Changes in Biochemical and Bread-Making Properties of Storage-Damaged Flour

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Damage to bread-making potentialities of stored wheat flour was accompanied by almost complete breakdown of free flour lipids and by a substantial decrease of bound lipids. Starch-gel electrophoresis patterns indicated that proteins of storage-damaged flour had undergone only minor changes. Mixing time of damaged flour was more than twice as long as that of sound flour. Gassing power of the damaged flour was comparable to that of the sound flour. Loaf volume potential and crumb grain, but not color, of damaged flour could be restored by adding polar or total lipids. Fractionation studies showed that the damage was due to the breakdown of the lipids rather than to changes in the gluten proteins or starch and water solubles.

The bread-making quality of freshly milled flour normally tends to improve for a time depending on the nature of the flour and conditions of storage (Zeleny, 1954). Subsequently, a point is reached where further aging no longer improves baking quality. Longer storage is accompanied by a gradual decline in bread-making quality. Presumably the initial improvement results from oxidation of certain wheat flour constituents. The deterioration that follows seems to result from the presence of oxidation products of unsaturated fatty acids (Sullivan *et al.*, 1936). Sinclair and McCalla (1937) studied changes in lipids in flours stored for up to 36 months in cotton bags or air-tight containers. They found that deterioration of bread-making quality was generally accompanied by substantial decreases in petroleum ether–extractable lipids, but no consistent changes were recorded in lipids soluble in an alcohol-ether mixture. Adding 5% fresh germ increased loaf volume of bread from storage-damaged flour but impaired baking quality of the original flour. An alcohol extract from germ had little effect on loaf volume; an alcohol extract from fresh flour somewhat increased loaf volume of bread baked from the original and from the damaged flours. Sullivan (1940) suggested that the beneficial effects of fresh germ on damaged flour observed by Sinclair and McCalla (1937) were due to glutathione in the germ. Lipids were postulated to damage stored flour primarily as a result of the formation of oxidized forms of unsaturated fatty acids; the beneficial effect of germ glutathione was attributed to the capacity of glutathione to reduce oxidized lipids.

Greer *et al.* (1954) noted a low level of petroleum ether–extractable fat in samples stored for 8 or more years. When fats from damaged old flours were added to normal flour, they reduced loaf volume and impaired bread crumb grain. Fat from fresh flour either had no adverse effect or gave slight improvement. After defatting, the stored flour still retained a deleterious factor, which was considered to be an insoluble condensation product of the oxidized fatty acids. Although a general tendency for low percentages of petroleum ether–extractable fat, resulting from adverse storage conditions, appeared to be associated with severe deterioration in baking quality, the correlation was by no means clear-cut.

Pomeranz *et al.* (1956) studied changes in stored damp wheat. A thousandfold increase in mold count was accompanied by a reduction of about 20% in free lipids and substantial damage to bread-making potentialities.

More recently, Daftary and Pomeranz (1965a) studied changes in lipids of soft and hard wheat stored at elevated moisture levels and high temperatures. Increase in mold count from 1000 to about 2,000,000 was accompanied by a 40% decrease in total lipid contents. Nonpolar lipids decreased about 25%; the damaged wheat contained only one third as much polar lipids as the sound wheat. Grain deterioration was accompanied by rapid disappearance of glycolipids and phospholipids. The breakdown of polar lipids was more rapid and more intensive than formation of free fatty acids or disappearance of triglycerides.

The purpose of this research was to determine, by fractionation and reconstitution techniques, the components responsible for storage-damaged flour and to establish means of restoring bread-making potentialities.

