FLOUR LIPIDS AND OXIDATION OF SULFHYDRYL GROUPS IN DOUGH[1]

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ABSTRACT

Flour dough lost its sulfhydryl groups faster than did defatted flour dough when mixed in oxygen or air for over 5 minutes. This difference between the normal flour dough and the defatted flour dough is attributed to the oxidation of lipids in dough. Oxidized flour lipids or oxidized methyl linoleate, when incorporated into doughs, increased the sulfhydryl oxidation and exerted an improving effect (increased extensigram height), as did such simple peroxides as t-butyl hydroperoxide, methyl ethyl ketone peroxides, and acetone peroxides. It thus appears that when enough oxygen is available in dough, it reacts with both sulfhydryl groups and lipids, and that oxidized lipids also oxidize sulfhydryl groups and thus exert an improving effect. The effects of peroxides, used as flour maturing agents, can be attributed to their oxidizing action on sulfhydryl groups.

Work from several laboratories had adequately demonstrated that oxidation of sulfhydryl groups in dough takes place during mixing in air (5,7,9). At the same time evidence is also accumulating for the involvement, although sometimes indirect, of flour lipids in this oxidation.

Smith, Van Buren, and Andrews (7) concluded from their studies, based on the uptake of oxygen by doughs, that polyunsaturated fatty acids, perhaps through the action of lipoxidase, were involved in both chemical and rheological effects in dough. Coppock and Daniels (1) showed by means of gas chromatography the presence of two unidentified oxidized lipid components in extracts from doughs treated with oxidizing agents in air. Tsen and Hlynka (10) presented titrimetric data on lipid extracts of doughs showing that lipid peroxides were formed during mixing in air or oxygen.

Tsen and Hlynka, on the basis of their work which was later supported by rheological studies of Narayanan and Hlynka (6), proposed the following phases of the interrelations among oxygen, flour lipids, and the sulfhydryl groups of wheat proteins: (I) the reaction of molecular oxygen with unsaturated flour lipids; (II) the reaction of oxidized lipids with flour protein –SH; and (III) the direct reaction of molecular oxygen with flour protein –SH as an alternate route to I and II.

[1]Manuscript received August 9, 1962. Paper No. 214 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Manitoba. Presented at the 47th annual meeting, St. Louis, Missouri, May 1962.

The present paper describes experimental data which further support the above hypothesis, with emphasis on the oxidation of sulfhydryl groups by oxidized flour lipids. It also reports the oxidative actions of oxidized linoleate and simpler peroxides on sulfhydryl groups in dough in order to elucidate further the reaction mechanism. The mechanism also helps to explain the action of acetone peroxides as flour improver (2). Extensigram data are presented to illustrate the effect of the oxidative changes in relation to corresponding changes in the rheological properties of dough.

Materials and Methods

Reagents. All chemicals used in this study were reagent grade. Tertiary butyl hydroperoxide and methyl ethyl ketone peroxides (Lupersol DDM) (60% as the peroxides) were kindly supplied by A. I. Lowell of the Lucidol Division, Wallace and Tiernan, Inc., Buffalo, N. Y. Acetone peroxides (Ketonox) (12.7% as the peroxides) was a gift from the J. R. Short Canadian Mills, Ltd., Toronto, and Tween-20 from the Atlas Powder Company, Canada, Ltd., Brantford, Ontario. Methyl linoleate (A Grade) was purchased from the California Corp. for Biochemical Research. Petroleum ether (Skellysolve F-95) was redistilled in an all-glass apparatus and the fraction with a boiling point range below 45°C. was used in this study. All distilled water was passed through a Deeminizer before use.

Flour and Dough. The Bakers' strong flour (protein, 15.3%; ash, 0.60%; petroleum ether extract, 1.27%) and the defatting procedure, used in this study, were those previously described (10). Doughs were prepared from 100 or 200 g. of flour (14% moisture) and sufficient water and salt to give an absorption of 59.2% and salt content of 1% (flour basis). They were mixed in the GRL mixer (3) under nitrogen, air, or oxygen. Flours for mixing in nitrogen or oxygen were purged with nitrogen or oxygen under alternate vacuum and pressure. The solutions were used "as is" for mixing the dough in air but were saturated with either nitrogen or oxygen for mixing in these gases. Unless otherwise stated, all the mixing was done in nitrogen.

