Changes in Wheat Flour Damaged by Mold During Storage

Effects on Lipid, Lipoprotein, and Protein[1]

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Flours, with 18% moisture, from four wheats were stored for 16 weeks at 23°, 30°, and 37° C. The original mold count of the samples was 50 to 200 colonies per g, and at the end of storage was 13,000 to 2,700,000 per g. The final counts were much higher in flour stored at 23 and 30° C than at 37° C. *Aspergillus niger* v. tiegh., *A. candidus* Lk ex Fr., and *A. versicolor* (Vuill.) tiraboschi were the predominant species present. Kjeldahl protein values increased slightly in damaged flours; protein determinations by a dye-binding method yielded decreased values in damaged flour samples stored at 37° C. Free lipids decreased more in samples stored at 23 than at 30 or 37° C; bound lipids decreased as the temperature of flour storage increased. Residual lipids in all storage-damaged flours contained markedly reduced amounts of polar components and of lipoprotein. The breakdown of bound lipids apparently was accompanied by transformation of polar to nonpolar-like components. The ratio of nonpolar to polar components in residual bound lipids increased as the storage temperature of the flour increased.

Previous studies from our laboratory (Daftary and Pomeranz, 1965a) have shown that deterioration in wheat damaged by fungi was accompanied by lowering of total free lipids (extractable with petroleum ether), gradual increase in free fatty acids, decrease in triglycerides, and rapid disappearance of polar lipid components. Subsequent studies on storage-damaged flour indicated also a substantial breakdown of bound lipids (extractable with water-saturated butanol, following petroleum ether extraction) (Pomeranz *et al.*, 1968). Starch-gel electrophoretic patterns indicated that proteins of storage-damaged flour had undergone only minor changes.

Moisture content and temperature are the main factors which determine the intensity of respiration and damage in stored grains and grain products (Milner and Geddes, 1954). The kinds of molds that grow in grain and grain products are influenced by the initial moisture, temperature, and oxygen concentration; by the direction and extent to which these and other factors (such as nutrient availability) change as molds grow; and by the inherent growth rates of the individual fungi (Semeniuk, 1954). The influence of those factors, except for moisture, on the kinds of molds growing in grain is little known. Even less is known about fungi present in wheat flours stored under various conditions (Hesseltine, 1968).

The purpose of this research was to determine the effects of temperature on the numbers and kinds of molds in wheat flours containing about 18% moisture, and on lipid, protein, and lipoprotein contents and composition.

EXPERIMENTAL

Flours. The flours used in this study were milled on an experimental Miag Multomat mill to an extraction of about 68% from two hard red winter (H.R.W.) and two hard red spring (H.R.S.) wheats harvested in 1965. Some chemical and breadmaking characteristics of the flours are described in Table I. Moisture content of the flours ranged from 11.9 to 12.2%, and was increased to about 18% by spreading the flours on trays and placing them for 4 days in a humidity cabinet at 99% relative humidity and 98° F. The flours were packed in 5-lb lots in polyethylene bags, with small beakers containing water in the center, and the bags were stored in closed cans at room temperature (about 23° C), 30° C, and 37° C. Control samples, with about 12% moisture, were stored at 4° C. The moisture contents of samples stored at elevated temperatures for 16 weeks increased slightly during storage as a result of metabolic changes; the increase was smallest in samples stored at 37° C (Table II).

After 16 weeks, the flours were removed from storage, the contents of the bags were crushed, mixed, and allowed to dry on large trays at room temperature for 4 days. The air-dried samples were reground on an experimental mill and bolted through a 9xx sieve.

Analytical Determinations. All analytical determinations, extractions, fractionations, and baking tests were made in duplicate. Moisture, ash, and Kjeldahl protein were determined by the AACC method (1962). Percent nitrogen was converted to percent protein by the factor 5.7. Protein by the dye absorption method was determined basically according to the procedure of Udy (1956). Free lipids were extracted exhaustively with petroleum ether (b.p. 35° to 60° C) in a Goldfish extractor. Petroleum ether in the flour was allowed to evaporate at room temperature, and bound lipids were extracted with water-saturated butanol as described previously (Daftary and Pomeranz, 1965b). The butanol extract was filtered, evaporated under reduced pressure, and redissolved in petroleum ether. The lipids were fractionated on silicic acid columns and by thin-layer chromatography (tlc) as described by Daftary and Pomeranz (1965b). The pure compounds used for identification of lipids included oleic acid, linoleic acid, trilinolein, phosphatidylcholine, phosphatidylethanolamine, monogalactosyl diglyceride, and digalactosyl diglyceride.