EXPERIMENTAL

Flour and Flour Fractions. Marquis spring wheat, composited from equal portions of wheat grown in 1964 at eight locations, was milled experimentally to an extraction of 68% on a Miag Multomat. Expressed on a 14%-moisture basis, the flour contained 0.47% ash and 13.7% total protein. Aliquots of the flour, containing 14.6% moisture, were stored in polyethylene bags for 14 months at 4° C., and for 8 months at 4° C. followed by 6 months at room temperature (27° C.). Compared to the freshly milled flour, no measurable changes in properties of the flour stored for 14 months at 4° C. were detectable. Flour stored for 6 months at room temperature, however, developed lumps, an objectionable color, and a musty odor. The original and damaged flours were fractionated into gluten and a mixture of starch and water solubles as described by Finney (1943). The fractions were frozen, lyophilized, and ground to pass a 60-mesh sieve on a micro-Wiley mill. Lyophilized samples were very low in moisture (about 1 to 2%). The ground samples were kept for 48 hours in a cabinet after grinding to attain a moisture of

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about 8%, and were stored at −20° C. in dark bottles until analyzed or used in breadmaking. All analytical determinations, extractions and fractionations, rheological measurements, and baking tests were made in duplicate.

Analytical Determinations. Moisture, ash, and Kjeldahl protein were determined by the AACC method (1962). Per cent nitrogen was converted to per cent protein with the factor 5.7.

Lipid Extraction and Fractionation. 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 the flour was re-extracted with water-saturated butanol as described previously (Daftary and Pomeranz, 1965b). The butanol extract (bound lipids) was filtered, evaporated under reduced pressure, and redissolved in petroleum ether. Total lipids were extracted directly with water-saturated butanol. The lipids were fractionated on silicic acid columns and by thin-layer chromatography as described elsewhere (Daftary and Pomeranz, 1965b). The pure compounds used for identification of lipid components included trilinolein, phosphatidyl chloline, phosphatidyl serine, phosphatidyl ethanolamine, monogalactosyl diglyceride, and digalactosyl diglyceride.

Extraction and Fractionation of Proteins. Proteins were extracted with 0.1*N* lactic acid, frozen, and lyophilized. The lyophilized extract was dissolved in pH 3.2 aluminum lactate–lactic acid buffer, containing 3*M* urea, and fractionated by vertical starch-gel electrophoresis. Separations of 0.050 ml. of a 10% protein dispersion were made. Acid-hydrolyzed potato starch (from Connaught Medical Research Laboratory, Toronto, Canada), 15% in excess of the manufacturer's recommendation, was gelatinized in buffered 3.0*M* urea. Electrophoresis was performed at 30 ma. and approximately 325 volts for 6 hours at room temperature. The gel preparations were sliced and stained in 0.1% Amido Black 10B, and excess stain was removed by several changes of distilled water.

Mixograms. Mixograms were determined with 35.0 grams of flour (14%-moisture basis) and optimum water absorption (Finney, 1964).

Gassing Power. Forg assing power determinations, pressure meters were used (Sandstedt and Blish, 1934). Gassing power was determined on doughs prepared from 10 grams of flour and all ingredients (except shortening) used in the baking formula. The water absorption of 100% was used. The doughs were fermented for 5 hours and the pressure was determined at 1-hour intervals.

Breadmaking. Baking tests were made on a laboratory scale from 10 grams or 100 grams of flour (14%-moisture basis). The formula included (on flour basis), sucrose, 6%; salt, 1.5%; yeast, 2%; 60° L malt sirup, 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-hour fermentation time at 30° C. were employed. Punching and panning were performed mechanically. Baking times were 15 and 24 minutes at 218° C. for the 10- and 100-gram methods, respectively. Loaf volumes were measured by dwarf rape seed displacement. Differences of 3 cc. and 25 cc. were significant at the 5% level in the 10- and 100-gram methods, respectively. After the loaves had cooled, they were cut and their crumb grains evaluated. The following code was employed: *S* = satisfactory, *Q* = questionable, and *U* = unsatisfactory.