For extensigraph tests, the dough was given a reaction time of 10 minutes, then rounded and shaped, and stretched after a rest period of 20 minutes. During the reaction time and rest period, the dough was kept in a cabinet maintained at 30°C. and 95% r. h.

Lipids. Flour lipids used in this study were extracted from Bakers' strong flour with petroleum ether. Oxidized flour lipids and oxidized methyl linoleate were prepared by ultraviolet irradiation of oxygenated lipids and linoleate.

The iodometric method of Lundberg and Chipault (4) was used to determine peroxide values of oxidized flour lipids and oxidized linoleate.

For the incorporation of oxidized flour lipids into flour, two methods were investigated: (a) Oxidized flour lipids were dissolved in petroleum ether; the solution was mixed into flour and the solvent was then evaporated off under nitrogen. (b) An emulsion of oxidized flour lipids and Tween-20 was prepared; the emulsion, after bubbling with nitrogen to remove its dissolved oxygen, was added to flour before mixing. After a trial of both methods, the emulsion method was found to be more satisfactory. The emulsion was prepared by blending oxidized flour lipids and Tween-20 solution in a Waring Blendor for 1 minute to give a system with Tween-20 content of 0.25%. The doughs were mixed in nitrogen for 5 minutes. The control dough was prepared under the identical condition, but with 0.25% Tween-20 solution instead of the emulsion. The same procedure was adopted for the addition of oxidized methyl linoleate, t-butyl hydroperoxide, or methyl ethyl ketone peroxides to flour. For acetone peroxides, the commercial preparation (a starch mixture) was used directly.

Determination of Sulfhydryl Content. Sulfhydryl contents in flour or dough were determined according to the method of Sokol, Mecham, and Pence (8,9) with two modifications: First, liquid nitrogen was used to freeze dough samples before lyophilization. The dried dough was ground in a micro Wiley mill (60-mesh screen) and stored at −40°C. for subsequent analysis. Second, a Goldfisch extraction beaker with a closely fitted cover was used as the titrating vessel. During the dispersion of the sample and the titration, an ice-water bath was not used; instead, nitrogen was continuously flushed into the beaker to minimize the oxidation by air. Each individual sulfhydryl value represents at least duplicated titrations and is reported as μeq. per g. of flour or dough, both on a dry basis.

Results and Discussion

Effect of Reaction Time on the Sulfhydryl Oxidation. Dough samples were mixed for 2.5 minutes in air or oxygen and were then put into a plastic container and placed in a cabinet maintained at 30°C. and 95% r. h. for a given length of time referred to as the reaction time. At the end of the desired reaction time, 0, 30, 60, or 90 minutes, the samples were frozen and lyophilized, and their sulfhydryl contents were determined.

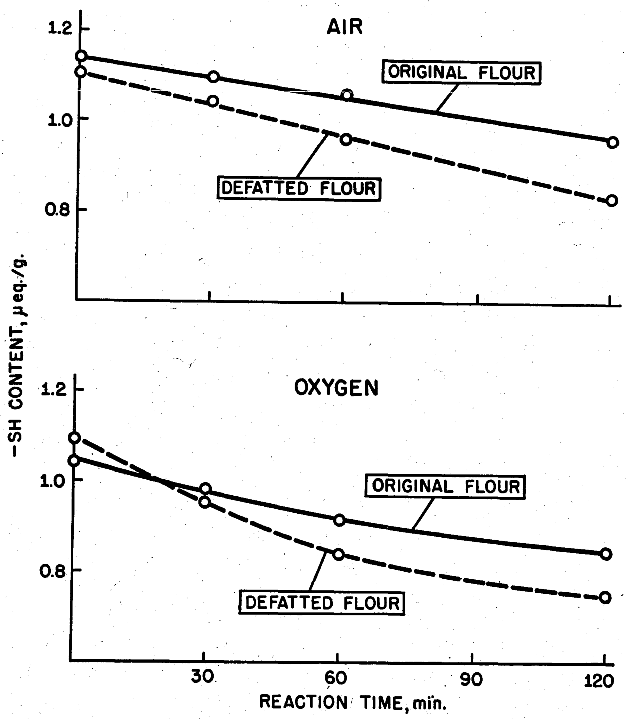

Fig. 1. Effect of extracting lipids on the sulfhydryl oxidation in resting doughs.

