Changes in Wheat Flours Damaged by Mold During Storage

Effects in Breadmaking

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Comanche wheat flour containing 18% moisture was stored at various temperatures for 4 months to produce mold damage. When 1.6% free lipids from sound flour were added to mold-damaged flours, loaf volume was improved but not restored to initial potential. Mixing times of doughs from damaged flours were longer than those of doughs from sound flour, and increased with increasing storage temperature or with lipid addition. The gluten from damaged flours was difficult to wash out and had impaired rheological properties. In damaged flour, the yield of gluten was reduced, and yield of the starch fraction was increased; the starch fraction was rich in proteins. Starch-gel electrophoretic patterns of gluten proteins from damaged flour showed a decrease in glutenin and an increase in gliadin-like components; the water-soluble fraction contained an unusual protein component migrating toward the anode and essentially no fast-moving components. Baking studies of reconstituted doughs indicated damage to lipids, gluten proteins, and water-solubles; no damage to starch was established.

Fractionation studies of mold-damaged flour (14% moisture) stored at room temperature indicated that most damage was due to the breakdown of lipids (Pomeranz *et al.*, 1968). Starch-gel electrophoretic patterns of gluten proteins indicated only minor changes. Loaf volume potential and crumb grain of the damaged flour could be restored by adding polar or total flour lipids from a sound flour. Mixing times of doughs from the damaged flour were substantially longer than those of doughs from the sound flour. Cuendet *et al.* (1959) studied flours that contained up to 14% moisture and were stored at 37.8° C. He reported that replacing lipids from aged flour with lipids from fresh flour did not restore breadmaking quality. According to Greer *et al.* (1954), deteriorated, defatted flour retains material that is detrimental in breadmaking, possibly an insoluble condensation product of oxidized fatty acids.

The purpose of our research was to determine by fractionation and reconstitution techniques the components responsible for damage to flour when stored at various temperatures.

EXPERIMENTAL

Flours and Flour Fractions. The hard red winter wheat Comanche flour has been described (Daftary *et al.*, 1970). The flour with 18% moisture was stored for 16 weeks at 23°, 30°, and 37° C. The original mold count of 200 increased up to 2,700,000 per g. During storage, increased mold count was accompanied by extensive breakdown of lipids (mainly polar) and lipoproteins, and by decrease in dye-binding of flour proteins.

The control and damaged flours were fractionated into gluten, starch, and water-solubles, as described by Shogren *et al.* (1969). The starch fraction was further manually fractionated into "prime" and "tailings" starch. Lipids were prepared as described previously (Pomeranz *et al.*, 1968).

Analytical Determinations. Moisture, ash, and Kjeldahl protein were determined by the AACC methods (1962). The method for starch-gel electrophoresis of flour proteins was described previously (Pomeranz *et al.*, 1968). All determinations (including baking tests) were made at least in duplicate.

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Breadmaking. The baking procedure of Shogren *et al.* (1969) for 10 g of flour was used. All results are expressed on a 14% moisture basis. Differences of 2 cc were significant at the 5% level.

RESULTS AND DISCUSSION

As indicated previously (Daftary *et al.*, 1970) mold-damage resulted in breakdown of most of the flour lipids. When 1.6% total free lipids from sound flour were added to mold-damaged flours, loaf volume was improved but not restored to that of the sound flour (Table I). Mixing times of doughs from damaged flours were substantially longer than mixing times of doughs from sound flours; adding 1.6% total free lipids further increased mixing time. Mixing times increased as storage temperature increased, and as damage to breadmaking potentialities increased.

To determine what fraction of flour was damaged, the sound flour and flour stored at 37° C were fractionated into gluten, starch, and water solubles. The yields and protein contents of the fractions are described in Table II. The yields of gluten from damaged flours (unextracted and petroleum-ether-extracted) were much lower than those from sound flour. At the same time, the damaged flours yielded more starch that contained relatively high concentrations of protein.

Changes in the water-soluble fraction were small; there was a decrease in yield and an increase in protein contents in the fractions from damaged flours.

The glutens from the damaged flours had impaired rheological properties and were difficult to wash out. Electrophoretic patterns of proteins in gluten and water-soluble fractions of sound and damaged (at 37° C) Comanche flours are shown in Figure 1. The gluten fraction from damaged flour appears to contain the same components but in different concentrations than those found in the sound gluten. In the gluten from the damaged flour, the amount of glutenin at the point of application is reduced, and the amount of gliadin-like components (in terms of electrophoretic mobility) is increased. It must be emphasized that the gluten from sound flour represented 85% of the total flour protein, while the gluten from damaged flour represented only 30% of the total flour protein. It is likely that the least damaged or unaltered part of the protein was recovered as gluten by the gluten washing technique. The water-soluble fraction of damaged flour shows an unusual component migrating toward the anode. Most fast-moving components in the water-soluble fraction dis-