RESULTS AND DISCUSSION

The damaged flour contained practically no free lipids, and bound lipids were reduced to about one third the amount in sound flour (Table I). Fractionation of the sound and damaged flours yielded comparable amounts of gluten and a mixture of starch and water solubles. Similarly, protein contents of the corresponding fractions from the two flours varied little. However, the gluten from the damaged flour had impaired rheological properties (lacking coherence and elasticity) and was difficult to wash out and handle. TLC (thin-layer chromatography) of nonpolar lipids of damaged flour (Figure 1) shows a substantial decrease in triglycerides and increase in free fatty acids, mono-, and diglycerides. The free polar lipids of damaged flour contained practically no digalactosyl glyceride or phospholipids. The concentrations of polar bound lipids were also substantially reduced in the damaged flour. The changes were accompanied by formation of substantial amounts of unidentified components with an R_f value approaching 1.0 in the chromatograms developed with the chloroform–methanol-water mixture. Deterioration of flour was accompanied by formation of at least three unidentified compounds which showed autofluorescence under ultraviolet light. Two of those compounds were in the petroleum ether extract, and one in the butanol extract. In interpreting the TLC in Figure 1, note that the chromatogram illustrates distribution, and not amounts, of free and bound lipids in sound and damaged flour. For example, monogalactosyl glyceride in free lipids of sound flour was about three times as concentrated as in free lipids of damaged flour. Since the sound flour contained about 13 times more free lipids than the damaged flour, it, therefore, had 40 times more of the glycolipid than the damaged flour.

There were only small differences in starch-gel electrophoresis patterns of proteins extracted with 0.1*N* lactic acid, and fractionated in the presence of 3*M* urea in pH 3.2 aluminum lactate–lactic acid buffer system (Figure 2).

Table I. Composition[a] of Sound and Damaged Flours and of Fractions Separated from the Flours

Description	Sound Flour	Damaged Flour
Whole flour		
Protein (N × 5.7), %	13.7	13.9
Free lipids, %	0.93	0.07
Bound lipids, %	0.75	0.24
Gluten		
Yield, %	18.8	19.4
Protein (N × 5.7), %	63.2	61.3
Mixture of starch and water-solubles		
Yield, %	81.0	80.0
Protein (N × 5.7), %	2.6	2.8

[a] On a 14%-moisture basis.

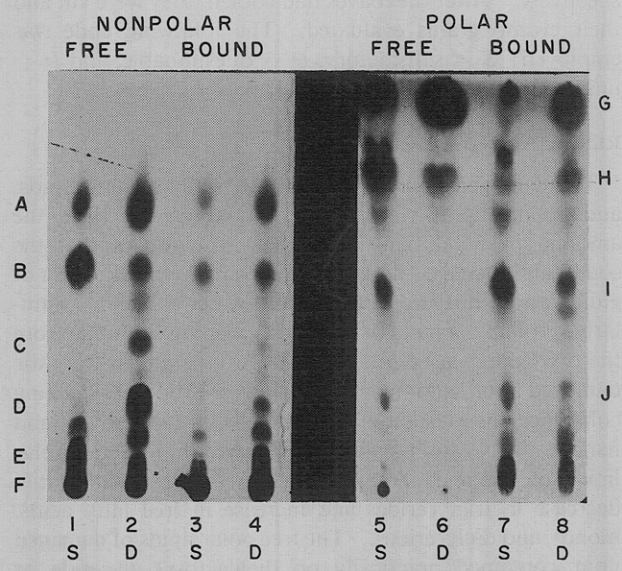

Figure 1. Thin-layer chromatography of lipids from sound (*S*) and damaged (*D*) Marquis flour

Spots 1 through 4 developed with chloroform, 5 through 8 developed with chloroform-m–thanol–water (65:35:4); 1, 2, 5, and 6 free lipids extracted with petroleum ether 3, 4, 7, and 8 bound lipids extracted with water-saturated butanol following petroleum ether. Spots of 100-μl. lipids; visualized by charring with sulfuric acid; picture taken under ultraviolet light. Tentatively identified as (*A*) mixture of hydrocarbons and steryl esters, (*B*) triglycerides, (*C*) free fatty acids, (*D*) diglycerides, (*E*) monoglycerides, (*F*) unresolved polar lipids, (*G*) unresolved nonpolar lipids, (*H*) monogalactosyl glyceride, (*I*) digalactosyl glyceride, (*J*) phosphatidyl choline.