Lipoproteins. Protein in petroleum-ether extracts was detected by a spot test (Hoseney *et al.*, 1970). The extracts were spotted on filter paper and the solvent allowed to evaporate. The paper was dipped in a 0.1% aqueous solution of Amido Black 10 B for 5 min. The paper was removed from the dye and repeatedly washed with water. The presence of protein was indicated by a dark spot on a white background.

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Table II. Moisture Contents of Flours Stored at Various Temperatures

Variety	Moisture Contents (%) in Flours: At beginning of storage	After Storage at 23° C	30° C	37° C
Comanche	17.9	18.8	18.6	17.6
Qv − Tm × Mql − Oro	17.7	19.4	18.7	18.4
Thatcher	18.1	19.5	18.5	18.2
Selkirk	18.2	19.5	19.2	18.6

Mold Counts. Dilution cultures were made using a method similar to that of Bottomley *et al.* (1952). The suspension medium was a sterile 0.2% agar solution, and the culture media were Difco malt agar and Difco malt agar with 6% NaCl added. As the malt-salt agar gave higher and more consistent mold counts, the malt agar counts are not included in data presented here.

Breadmaking. Baking tests were made on a laboratory scale from 100 g of flour (14% moisture basis). The formula included (on flour basis) sucrose, 6%; salt, 1.5%; yeast, 2%; 60° L-malt syrup, 0.5%; nonfat milk solids, 4%; commercial vegetable shortening, 3%; water as needed; and optimum potassium bromate. An optimum mixing time with the straight-dough procedure and a 3-hr fermentation time at 30° C were employed. Punching and panning were performed mechanically. Baking time was 24 min at 218° C. Loaf volumes were measured by dwarf rape seed displacement. Differences of 25 cc, were significant at the 5% level.

RESULTS AND DISCUSSION

The initial mold count of the flour, before wetting, ranged from 50 to 200 colonies per g, and after 16 weeks of storage was as high as 2,700,000 per g (Table III). The highest mold counts were from the flours stored at room temperature, while those from flours at 37° C were relatively low. At 37° C mold populations may have increased initially and then decreased as conditions became unfavorable for continued growth.

At 23° and 30° C the most abundant fungi were the *Aspergillus niger*, *A. candidus*, and *A. versicolor* groups. *A. flavus* was the only species as abundant at 37° C as at the other temperatures.

No statistically significant change in ash contents were determined in stored samples. Protein content determined by the Kjeldahl method was slightly, but consistently, higher in mold-damaged samples than in the corresponding controls (Table IV). The relative increase on a percentage basis can be explained by respiratory losses of carbohydrates. In samples stored at room temperature and at 30° C, a somewhat smaller increase was observed when protein was determined by the dye-binding method. In the samples stored at 37° C, the dye-binding method yielded a substantially decreased protein value. The reason for that decrease is unknown. Dye binding is decreased in toasted or overheated soybean flours (Moran *et al.*, 1963; Pomeranz, 1965). Such severe heating for 1 hr or less apparently decreases availability of the ε-amino group of lysine to acid orange 12 binding.

Levels of free lipids (extractable in petroleum ether) (Table V) were substantially lower in storage-damaged than in con-

Table I. Chemical and Breadmaking Characteristics[a] of Flours before Storage

Variety	Class	CI No.	Ash, %	Protein (N × 5.7), %	Water Absorption, %	Mixing Time, min	Bromate Requirement, PPM	Loaf Volume, cc
Comanche	H.R.W.	11673	0.42	13.1	64.2	3–5/8	20	919
Qv − Tm Mql − Oro	H.R.W.	12995	0.42	12.5	63.2	5–1/8	10	922
Thatcher	H.R.S.	10003	0.54	13.2	64.3	3–1/4	20	920
Selkirk	H.R.S.	13100	0.54	13.4	64.1	2–7/8	30	922

[a] On 14% moisture basis.