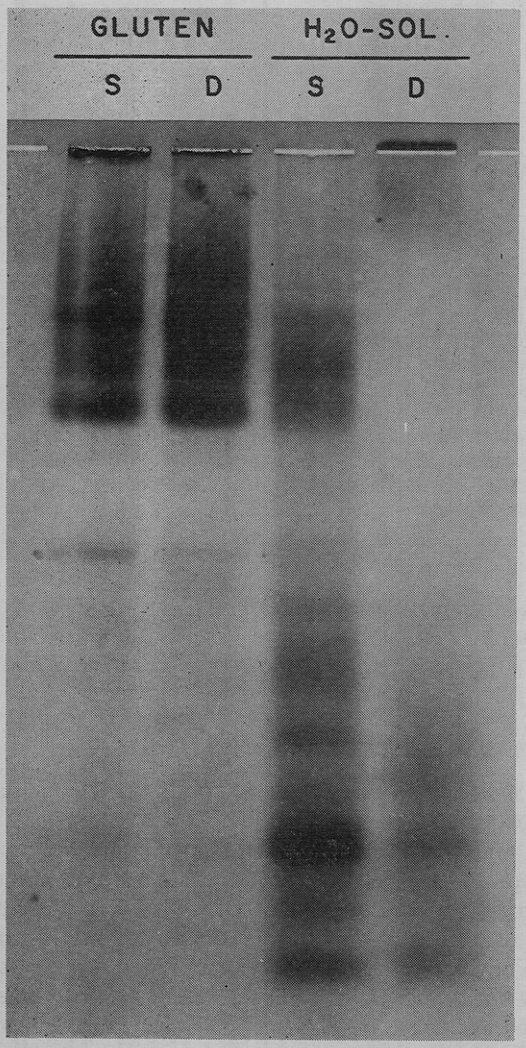

Figure 1. Starch-gel electrophoretic patterns of gluten and water-soluble proteins of sound (S) and damaged (D) Comanche (37° C) flours

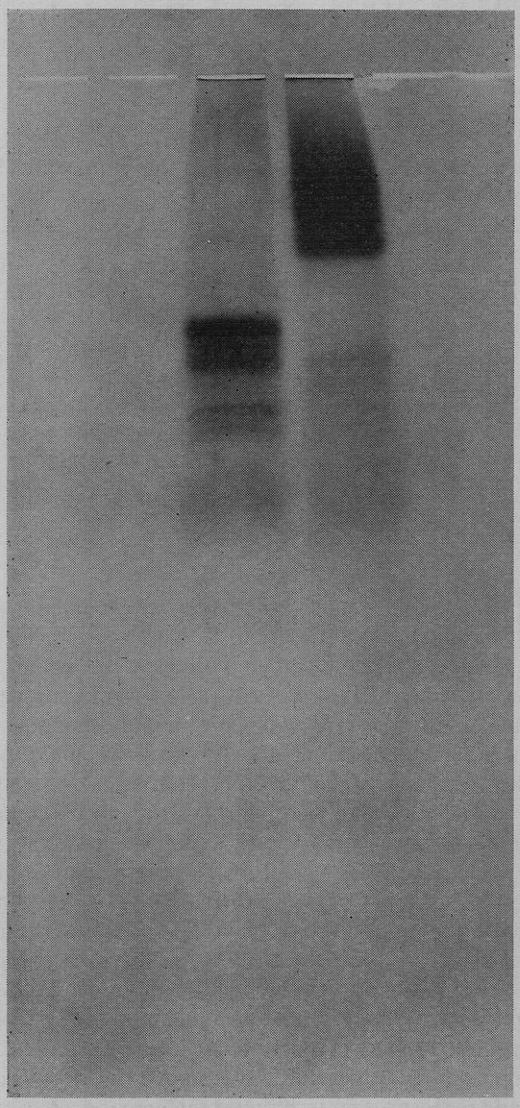

Figure 2. Starch-gel electrophoretic patterns of proteins extracted with 0.1 N lactic acid from the starch "tailings" fractions of sound (left) and damaged (right) Comanche flours

Table I. Effects of Total Free Lipids from Sound Flour on Bread Baked from Damaged Flour

Storage Temperature, °C	Mixing Time, min	Loaf Volume, cc
	Control	
4	4	74
23	10 3/4	44
30	12	45
37	18	23
	Reconstituted with 1.6% Free Lipids	
23	11 1/2	58
30	13	58
37	23	43

Table II. Yields and Protein Contents in Fractions from Sound and Damaged Flours

	Gluten		Starch		Water Solubles		
Flour	Yield, %	Protein, %	Yield, %	Protein, %	Yield, %	Protein, %	Total Yield, %
Sound	19.1	58.0	67.0	1.4	3.8	27.0	89.9
Sound, *p*-ether-extracted	18.9	61.0	66.2	0.9	3.8	25.0	88.9
Damaged	5.2	76.0	84.9	8.7	2.8	31.0	92.9
Damaged, *p*-ether-extracted	6.0	69.0	75.6	8.6	3.5	29.0	85.1

appeared, including a prominent band presumed to be glycoprotein.