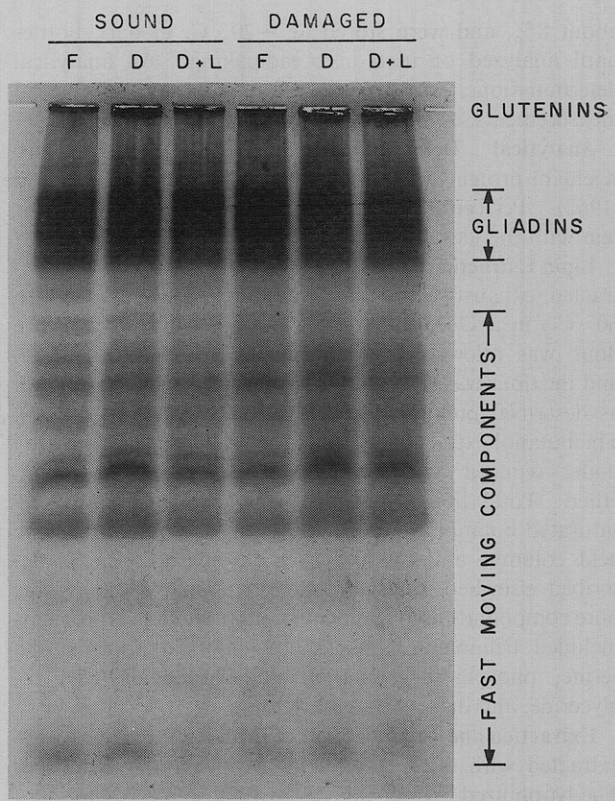

Figure 2. Starch-gel electrophoretic patterns of proteins extracted with 0.1*N* lactic acid from sound and damaged flours (*F*), doughs (*D*), doughs mixed with 0.5% polar lipids from sound flour (D + L)

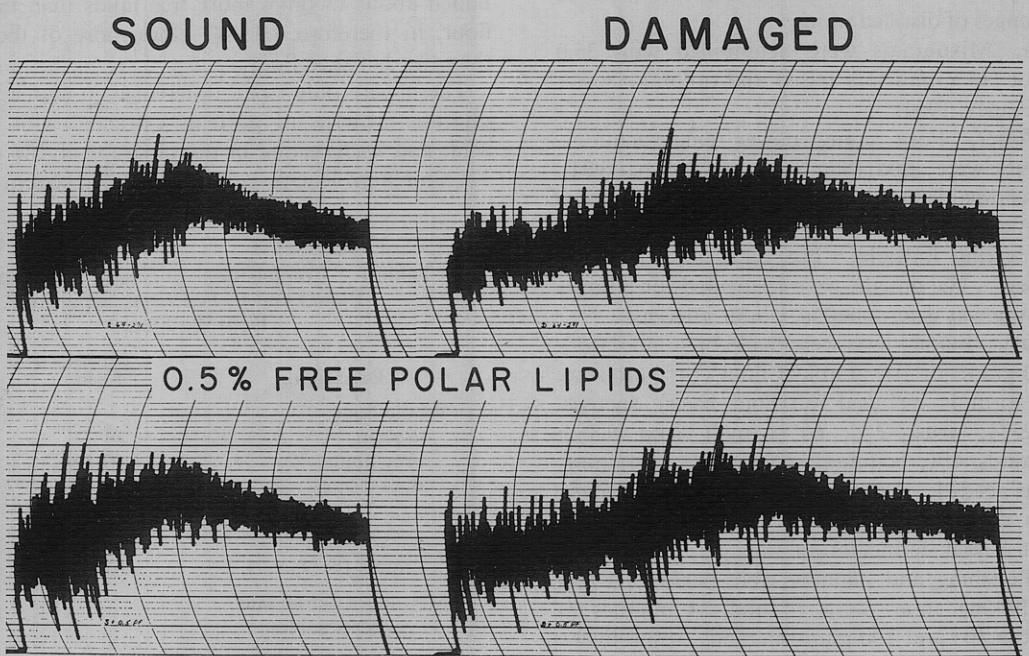

Figure 3. Mixograms of doughs from sound (left) and damaged (right) Marquis flours

Top row: unsupplemented flours; bottom row: flours mixed with 0.5% free polar lipids from sound flour

The damaged flour contained reduced amounts of minor, fast moving components; the amounts of major gliadin and glutenin components were unaffected. Proteins extracted from doughs contained consistently more high molecular weight compounds (unfractionated and retained at the point of application) than proteins extracted from flours. There was, however, no consistent difference in the amount of the high molecular weight protein extracted from doughs of sound and damaged flours. The electrophoretic patterns of protein extracts were affected little, if any, by adding to doughs 0.5% polar wheat flour lipids from the sound flour.

