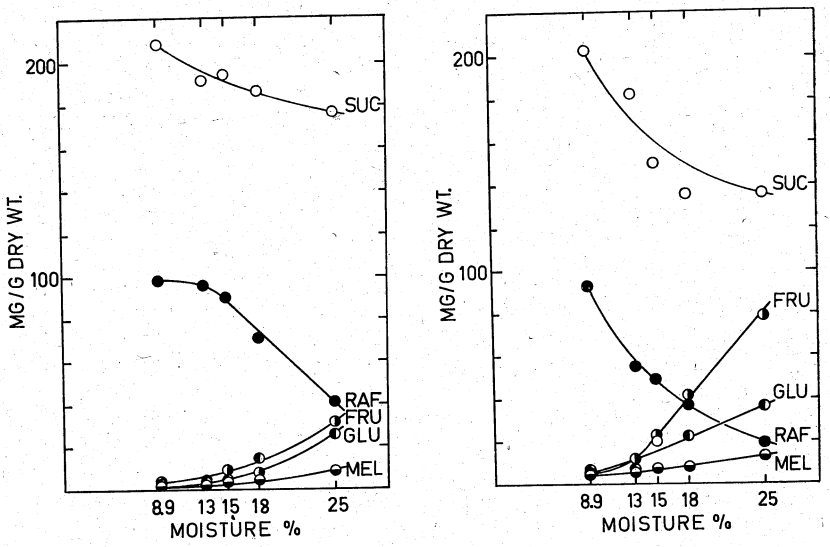

Fig. 2. Effect of moisture content on the change in the amounts (moisture-free basis) of sugars in commercial wheat germ stored 8 days at 35°C. (left), and 50°C. (right).

detail the effect of moisture content at 35° and 50°C. on the concentrations of soluble sugars, indicating that nonreducing sugars decrease and reducing sugars increase with increasing moisture content.

Table II shows the degree of browning of these samples as measured by the fluorescence (3,4). Browning increases when moisture and temperature increase. The primary browning reaction products are not, however, fluorescent; visually detectable browning of the germ occurs noticeably before any significant increase in fluorescence value. A marked increase in fluorescence appears only at moisture levels above 15%. In general, decrease in nonreducing sugars is followed by

TABLE II

EFFECT OF TEMPERATURE AND MOISTURE ON DEVELOPMENT OF FLUORESCENCE IN WHEAT GERM DURING 8 DAYS OF STORAGE

(911 mg.; moisture-free basis)

H_2O	Fluorescence Units			
	29°C.	35°C.	45°C.	50°C.
%				
8.9	4	3	6	7
13	4	4	10	15
15	4	4	19	66
18	65	79	94	6,800
25	92	3,200	5,000	17,000

increases in browning. Increases in glucose and fructose are not so great as might be expected from the breakdown of sucrose and raffinose alone. This is most likely due to the reaction of reducing sugars with free amino acids to form the intermediates of nonenzymatic browning.

As shown in Fig. 3, commercial wheat germ contains, in addition to the above-mentioned sugars, three other sugarlike compounds, one of which coincides with lactose. While the chromatographic evidence for the possible presence of lactose in wheat germ is only presumptive, work is in progress to isolate this compound in crystalline form. The two other compounds are marked as spots 100 and 101. During 8 days' storage at 8.9% moisture, two additional unknown compounds, 102 and 103, appear. When the moisture content increases, compound 101 rapidly disappears, whereas compounds 100, 102, and 103 increase very markedly. A small amount of galactose also appears at higher moisture levels. Glass and Geddes (7) recently isolated D-galactose, as well as D-glucose, D-fructose, glycerol, and myo-inositol, from wheat which had been stored under nitrogen at 16 to 18% moisture for 24 weeks at 30°C. Compounds 102 and 103 reach a maximum at 15% moisture level, after which they begin to decrease. On the other hand, several additional unknown spots appear on chromatograms above this moisture level. Because fluorescence also has a sharp increase at this point, it is likely that some of the unknowns may be labile primary intermediates in the nonenzymatic browning reaction. The position of the above-mentioned unknowns on the chromatogram, with the exception of the "lactose" spot, does not coincide with any of the known simple carbohydrates. Work is in progress to establish their identity.

For comparison, changes in soluble carbohydrates during germination of intact wheat grains were also studied. Figure 4 shows that fruc-

tose, glucose, and maltose are the major soluble carbohydrates in the intact wheat grains. Raffinose disappears at an early stage of germination, whereas fructose, glucose, and maltose greatly increase. After a few days, a series of oligosaccharides appear at the upper right hand corner of the chromatograms. Compound 100 was the only one of the

