

## GRAIN STORAGE STUDIES

### XXXI. Changes Occurring in Low-Molecular-Weight Compounds in Deteriorating Wheat<sup>1</sup>

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#### ABSTRACT

D-galactose, myo-inositol, and glycerol were isolated and characterized from the monosaccharide fraction obtained from a sample of wheat which had deteriorated under an atmosphere of nitrogen for 24 weeks at 18% moisture and 30°C. In addition, D-glucose and D-fructose were obtained in chromatographically pure form. The control wheat sample yielded D-glucose and D-fructose together with trace amounts of what appeared to be D-galactose and myo-inositol.

In a recent paper in this series (5), marked changes in reducing and nonreducing sugars were reported in wheat stored at 16–18% moisture in an atmosphere of nitrogen. Nonreducing sugars (expressed as sucrose) decreased greatly and to an extent which was almost exactly compensated for by an increase in reducing sugars (expressed as maltose). When damp wheat was stored in air, and hence became exceedingly moldy, the increase in reducing sugars was only about one-fourth as great as the decrease in nonreducing sugars. Apparently sugars were extensively utilized by molds.

Expressing the nonreducing and reducing sugars as sucrose and maltose, respectively, is purely arbitrary and no indication of the actual sugars involved is implied.

Sucrose, along with raffinose, is a prominent constituent of wheat

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germ (3) but is only a minor component (0.1 to 0.2%) of wheat flour (8,10). Wheat flour contains about 1% of glucofructans which are readily hydrolyzed with dilute acids and hence contribute markedly to the nonreducing sugar values, as determined by conventional methods using acid hydrolysis (1). Although the reducing sugars in wheat are usually reported as maltose for convenience, it is well known that glucose and fructose are also present (6,8,10).

The objective of the present study was to determine the nature of the reducing sugars which are formed when moist wheat is stored in an atmosphere of nitrogen. In the course of this investigation, the substances present in a monosaccharide fraction obtained from a sound and a deteriorated sample of wheat by elution from charcoal were studied.

### Materials and Methods

Two samples from the same original lot of Marquis wheat were used. One had been stored for 24 weeks at 30°C. and 18% moisture under an atmosphere of nitrogen (containing 0.04% oxygen); the other was a control sample which was stored at -10°C. during the same period. The viability, nonreducing and reducing sugar contents were:

	Viability	Sugars per 10 g. wheat (dry basis)	
		Nonreducing (as sucrose)	Reducing (as maltose)
		mg	mg
Control	97	232	37
Stored under nitrogen	0	75	120

*Extraction of Sugars:* Each sample, air-dried and ground to pass a 30-mesh screen, was defatted by extraction with petroleum ether. Eleven hundred grams of the defatted material were suspended in boiling 70% (v/v) aqueous ethanol and refluxed for 2 hours to inactivate the enzymes. The centrifuged material was extracted three times with successive 3-liter portions of warm (70°C.) 70% ethanol. All extracts were pooled and evaporated under reduced pressure at 40°C. to a thick syrup. The syrup was dissolved in 600 ml. distilled water and dialyzed against 2 liters of distilled water at approximately 5°C. for 72 hours. The dialysate was then decreased in volume to 100 ml. under reduced pressure at 40°C. and deionized by passage through an Amberlite MB-3 resin<sup>2</sup> column.

<sup>2</sup> Fisher Scientific Co., Pittsburgh, Pa.

*Preparation of the Monosaccharide Fraction with Charcoal* (11). One hundred grams each of charcoal (Darco G60)<sup>3</sup> and Celite 535<sup>4</sup> were suspended in 2.5% aqueous ethanol<sup>5</sup> and the slurry poured into a chromatographic column to give, when settled, a column of adsorbant 35 mm. × 350 mm. A 25-ml. aliquot of the sugar concentrate (adjusted to a concentration of 2.5% ethanol by the addition of absolute ethanol) was added to the column and developed by the addition of 2 liters of 2.5% aqueous ethanol, after which an essentially negative Molisch test was obtained on the eluate. The material was then evaporated to a syrup and stored at -10°C. until used.

*Paper Chromatography.* Paper chromatograms of the material eluted from the charcoal by 2.5% ethanol, along with known sugars, were obtained by spotting on strips of Whatman No. 1 filter paper and developing in the descending manner for periods ranging from 24 to 48 hours, using the solvent system 1-butanol-pyridine-water (6:4:3) (v/v). The positions of the sugars were located by spraying the air-dried chromatograms with ammoniacal silver nitrate and heating in the usual manner.

