GRAIN STORAGE STUDIES

XXXII. Quantitative Changes Occurring in the Sugars of Wheat Deteriorating in the Presence and Absence of Molds[1]

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ABSTRACT

Sound wheat (germination 98%) was stored in atmospheres of air, nitrogen, or carbon dioxide for 8 weeks at 30°C. and 20% moisture. By most of the criteria used, those samples stored in anaerobic conditions, and hence without mold growth, deteriorated almost as rapidly and equally in degree as did a sample stored in aerobic conditions. All samples exhibited zero germination, had undergone extensive changes in sugar content, and gave flour of extremely poor baking quality after 8 weeks. The exception was fat acidity, which remained constant under anaerobic conditions while increasing tenfold under aerobic conditions.

In air, the sucrose content of wheat decreased markedly, whereas little change occurred in glucose, fructose, galactose, or maltose content. In carbon dioxide and nitrogen, the sucrose content decreased and rather large increases in glucose, fructose, and galactose occurred.

It is generally conceded that molds are very much involved in the various deteriorative changes observed in grain stored at moisture levels above 14% (12,13). Also apparent is the fact that damp grain itself is a system capable of extensive biochemical alterations which, if allowed to continue, result in germination. It is probably impossible to separate completely the effects of molds from that of wheat enzymes, because both are active under the same conditions of moisture and temperature. Environmental changes which affect one system would be likely to affect the other. Some success has been gained in differentiating between the two systems.

Milner, Linko, and their co-workers have investigated the biochemical changes occurring in moistened excised wheat embryos, changes which occur in a few hours or days and can be attributed to wheat enzymes (8,9). In this laboratory it was observed that wheat stored at 18% moisture and 30°C. in an atmosphere of nitrogen underwent a number of deteriorative changes not involving molds (5). A decrease in nonreducing sugars and a corresponding increase in reducing sugars indicated the development of an active glycosidase system under the storage conditions used.

This paper presents quantitative data obtained when wheat was

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allowed to deteriorate in an anaerobic environment, changes which apparently are the result of the action of wheat enzymes only. Of particular interest in this study is the effect of the storage conditions on the sugars maltose, sucrose, glucose, fructose, and galactose, all of which have been shown to be present in sound or deteriorated wheat (4,7).

Materials and Methods

Wheat. Good-quality hard red spring wheat Ceres was used. It had been grown under irrigation in Montana, was virtually free of mold infestation, and had a germination of 98%. It was divided into lots of 700 g., each of which was placed in a 1-liter round-bottomed flask, and adjusted to 18% moisture by addition of the calculated amount of distilled water.

All flasks were fitted with ground-glass connections, which permitted convenient aeration and withdrawal of samples of the interseed gas for analyses. Each was flushed with the appropriate gas (air, nitrogen, or carbon dioxide) which had been brought to a relative humidity of 95% by passage through a 10% aqueous sulfuric acid solution.

The flasks were kept in a water bath maintained at 30°C. At weekly intervals samples were removed for analyses, and the flasks flushed with the respective gases. In the case of the air-stored sample, the rapid carbon dioxide production necessitated daily flushing at the beginning of the experiment and twice-daily flushings toward the end.

Analytical Methods. Moisture content was determined by the two-stage air-oven method (1).

Seed viability was determined by the Minnesota State Seed Testing Laboratory. One hundred seeds were placed between damp blotting papers and held at 20°C. Those seeds which had normal-appearing sprouts after 7 days were considered viable.

Fat acidity was measured on a diethyl ether extract (6 hours) of 3 g. of ground meal (micro-Wiley mill, No. 40 screen) according to the method of Hunter *et al.* (6).

Reducing and nonreducing sugars were determined by the alkaline ferricyanide method (1) and are expressed as mg. of maltose and sucrose, respectively, per 10 g. of dry wheat.

Inorganic phosphorus was measured using the method described by McCance and Widdowson (10).

Quantitative paper chromatography of the various sugars carried out as described by Koch *et al.* (7) gave excellent reproducibility and good recovery of sugars on standard chromatograms using the sol-

TABLE I

DETERIORATIVE CHANGES IN WHEAT STORED IN AIR, NITROGEN, OR CARBON DIOXIDE AT 30°C.[a]

TIME	VIABILITY			FAT ACIDITY[b]			INORGANIC PHOSPHORUS[c]			REDUCING SUGARS[d]			NONREDUCING SUGARS[e]		
	Air	N_2	CO_2	Air	N_2	CO_2	Air	N_2	CO_2	Air	N_2	CO_2	Air	N_2	CO_2
weeks	%	%	%												
0	98	98	98	15	15	15	31	31	31	41	41	41	190	190	190
1	80	90	94	64	11	13	30	32	31	40	39	43	160	180	185
2	47	95	95	59	13	12	29	32	37	36	..	77	100	155	160
3	36	82	93	51	14	17	31	35	37	65	92	80	106	150	140
4	24	76	50	73	12	13	37	42	38	64	101	85	..	130	145
5	24	43	4	74	19	21	39	37	40	65	108	92	90	91	143
6	16	2	0	84	16	16	33	42	49	51	108	93	62	85	140
7	4	0	0	115	23	16	33	48	45	47	106	114	50	77	107
8	0	0	0	150	21	19	33	46	45	41	117	117	43	100	115

[a] All results are averages of at least two determinations.
[b] Fat acidity as mg. potassium hydroxide per 100 g. wheat.
[c] Inorganic phosphorus as micrograms per g. wheat.
[d] Reducing sugars as mg. maltose per 10 g. wheat.
[e] Nonreducing sugars as mg. sucrose per 10 g. wheat.

vent system pyridine, ethyl acetate, water (2:5:5, v/v).