The results (Fig. 1) show that during the reaction period doughs prepared from the defatted flour lose their sulfhydryl groups faster than doughs from the original flour. It is suggested that lipids compete for the available oxygen in the dough and that removal of lipids thus increases the sulfhydryl oxidation. Data for 0- and 3-hr. reaction periods, presented by Smith, Van Buren, and Andrews (7), record a similar observation for a second clear flour.

Effect of Mixing on the Sulfhydryl Oxidation. Further study was made to investigate the effect of lipids on the sulfhydryl oxidation in dough in which more oxygen was made available by continuous mixing. The original flour dough and the defatted flour dough were mixed in air or oxygen for 2.5, 5, 10, and 20 minutes, and their sulfhydryl contents were measured.

The results (Fig. 2) show that during mixing it is the original rather than the defatted flours that lose sulfhydryl groups most rapidly. Similar results are also reported by Smith *et al.* (7) for a second clear flour, though not for a patent flour.

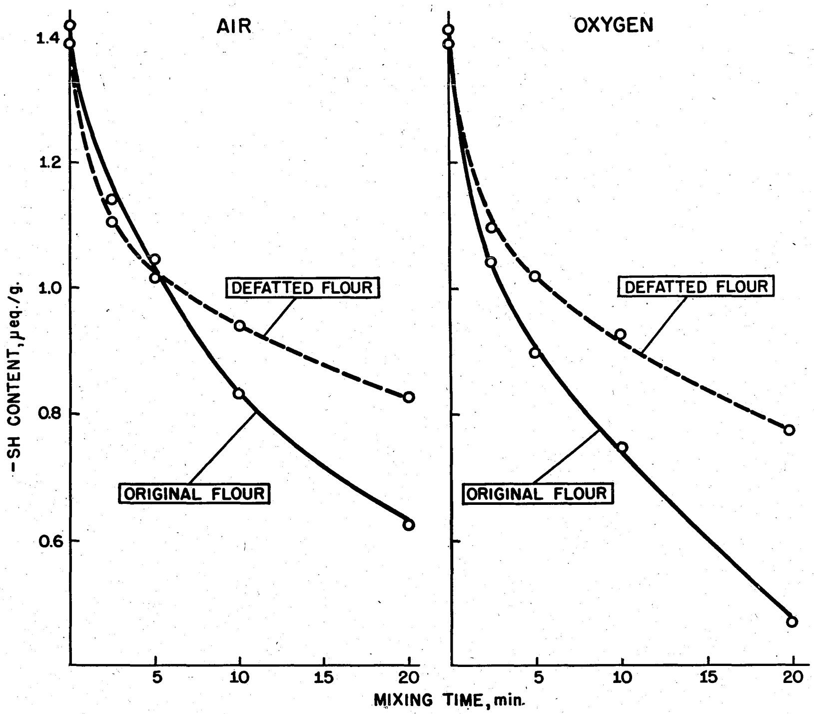

Fig. 2. Effect of extracting lipids on the sulfhydryl oxidation during mixing.

The contrast between the results obtained during continued mixing up to 20 minutes (Fig. 2), and during the reaction period after 2.5 minutes of mixing (Fig. 1), can be explained in terms of our unified hypothesis of the role of lipids. During mixing, oxygen is continuously incorporated into the dough and made available at reaction sites in excess of requirements.

In the original flour the sulfhydryl groups are oxidized by two reactions; namely, by direct reaction with oxygen and by reaction with lipid peroxides that are formed during mixing. Under the condition of excess oxygen their effect is additive. The loss of sulfhydryl is thus more rapid in the original flour than in the defatted flour in which only the direct reaction with oxygen is involved. By contrast, in dough resting during the reaction period the availability of oxygen is limited and direct competition occurs. (The oxygen reacts either with the sulfhydryl group and/or with oxidizable lipid.) Accordingly the loss of sulfhydryl under the condition of limited oxygen is more rapid when the fat is removed.