*Column Chromatography.* The compounds present in the fraction obtained from the nitrogen-stored wheat were isolated by chromatography using a column of cellulose powder (Whatman No. 1). Approximately 400 mg. of the syrup were dissolved in a minimum of 1-butanol-pyridine-water (6:4:3) (v/v) and applied to the column (35 mm. × 600 mm.); development of the column was carried out using this solvent system. Fractions (4 ml. per tube) were collected by means of an automatic fraction collector. Those tubes which contained material reacting with ammoniacal silver nitrate were detected by spotting a drop from each tube on a piece of filter paper, spraying with the reagent, and heating. The material in the tubes containing identical compounds, as determined by paper chromatography, were pooled and the solvent was removed by evaporation under reduced pressure at 40°C.

## Results and Discussion

The material obtained by extraction of sound and spoiled wheat and partial fractionation on charcoal gave, when subjected to paper chromatography, the following results.

<sup>3</sup> Atlas Powder Co., Wilmington, Del.

<sup>4</sup> Johns-Manville Co., New York, N. Y.

<sup>5</sup> Dilute aqueous ethanol (2.5%) rather than water was used, since this gave a better separation of mono- and disaccharides.

Sample	Component Number						
	1	2	3	4	5	6	7
	Rg value <sup>a</sup>						
Control	... <sup>b</sup>	0.61	0.77	... <sup>b</sup>	1.0	1.16	...
Stored under nitrogen	0.26	0.61	0.77	0.85	1.0	1.16	1.75

<sup>a</sup> Rg represents the mobility of the compound in question with relative respect to that of glucose.

<sup>b</sup> Present but in barely detectable amounts.

Of the seven substances detected in more than trace amounts in the deteriorated wheat, only four appeared in the control sample. Comparison with known sugars indicated components 2, 3, 5, and 6 to be maltose, sucrose, glucose, and fructose respectively, sugars which have been shown to be present in wheat flour (6,8,10). Components 1, 4, and 7 from the sample stored under nitrogen were obtained in chromatographically pure form by separation on the cellulose column and were identified as follows:

*Component 1.* Crystallized from methanol. Identified as myo-inositol by m.p. 225°C., mixed m.p. with myo-inositol 225°C. Preparation of hexaacetate by treatment with acetic anhydride in anhydrous pyridine. Melting point and mixed melting point 219°–220°C.

*Component 4.* Crystallized from methanol. Identified as galactose by m.p. and mixed m.p. with authentic sample 162°–164°C.; N-methylphenylosazone prepared by treatment with N-methylphenylhydrazine, m.p. and mixed m.p. 192°C.

*Component 7.* A viscous, slightly brown liquid which gave an odor of acrolein when heated with sodium bisulfate. Identified as glycerol by preparation of glyceryl tri-*p*-nitrobenzoate by treatment with *p*-nitrobenzoyl chloride in pyridine; m.p. and mixed m.p. 195°C.

D-galactose, myo-inositol, and glycerol obtained from the deteriorated wheat have not, to the authors' knowledge, been reported previously to be present in the free state in sound wheat. Subsequent to the completion of this work, Linko *et al.* (7), in storage experiments with wheat germ and intact wheat, observed characteristic increases in reducing sugars, principally fructose, glucose, and galactose, and a decrease in nonreducing sugars, primarily raffinose. They also observed a transient appearance of several other unidentified sugarlike compounds. Galactose has also been reported to be present in autolytic extracts of barley (9). Chromatographic evidence in the present study indicated that D-galactose and myo-inositol are present in sound wheat, but in such small amounts that no attempt was made to isolate them for more positive identification.

Some wheat enzymes are quite active at moisture contents of 18% and 30°C. (5), and wheat contains phytase and lipase. The hydrolysis

of phytic acid would result in free myo-inositol and an increase in inorganic phosphate. The latter has been reported in a previous paper (4). The presence of free glycerol indicates that wheat lipase can catalyze the hydrolysis of triglycerides in both the alpha- and beta-position of the glycerol moiety.

The source of free galactose in this wheat is less certain. Several galactose-containing compounds are known to be present in wheat. These include raffinose (3), and galactosyl glycerides (2). The galactose found here could conceivably have originated from either of these compounds. A demonstration of the presence of enzymes in wheat capable of hydrolyzing these compounds would help clarify this point.

Glucose, fructose, sucrose, and maltose have been isolated and identified from wheat flour by other workers (6,8,10) and were not further identified here.

A quantitative study of the changes in individual sugar levels in deteriorated wheat is currently underway. Visual comparison of chromatograms of extracts of sound and deteriorated wheat in the present study indicate that little or no change has occurred in the maltose and sucrose levels.

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