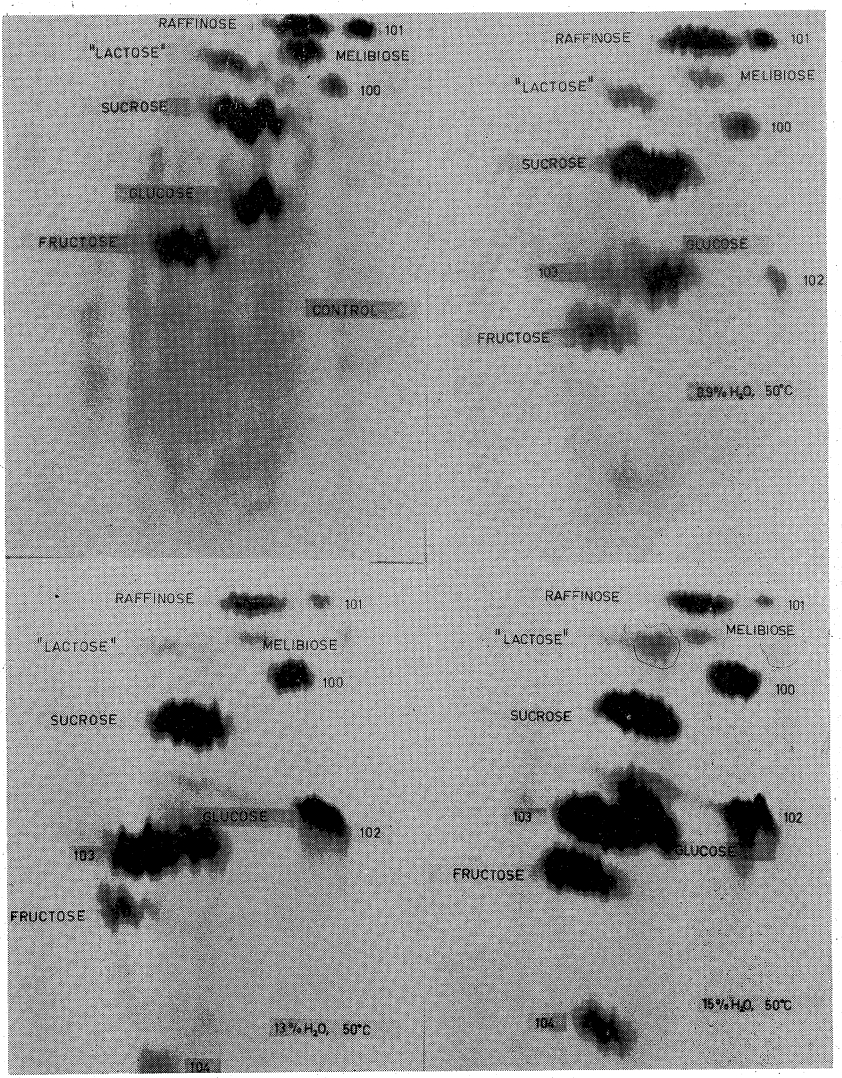

Fig. 3. Two-dimensional paper chromatograms of soluble carbohydrates in commercial wheat germ stored 8 days at 50°C. and at various moisture levels. Amounts used for each chromatogram correspond to 5 mg. germ (moisture-free basis). Spots are identified with silver nitrate. (See also continuation, opposite page.)

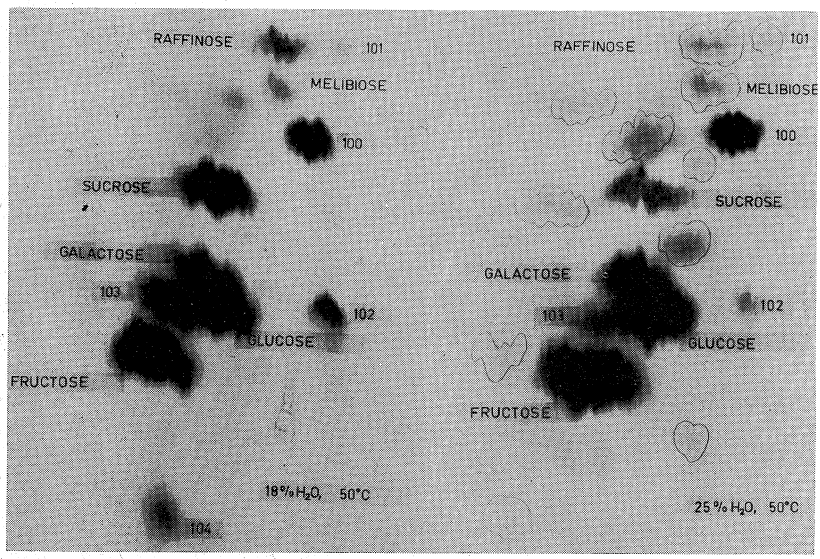

Fig. 3 (Continuation).

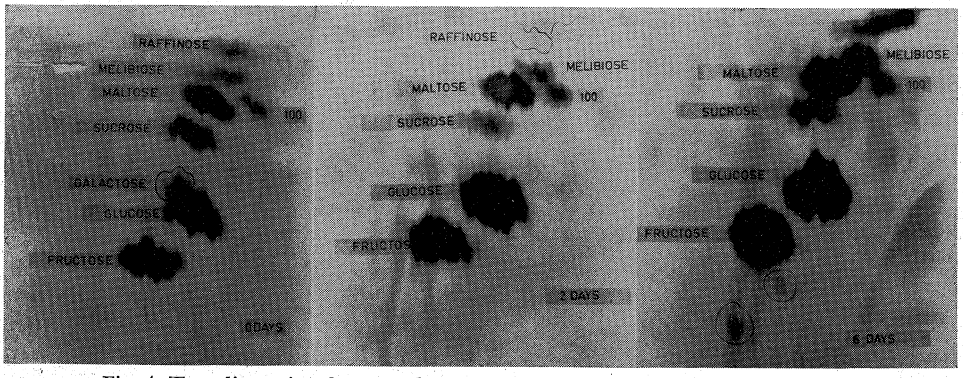

Fig. 4. Two-dimensional paper chromatograms of soluble carbohydrates of intact wheat grains after various germination periods. Amounts used for each chromatogram correspond to 18 mg. of grain (moisture-free basis). Spots are identified with silver nitrate.

unknowns found at any stage of germination. This suggests that the unknown compounds apparently are not normal metabolites, but rather compounds formed during storage after the embryo has lost its viability; these appear to be concerned with the browning reaction characteristic of "sick" wheat.

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