Effect of Oxidized Flour Lipids and Oxidized Linoleate. It has been shown that lipid peroxides are formed during dough mixing (10) and postulated that these peroxides oxidize sulfhydryl groups and thus affect the rheological properties of dough. Direct verification was sought by adding oxidized flour lipids and oxidized linoleate to defatted flour and determining the resulting change in the sulfhydryl content. Linoleic acid comprises about 60% of the total fatty acids in flour lipids. Doughs were mixed in nitrogen for 5 minutes. The dough samples for sulfhydryl determination were taken immediately after mixing, and extensigrams were also prepared.

TABLE I
EFFECT OF OXIDIZED FLOUR LIPIDS AND OXIDIZED LINOLEATE ON -SH CONTENT AND EXTENSIGRAM HEIGHT OF DOUGHS

Additive			Dough Properties	
Name	Amount Added	Peroxide Value [a]	-SH Content	Extensigram Height
	% of flour	*µeq./g. flour*	*µeq./g. dough*	*B.U.*
Oxidized flour lipids	0	—	1.31	320
	0.15	1.1	1.04	410
	0.30	2.2	0.92	510
	0.60	4.4	0.89	630
	1.20	8.8	0.72	870
Oxidized linoleate	0	—	1.31	320
	0.075	0.8	1.18	460
	0.15	1.6	1.04	600
	0.30	3.2	0.84	860
	0.60	6.6	0.61	over 1050
	1.20	13.2	0.42	over 1050

[a] Calculated from determination made on the original emulsion.

The results presented in Table I show that increasing amounts of both additives cause progressive decreases in sulfhydryl content and increases in extensigram height. However, at comparable or equal sulfhydryl contents, the extensigram heights of doughs treated with oxidized flour lipids differ from those of doughs with oxidized linoleate. This difference is probably due to a number of factors: (a) Oxidized flour lipids are much more complex than oxidized linoleate. Some components of the oxidized flour lipids could also influence dough rheology as expressed by the extensigram heights in this study. (b) Oxidation of sulfhydryl groups, important as it undoubtedly is, may not be the only factor exerting the improving effect.

Figure 3 shows sulfhydryl content plotted against the amount of oxidized linoleate added in terms of peroxide values. The first part of the curve reveals a linear relation between the sulfhydryl loss and

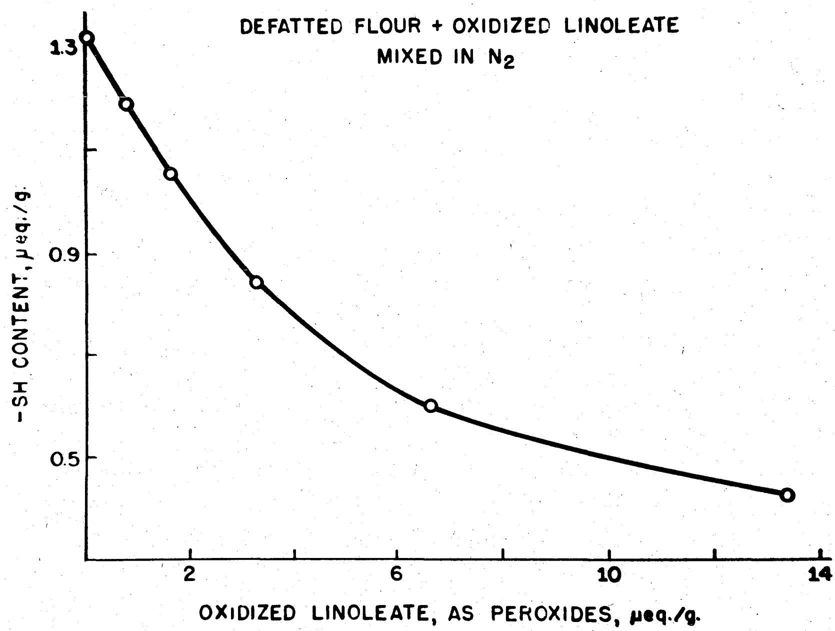

Fig. 3. Relationship between added oxidized linoleate in terms of peroxide values and the sulfhydryl oxidation in dough.