The proteins not recovered by gluten washing were in the starch fraction (Table II). Therefore the starch fractions of damaged and sound flours (petroleum-ether-extracted) were further fractionated into "prime" and "tailings" starch. The results are shown in Table III; the "prime" and "tailings" starches are based on unfractionated starch. The high yield and protein content of the "tailings" fraction from damaged flour indicate that the fraction contains the unrecovered gluten protein. A starch-gel electrophoretic pattern (Figure 2) of the extract from the damaged "tailings" fraction was characterized by a high concentration of gluten proteins (mainly gliadins). A comparable pattern of sound flour "tailings" contained mainly fast-moving bands (globulin proteins) normally associated with the "tailings" fractions.

The gluten, water-soluble, and "prime" starch fractions were tested by reconstituting and baking. The results are summarized in Table IV. The data indicate that both gluten and water-soluble fractions of damaged flours were impaired. Starch from damaged flour was not affected during storage.

The impairment of the water solubles from damaged flour apparently resulted from enzymatic modifications of the

Table III. Yields and Protein Contents of "Prime" and "Tailings" Starch Fractions from Petroleum-Ether-Extracted Flours

Starch	From Sound Flour		From Damaged Flour	
	Yield, %	Protein, %	Yield, %	Protein, %
Unfractionated	66.2	0.9	75.6	8.6
"Prime" fraction	66.0	0.5	52.1	0.9
"Tailings" fraction	29.0	1.6	41.3	9.7

Table IV. Loaf Volume of Reconstituted Flours Baked with Fractions from Nonextracted Sound (S) Flour and from Damaged (D) Flour Extracted with Petroleum Ether

Composition of Reconstituted Flours			Loaf Volume, cc
Starch	Gluten	Water solubles	
S	S	S	75
D	D	D	23
S	S[a]	D	67
D	S[a]	S	76
S	S[b]	S	73
D	D[b]	D	43
S	D[b]	S	61

[a] Gluten from sound flour contained lipids and, therefore, required no supplementation.
[b] Supplemented with 1.6% total free lipids.

water-soluble proteins. That is indicated by the loss of the fast-moving bands and the appearance of an unusual band migrating to the anode (Figure 1).

The impairment of the gluten proteins' breadmaking ability does not appear to be primarily the result of enzymatic hydrolysis. A breakdown of the gluten proteins would increase smaller molecular weight proteins which presumably would have greater solubility and electrophoretic mobility. Instead, the electrophoretic patterns (Figure 1) showed no increase in fast-moving bands. In addition, when flour was extracted with lactic acid (0.1N), 92% of the proteins from sound flour and only 66% from damaged flour were solubilized. Thus, mold-damage decreased protein solubility, thereby suggesting protein denaturation. Work with pre-ripe wheat has shown that, at increased moisture levels, wheat proteins are highly susceptible to denaturation at elevated temperatures (Finney *et al.*, 1962).

The results of this and previous studies indicate that wheat flour lipids are most susceptible to mold-damage and are, apparently, broken down before other wheat flour components (Daftary and Pomeranz, 1965). Damage to gluten and water solubles varies with storage conditions. Under conditions described previously (Pomeranz *et al.*, 1968) gluten proteins were not damaged, and adding lipids from sound flour practically restored loaf volume. Chemical analyses (Daftary *et al.*, 1970) and breadmaking tests of flours used in this study indicate damage to wheat flour proteins (both in the water solubles and in the gluten). The starch seems to be most resistant and no damage to functional properties was recorded in any of our investigations. Acker (1962) indicated that such damage was possible, though probably only at most advanced stages of mold damage. While our results suggest a "Sequence" of deterioration related to the overall extent of mold damage, different types of damage could take place under various storage conditions resulting in qualitative and quantitative changes in fungal populations.

LITERATURE CITED

Acker, L., *Advan. Food Res.* **11**, 263 (1962).
American Association of Cereal Chemists, St. Paul, Minn., "Cereal Laboratory Methods," 7th ed. (Methods 08-01, 44-15, and 46-10) 1962.
Cuendet, L. S., Larson, E., Norris, C. G., Geddes, W. F., *Cereal Chem.* **13**, 362 (1959).
Daftary, R. D., Pomeranz, Y., J. AGR. FOOD CHEM. **13**, 442 (1965).
Daftary, R. D., Pomeranz, Y., Sauer, D. B., J. AGR. FOOD CHEM. **18**, 613 (1970).
Finney, K. F., Shogren, M. D., Hoseney, R. C., Bolte, L. C., Heyne, E. G., *Agron. J.* **54**, 244 (1962).
Greer, E. N., Jones, C. R., Moran, T., *Cereal Chem.* **31**, 439 (1954).
Pomeranz, Y., Daftary, R. D., Shogren, M. D., Hoseney, R. C., Finney, K. F., J. AGR. FOOD CHEM. **16**, 92 (1968).
Shogren, M. D., Finney, K. F., Hoseney, R. C., *Cereal Chem.* **46**, 93 (1969).

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