Table II. Effects of Lipids from Sound Flour on Bread Baked from Sound and Damaged Marquis Flour with 3 Grams of Shortening per 100 Grams of Flour

Lipid Description	Lipid Level, Grams	Sound Flour		Damaged Flour	
		Loaf volume, cc.	Crumb grain	Loaf volume, cc.	Crumb grain
None	...	972	*S*	647	*U*
Total	0.5	...		650	*U*
Total	1.0	975	*S*	700	*U*
Total	1.5	950	*S*	870	*Q–U*
Free	0.8	...		685	*Q–U*
Free	1.5	950	*S*	850	*Q–U*
Nonpolar free	0.5	885	*Q–U*	635	*U*
Polar free	0.5	1043	*S*	835	*Q–U*
Polar free	1.0	1030	*S*	865	*Q–U*
Polar free	2.0	...		950	*Q*

Mixing times of doughs from damaged flours were consistently and substantially longer than those of doughs from sound flour (Figure 3). Adding 0.5% polar lipids had no effect on mixing time or tolerance of sound or damaged flour. The damaged flour had normal gas production as measured by pressure meters and by proof height. Bread baked from damaged flour was substantially lower in loaf volume and poorer in crumb grain than bread baked from sound flour (Table II and Figure 4). Adding free or total lipids largely restored the loaf volume of bread baked from damaged flour. Nonpolar lipids had a deleterious effect, and polar lipids had the largest improving effect. Bread baked from damaged flours supplemented with total, free, or polar lipids, however, still had impaired crumb color and flavor.

Baking results of control and reconstituted doughs (Table III) indicated that lipids from sound flour restored