Table III. Mold Count (Thousands per Gram) in Flours Stored at Various Temperatures

Storage Temp., ° C	Total Count	*Penicillium spp.*	*A. niger*	*A. glaucus*	*A. candidus*	*A. versicolor*	*A. flavus*
			Comanche				
23	2697	60	225	30	275	2100	7
30	1585	30	200	10	125	1200	20
37	31	2	2	1	10	14	2
			C.I. 12995				
23	629	17	290	42	200	75	5
30	420	35	250	55	30	45	5
37	40	4	1	1	10	9	15
			Thatcher				
23	2695	25	105	85	275	2200	5
30	1255	5	65	5	175	1000	5
37	21	...	1	...	10	5	5
			Selkirk				
23	1279	52	175	1	300	750	1
30	397	25	62	...	90	215	5
37	13	1	1	...	6	1	4

trol samples. The decrease was, however, consistently smaller in samples stored at 30° or 37° C than at room temperature (about 23° C). The decrease of polar lipids was much greater than the decrease of nonpolar lipids. In the residual lipids, relative concentrations of nonpolar components increased. The recovery of free lipids fractionated by silicic acid column chromatography was lower in controls than in damaged samples. The lower recovery in lipid of control samples probably resulted from the presence of nonlipid components (presumably lipoproteins) that were not eluted by the solvents used. This was confirmed by two findings. Fractionation of free lipids by tlc with chloroform-methanol-water showed that whereas sound flours contained a component that did not migrate, the component was absent in storage-damaged flours (Figure 1). The component retained at the point of origin gave an orange-red color after the plates were sprayed with ninhydrin and heated. Spot tests for protein in petroleum-ether extracts indicated disappearance of lipoproteins in all storage-damaged flours.

Almost complete breakdown of polar lipids (Figure 1) in flours stored at various temperatures confirms previous findings from this laboratory on changes in free lipids of mold-damaged wheat and flour (Daftary and Pomeranz, 1965a; Pomeranz *et al.*, 1968).

Fractionation of nonpolar free lipids by tlc with chloroform shows (Figure 2) that the residual lipids in storage-damaged flours contained more free fatty acids and diglycerides and less triglycerides than lipids in sound flour. In addition, several unidentified compounds, absent in sound flour, were present in storage-damaged flours. The formation of those compounds was temperature-dependent.

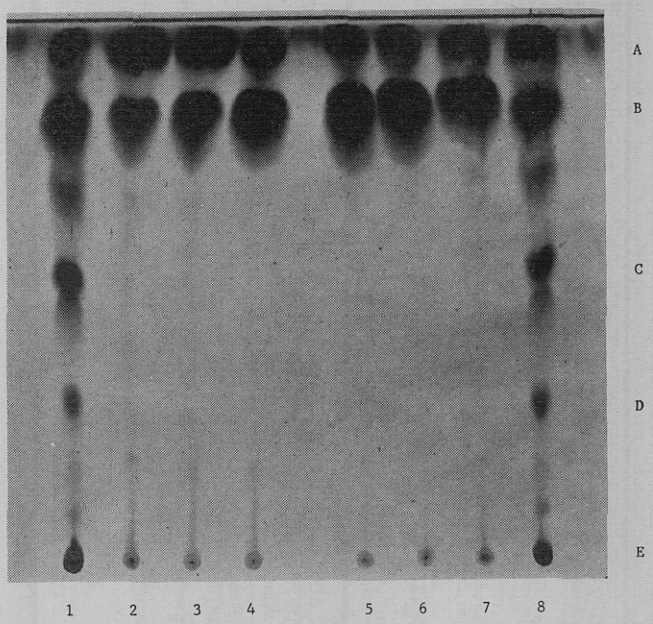

Figure 1. Tlc of free lipids from Comanche (spots 1 to 4) and Selkirk (spots 5 to 8) flours

Spots 1 and 8 lipids from sound flours; 2 and 7, 3 and 6, and 4 and 5 lipids from flours stored at 23°, 30°, and 37° C, respectively. Spots of 300-μg developed with chloroform-methanol-water (65:25:4), visualized by charring at 180° C for 30 min of plates sprayed with a saturated solution of $K_2Cr_2O_7$ in 70% (by volume) aqueous sulfuric acid. Picture taken under ultraviolet light. Tentatively identified as (A) mixture of nonpolar lipids, (B) free fatty acids and monogalactosyl diglyceride, (C) digalactosyl diglyceride, (D) phosphatidylcholine, and (E) nonmigrating components including lipoprotein

Table IV. Protein Contents (%) of Flours Stored at Various Temperatures

Variety	Kjeldahl Method				Dye-Binding Method			
	At beginning	After Storage at 23° C	After Storage at 30° C	After Storage at 37° C	At beginning	After Storage at 23 °C	After Storage at 30° C	After Storage at 37° C
Comanche	13.1	13.6	13.6	13.5	13.0	13.4	13.2	12.5
Qv − Tm × Mql − Oro	12.5	13.0	13.0	13.1	12.3	12.5	12.4	11.3
Thatcher	13.2	13.6	13.8	13.9	13.4	13.9	13.8	12.6
Selkirk	13.4	13.9	14.1	14.1	13.5	14.0	13.7	12.7