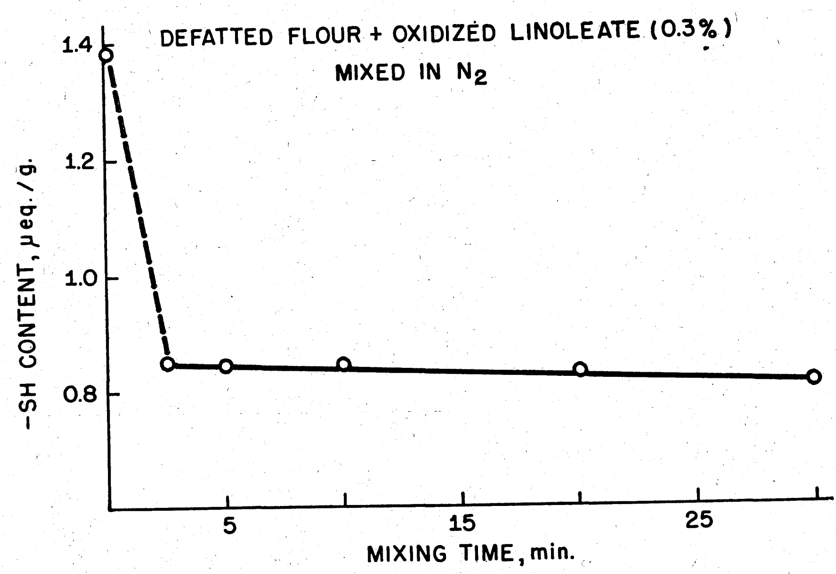

Fig. 4. Rate of the oxidation of sulfhydryl groups in dough by oxidized linoleate.

peroxides added. It also suggests virtual completion of the reaction within the 5 minutes of mixing. The rate of the oxidation was confirmed in an experiment in which doughs containing 0.3% of oxidized linoleate were mixed in nitrogen for various times. The results, given in Fig. 4, show that the oxidation is rapid and almost complete within 2.5 minutes or less. The further decrease in sulfhydryl content with additional mixing is relatively insignificant.

Effect of t-Butyl Hydroperoxide, Methyl Ethyl Ketone Peroxides, and Acetone Peroxides. Since variations in the sulfhydryl content and the extensigram height were closely related to the changes in peroxide values of oxidized flour lipids and linoleate, the experiments were extended to include three simple peroxides listed above. The original flour, rather than the defatted flour, was used, and doughs were again mixed in nitrogen for 5 minutes.

The results, given in Table II, show that all three compounds, when added in increasing amounts, decreased the sulfhydryl content of the dough. There were corresponding increases in extensigram height for both t-butyl hydroperoxide and acetone peroxides. However, extensigram data for methyl ethyl ketone peroxides show increasing height up to additions of about 0.25%, which reduce

TABLE II
EFFECT OF t-BUTYL HYDROPEROXIDE, METHYL ETHYL KETONE PEROXIDES, AND ACETONE PEROXIDES

Additive		Dough Properties	
Name	Amount Added	-SH Content	Extensigram Height
	% of flour	*µeq./g. dough*	*B.U.*
t-Butyl hydroperoxide	0	1.11	300
	0.025	1.03	420
	0.125	0.94	860
	0.625	0.71	over 1050
	1.25	0.48	over 1050
Methyl ethyl ketone peroxides	0	1.11	300
	0.005	0.95	710
	0.025	0.63	over 1050
	0.125	0.52	1040
	0.625	0.21	980
	1.25	0.07	930
Acetone peroxides (commercial preparation — Ketonox)	0	1.11	300
	0.02	1.09	440
	0.50	0.61	930
	1.00	0.37	1020
	2.00	0.29	over 1050
	4.00	0.17	over 1050

sulfhydryl content to about 0.63 μeq. per g. flour, followed by a slight decrease in height with substantially larger additions. This is probably due to overoxidation, resulting in dough breakdown. At comparable sulfhydryl contents, the extensigram heights still vary with the different added peroxides. This variation again indicates that in addition to oxidation of sulfhydryl groups, some other factor(s) is (are) involved in the change of dough properties. Nevertheless, the sulfhydryl oxidation appears to be mainly responsible for the change.

In general, the results obtained with the simpler peroxides confirm those obtained with oxidized flour lipids and oxidized linoleate. Very recently Ferrari and Higashiuchi proposed the use of acetone peroxides as flour maturing and bleaching agents (2); the mechanism of the maturing action of organic peroxides involves their reaction with sulfhydryl groups of flour proteins.

Acknowledgment

It is a pleasure to acknowledge the competent technical assistance of P. B. Mazur.

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