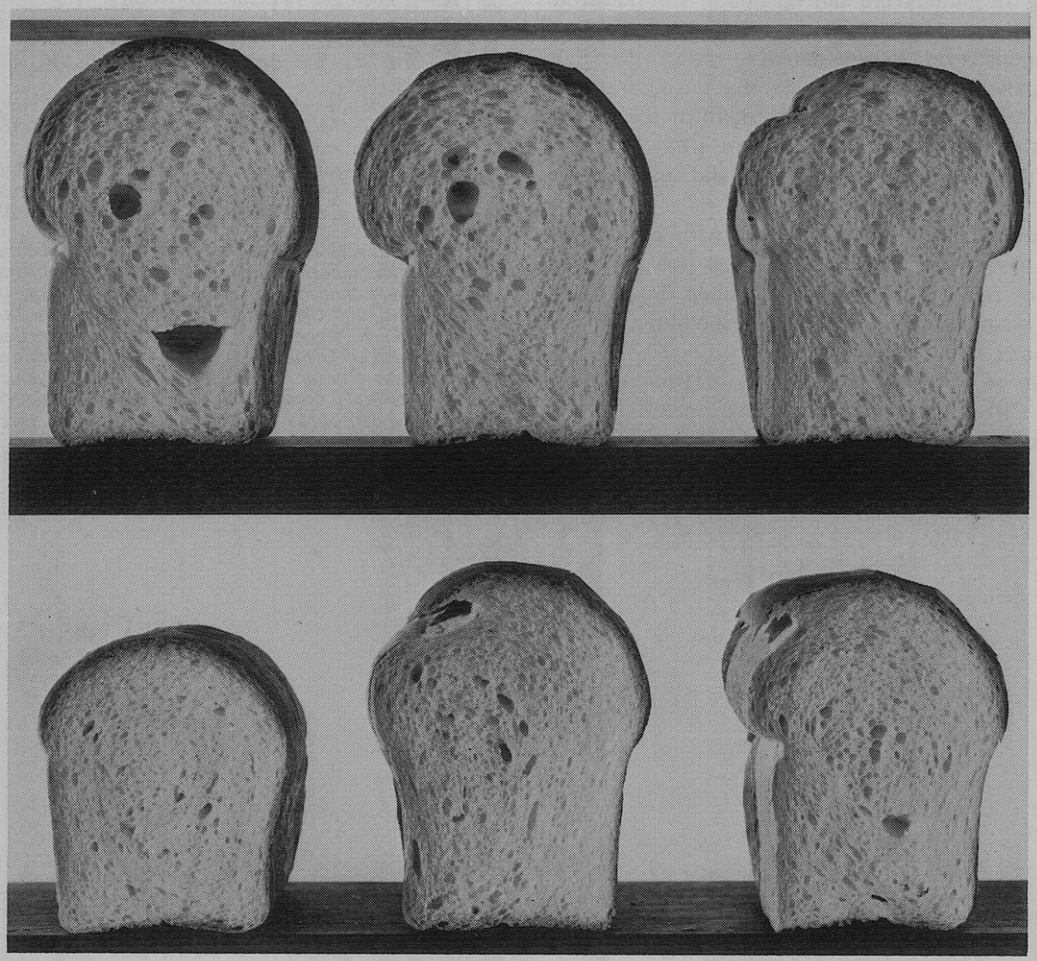

Figure 4. Bread baked from 100 grams of flour

From left to right, top row—sound flour, sound flour and 1.0 grams of total lipids, sound flour and 1.5 grams of total free lipids; bottom row—damaged flour, damaged flour and 1.5 grams of total lipids, and damaged flour and 1.5 grams of free lipids

Table III. Bread-Making Characteristics of Total and Reconstituted Sound and Damaged Flours Baked by the 10-Gram Method

Composition	Total Lipids Added, Gram	Baking Absorption, %	Mixing Requirement, Min.	Loaf Volume, Cc.
Sound flour (*S*)	...	65.5	3 3/4	83
Damaged flour (*D*)	...	69.0	9 1/8	66
Damaged flour (*D*)	0.12	68.5	9 3/8	82
Gluten (*S*) + starch[a] (*S*)	...	68.0	4	82
Gluten (*D*) + starch (*D*)	...	68.0	5 7/8	69
Gluten (*D*) + starch (*D*)	0.12	67.5	5 3/8	82
Gluten (*D*) + starch (*S*)	...	67.0	6	68
Gluten (*D*) + starch (*S*)	0.12	68.5	6	83
Gluten (*S*) + starch (*D*)	...	67.0	4 1/4	81

[a] Mixture of starch and water-solubles.

the loaf volume potential of damaged flour. Bread crumb grain of the damaged flour was nearly restored by the addition of lipids.

Replacing the gluten from damaged flour with that from the sound flour simultaneously restored the lipids that were decomposed in storage-damaged flour. Previous studies have shown (Chiu *et al.*, 1968) that during fractionation 85 to 90% of flour lipids are found in the gluten. Replacing the starch and water-solubles from damaged flour with those from the sound flour did not improve loaf volume or crumb grain.

The crumb grain of damaged flour, with or without lipids, had a creamy-brown color. Color was improved to creamy by replacing the damaged gluten or damaged starch and water-solubles with the corresponding fraction from the sound flour.

The results of our study are thus far of theoretical interest only. Restoration of loaf volume potentialities in damaged flour was not accompanied by comparable improvement of bread crumb flavor and color. In addition, the damaged flour may be inferior nutritionally and contain objectionable products of microbial metabolism. The results are of interest, however, as they confirm the previously established major role in bread making of flour lipids (Daftary *et al.*, 1967; Pomeranz *et al.*, 1965, 1966, 1968). Bread-making potential of flour is impaired by either solvent extraction of lipids or by breakdown of the lipids by microorganisms. In each case, the potential can be nearly restored by adding lipids from sound flour.

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Received for review August 10, 1967. Accepted October 24, 1967. Cooperative investigations between the Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan, and Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Contribution No. 611, Department of Grain Science and Industry, Kansas Agricultural Experiment Station. Mention of a trade product, equipment, or a commercial company does not imply its endorsement by the U. S. Department of Agriculture over similar products or companies not mentioned.