Table V. Lipids in Flours Stored at Various Temperatures

Variety and Storage Temp., ° C	Free Lipids, %				Bound Lipids, %			
	Total	Nonpolar	Polar	Recovery	Total	Nonpolar	Polar	Recovery
				Comanche				
4	0.75	61.7	34.4	96.1	0.74	15.7	79.0	94.7
23	0.17	93.5	8.1	101.6	0.70	25.4	70.3	95.7
30	0.21	96.7	4.5	101.2	0.67	36.1	60.5	96.6
37	0.22	96.2	5.2	101.2	0.48	65.1	34.5	99.6
				C.I. 12995				
4	0.79	62.3	32.5	94.8	0.66	11.3	84.0	95.3
23	0.18	92.4	8.5	100.9	0.61	26.2	68.9	95.1
30	0.21	95.7	5.8	101.5	0.53	34.7	62.2	96.9
37	0.25	96.1	5.1	101.2	0.39	69.2	30.0	99.2
				Thatcher				
4	0.89	69.1	26.2	95.3	0.86	8.2	86.3	94.5
23	0.19	96.5	6.0	102.5	0.85	31.8	63.1	94.9
30	0.25	96.5	4.8	101.3	0.76	39.2	56.1	95.3
37	0.30	97.4	4.1	101.5	0.50	71.3	26.6	97.9
				Selkirk				
4	0.87	69.4	26.3	95.7	0.83	8.7	86.8	95.5
23	0.24	95.8	5.9	101.7	0.74	33.1	62.1	95.2
30	0.36	97.7	4.6	102.3	0.71	44.2	53.2	97.4
37	0.31	97.7	4.0	101.7	0.50	71.2	27.1	98.3

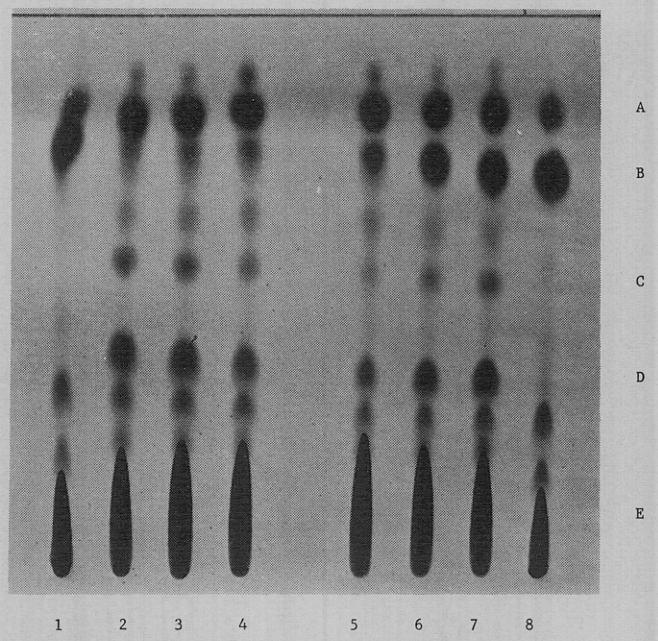

Figure 2. Tlc of free lipids from Qv-Tm × Mql-Oro (spots 1 to 4) and Thatcher (spots 5 to 8)

Spots of 200-μg developed with chloroform. Storage conditions and tlc visualization as in Figure 1. Tentatively identified as (A) hydrocarbons, sterol esters, and other nonpolar components, (B) triglycerides, (C) unidentified components, (D) diglycerides and (E) free fatty acids

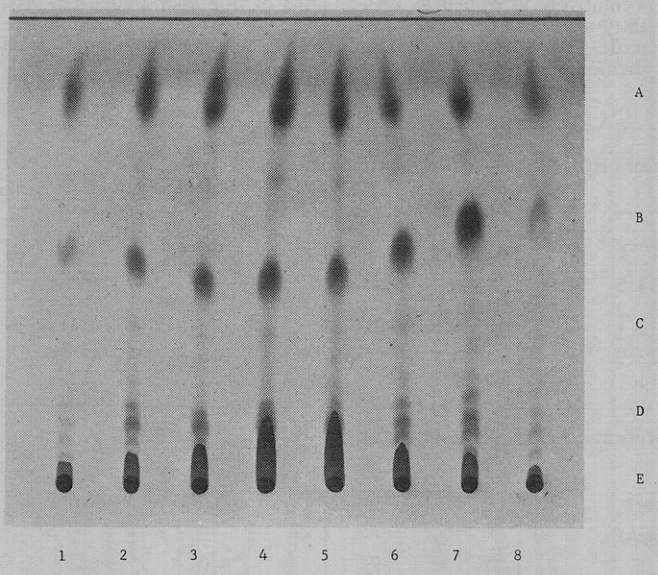

Figure 3. Tlc of bound lipids. Legend as in Figure 2

The decrease in damaged flours of bound lipids was smaller than the decrease of free lipids (Table V). The amount of bound lipids was smallest in flours stored at 37° C. The data indicate that the decrease was accompanied by changes in composition of the bound lipids. The changes resulted, in part at least, from the transformation of polar to nonpolar components (as assessed by their elution from silicic acid columns).