Mold counts were made by comminuting 5 g. of grain in 500 ml. of sterile 0.15% agar solution in a Waring Blendor; 5-ml. aliquots were further diluted in the same suspension medium and replicate 1-ml. portions cultured in malt-salt agar (2).

Results

The air-stored sample (Table I) began to deteriorate almost immediately, as indicated by germination tests. Those stored in carbon dioxide and nitrogen retained their original viability for about 3 weeks, after which a very rapid decrease began. Fat acidity, a widely accepted criterion of grain condition, showed a ten-fold increase in the sample stored in air. Fat acidity for the sample stored in nitrogen and carbon dioxide remained constant and low. Inorganic phosphorus, previously shown (3) to increase in stored damp wheat, increased (by 50%) only in anaerobic storage.

In air storage the reducing sugars underwent a transient increase at the expense of nonreducing sugars. However, the final value for reducing sugars was identical to the initial value. The nonreducing sugars decreased by 147 mg. per 10 g. dry wheat (expressed as sucrose). In contrast, the reducing sugars in the samples stored in nitrogen and carbon dioxide each increased by 76 mg. per 10 g. wheat (expressed as maltose). The nonreducing sugars decreased, although not as markedly as in aerobic storage, and by an amount which, in general, closely approximated the increase in reducing sugars.

Individual sugars underwent the expected changes as determined by quantitative paper chromatography (Table II). In aerobic storage sucrose decreased by about 60% (33 mg. per 10 g. wheat), the

TABLE II

CHANGES IN THE SUGAR CONTENT OF WHEAT DURING STORAGE FOR EIGHT WEEKS AT 30°C.

SUGAR	ZERO TIME CONTROL	STORAGE ATMOSPHERE		
		Air	Nitrogen	Carbon Dioxide
Fructose[a]	6	5	18	16
Galactose[a]	2	3	9	9
Glucose[a]	8	7	24	23
Maltose[a]	5	1	4	3
Sucrose[a]	54	21	39	36
Reducing sugars[b]	35	29	101	94
Reducing sugars[c]	41	41	117	117
Nonreducing sugars[d]	190	43	100	115

[a] Individual sugars as mg. per 10 g. wheat.
[b] Obtained by paper chromatography and expressed in terms of mg. maltose per 10 g. dry wheat.
[c] Obtained by ferricyanide procedures. Results expressed as mg. maltose per 10 g. dry wheat.
[d] Obtained by ferricyanide procedures. Results expressed as mg. sucrose per 10 g. wheat.

monosaccharides remained about the same, and maltose virtually disappeared. In the samples stored under anaerobic conditions sucrose decreased, the monosaccharides all increased markedly, and maltose decreased slightly.

Mold counts (Table III) were indicative of the great difference in the two types of storage. For practical purposes the samples under anaerobic storage can be considered devoid of fungal activity. The air-stored sample was heavily infested, with the mold plainly visible to the naked eye.

Baking tests performed on flour milled from the original, air-stored, and the nitrogen-stored wheats resulted in loaves of bread with volumes of 730, 515, and 510 cc. respectively. As compared to the original wheat, the flours from the two deteriorated wheats gave doughs with equally poor handling properties, being very sticky.

TABLE III

EFFECT OF STORAGE ATMOSPHERE ON THE MOLD POPULATION OF WHEAT STORED UNDER VARIOUS ATMOSPHERES FOR EIGHT WEEKS AT 20% MOISTURE AND 30°C.

STORAGE ATMOSPHERE	PERCENT SURFACE-DISINFECTED SEED YIELDING:					MOLD COUNT
	Aspergillus glaucus	*Aspergillus candidus*	*Aspergillus ochraceus*	*Aspergillus flavus*	*Alternaria*	
						thousand/g
Original[a]	2	0	0	0	66	4
Air	0	14	4	100	100	547,000
Nitrogen	2	0	0	0	0	<0.1
Carbon dioxide	2	0	0	0	0	<0.1

[a] The original was a sample held at approximately 12% moisture and at —10°C. while the experiment was being conducted.

Discussion

Data show that damp (20% moisture) grain stored at 30°C. temperatures is an unstable system with a short storage life even in the absence of molds. That a large percentage, if not all, of the deteriorative changes observed under aerobic storage was the result of mold activity has been repeatedly shown by other workers.

Storage under anaerobic conditions does not appear to be a solution to the problem of preserving, except for a few days, grain whose moisture content would also make it unsafe for aerobic storage.

The extensive deteriorative changes observed in this wheat can only be presumed to be the result of an abnormal seed metabolism proceeding in the absence of oxygen. A significant activity of anaerobic microorganisms is excluded, for the present, primarily because of the observation that no net change occurred in sugar content even though large changes in the distribution of the sugars occurred. The

stable level of fat acidity also indicates that this is true. A microbiological system capable of causing such extensive alteration in the seeds must derive its energy somewhere, and until this is observed such a system must be presumed absent.

The increase in monosaccharides and decrease in sucrose suggests a rather extensive development of glycosidases in wheat prior to the occurrence of external signs of germination. The data in Table II indicate that only a portion of the glucose and fructose originated from sucrose. The appearance of galactose in the anaerobic samples could conceivably be a result of raffinose degradation. This, however, has been reported (11) to be an aerobic process. A possible alternate source of galactose is the galactosyl glycerides present in wheat. Also apparent from Table II is the discrepancy between sugar content as determined by the ferricyanide method and by the paper chromatographic method. This can be largely accounted for in the case of the reducing sugars by correcting for the molecular-weight differences between maltose and the monosaccharides. The large difference between sucrose content and nonreducing sugars expressed as sucrose is the result of determining only sucrose in the chromatographic procedure, whereas in the ferricyanide method raffinose and the various fructans present in wheat are also measured.

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