Recoveries from silicic acid columns were higher at the higher storage temperatures (Table V). Increase in storage temperature was also accompanied by an increase in the ratio of nonpolar to polar components in residual bound lipids.

The bound lipids in damaged flours contained substances with R_f values corresponding to those of triglycerides and fatty acids (Figure 3). The increase was mainly in free fatty acids. The large increase in fatty acids in bound lipids (Figure 3) indicates that some changes may have resulted from binding of components that were originally extractable with petroleum ether or were formed during storage damage. The large decrease in bound polar lipids of flours stored at 37° C (Table V) resulted from breakdown of both glycolipids and phospholipids (Figure 4).

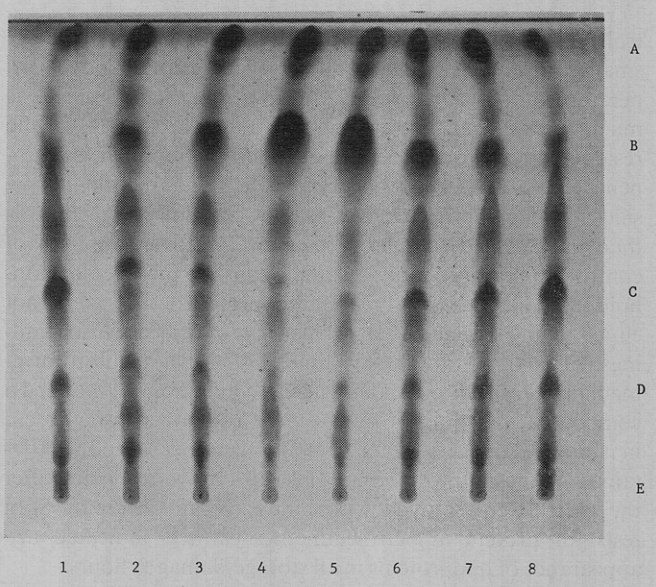

Figure 4. Tlc of bound lipids. Legend as in Figure 1

The results of this study confirm previous findings concerning rapid breakdown of free polar lipids in mold-damaged flours. Changes in bound lipids are more complicated and involve, in addition to breakdown of polar components, the modification of the latter to nonpolar-like components. All those changes are affected by the storage temperature. In addition, lipoproteir in free lipids is degraded during storage-deterioration. The multitude of changes in lipids of storage-damaged flours gives rise to numerous metabolites that can be used to detect incipient deterioration. Identification of some of these metabolites will be reported shortly.

LITERATURE CITED

American Association of Cereal Chemists, St. Paul, Minn., "Cereal Laboratory Methods," 7th ed., 1962.
Bottomley, R. A., Christensen, C. M., Geddes, W. F., *Cereal Chem.* **29,** 53 (1952).
Daftary, R. D., Pomeranz, Y., J. AGR. FOOD CHEM. **13,** 442 (1965a).
Daftary, R. D., Pomeranz, Y., *J. Food Sci.* **30,** 577 (1965b).
Hesseltine, C. W., *Baker's Digest* **42**(3), 40 (1968).
Hoseney, R. C., Pomeranz, Y., Finney, K. F., *Cereal Chem.* in press, 1970.
Milner, M., Geddes, W. F., "Storage of Cereal Grains and Their Products," Amer. Ass. Cereal Chemists, St. Paul, Minn., 1954.
Moran, E. T., Jensen, L. S., McGinnis, J., *Nutrition* **79,** 239 (1963).
Pomeranz, Y., *J. Food Sci.* **30,** 307 (1965).
Pomeranz, Y., Daftary, R. D., Shogren, M. D., Hoseney, R. C., Finney, K. F., J. AGR. FOOD CHEM. **16,** 92 (1968).
Semeniuk, G., "Storage of Cereal Grains and Their Products," Amer. Ass. Cereal Chemists, St. Paul, Minn., 1954.
Udy, D. C., *Cereal Chem.* **33,** 190 (1956).

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