OBSERVATIONS ON THE REACTIVITY OF SULFHYDRYL GROUPS IN WHEAT FLOUR[1]

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ABSTRACT

In flour samples dispersed in urea solutions (pH 7.0–7.5), sulfhydryl groups reacted faster and more extensively with mercuric chloride and N-ethylmaleimide than with iodoacetamide; with fourfold excesses of these three reagents, 100, 70, and 10%, respectively, of the total sulfhydryl groups reacted in 20 minutes. Very little or no effect of potassium bromate on the sulfhydryl content of doughs mixed in air was found at normal bromate dosages with straight-grade flours. However, with a fifth-break flour, a small decrease in sulfhydryl content resulted from bromate additions to fermented and nonfermented doughs. During prolonged mixing in air, about half the sulfhydryl groups of a spring wheat flour were lost. Additions of an anionic detergent of the alkyl sulfate type did not affect this loss, although the expected stabilization of a recording mixer curve was observed.

The action of flour improvers has often been postulated as an oxidation of sulfhydryl groups in flour proteins to form intermolecular disulfide bonds. These linkages would then account for increased toughness and gas retention in doughs and larger loaf volume in bread. On this basis, sulfhydryl-disulfide relationships in wheat products have interested cereal chemists for many years. The available information has been reviewed at intervals (1, 12, 13) so that no elaboration is needed here. Evidence also has been presented that sulfhydryl groups may be a factor in determining the mixing characteristics of doughs (10). Nevertheless, the participation of sulfhydryl groups as a critical factor in either dough mixing or the action of oxidizing improvers has not been positively demonstrated.

A principal obstacle has been the low level at which sulfhydryl groups occur in flours and the consequent analytical difficulties in tracing changes in sulfhydryl content. However, the silver titration method of Benesch, Lardy, and Benesch (3) has been applied to flour and flour constituents (9, 13), and found sufficiently sensitive and precise to encourage a study of the reactivity of sulfhydryl groups in flour. Before devoting particular attention to either dough oxidation or mixing in relation to sulfhydryl changes, a survey was made of the general reactivity of sulfhydryl groups in flour, including their reactivity with various specific sulfhydryl reagents. These findings will be reported here.

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Materials and Methods

The flours used were selected to represent quite different types. They were described in a previous publication (13).

The iodoacetamide (IAC) was a laboratory preparation; *p*-chloromercuribenzoate (PCMBA) was from Sigma Chemical Co., 3500 DeKalb Street, St. Louis, Mo.[2]; N-ethylmaleimide (NEMI) from Mann Research Laboratories, 136 Liberty St., New York 6, N. Y.; and Duponol "R" (mixture of fatty alcohol sulfates) from E. I. du Pont de Nemours & Co., Wilmington 98, Dela.

The method employed for the determination of sulfhydryl groups in flour, dough, and flour fractions was essentially the silver amperometric titration of Benesch, Lardy, and Benesch (3). Precautions to be observed and the particular procedures followed in applying the method to flour have been described (13). Dispersion of samples and titration at 2°C., addition of ethylenediaminetetraacetate (EDTA), and titration of dispersions rather than extracts or digests were found to be required for maximum recovery of sulfhydryl (13). In addition, each result was obtained from at least three individual titrations.

Difficulties and inconveniences were encountered in applying the method directly to doughs and flour-water slurries. Well-mixed doughs required prolonged exposure (up to 16 hours) to the titrating medium for complete dispersion, and, after such an interval, the sulfhydryl titer of the sample was reduced almost to zero. Unless freshly prepared for each titration, slurries also showed a significant decline in sulfhydryl content in successive titrations.

To minimize these losses, doughs and slurries were dried by lyophilization. Doughs were frozen rapidly by spreading them in thin layers on dry ice, and slurries were shell-frozen in balloon flasks in a dry ice and acetone bath. After lyophilization, grinding, and moisture equilibration, the dried doughs and slurries were handled like ordinary flour samples.

A small loss of sulfhydryl content occurred during the lengthy lyophilization. This loss was detected by a comparison of flour-water slurries with lyophilized portions of the same slurries. The slurries were made by blending three parts by weight of flour with four parts of distilled water in a food blender. After a few minutes of mixing, a portion was immediately frozen and lyophilized, and another portion of the slurry was immediately titrated. Comparisons of the amounts of titrant used for three separate slurry preparations and their lyophilized counterparts are given below:

[2] Mention of trade names, equipment, or suppliers does not constitute endorsement by the Department of Agriculture over others not mentioned.

Sample	*Dry Weight of flour*	*0.001N Silver Nitrate*
	g	*ml/g dry flour*
Slurry	0.533	0.71
After lyophilization	0.535	0.66
Slurry	0.675	0.71
After lyophilization	0.677	0.62
Slurry	1.032	0.64
After lyophilization	1.035	0.61

Sulfhydryl contents of doughs given in this paper, therefore, are probably 5 to 10% low. However, differences between samples should be reflected reasonably well, since all were dried by the same procedure. No loss of sulfhydryl groups has been observed in flours during storage for a month at room temperature (about 24°C.), although in one instance the sulfhydryl content of a flour decreased slightly in 2 months at room temperature. Lyophilized doughs are stable for at least 1 week at room temperature. No changes have been observed in flours stored at −18°C. for at least 2½ months.

Results and Discussion

Reactivity with Specific Sulfhydryl Reagents. The reactivity of flour sulfhydryl groups with two organic reagents (NEMI and IAC) and with mercuric ions was compared under the conditions previously found to permit titration of maximum amounts of sulfhydryl groups with silver – that is, in urea-containing suspensions at 2°C. Results are presented in Table I. Reaction proceeded much more readily with NEMI and mercuric chloride than with IAC; the maximum extents of reaction observed were, respectively, 90, 100, and 30%. The rate

TABLE I

REACTION OF N-ETHYLMALEIMIDE, MERCURIC CHLORIDE, AND IODOACETAMIDE WITH SULFHYDRYL GROUPS IN A COMMERCIAL HARD RED SPRING FLOUR[a]

REAGENT	TIME	SULFHYDRYL BLOCKED		
		CONCENTRATION OF REAGENT (equiv. per equiv. sulfhydryl)		
		2	4	8
	minutes	%	%	%
NEMI	20	40	70	70
	90	90	90	90
IAC	20	10	10	10
	90	30	30	30
Mercuric chloride	20	100[b]		

[a] Flour sample suspended in 6.4 *M* urea, 0.1 *M* tris (hydroxymethyl) aminomethane, pH 7.5. Values are averages of three titrations.
[b] No EDTA used in titration.

of reaction of sulfhydryl groups with IAC was not changed by increasing the IAC concentration; whereas a change was noted with increased NEMI concentrations for the 20-minute reaction time. IAC is known to be a slow-reacting sulfhydryl reagent. Lontie and Beckers (8) showed that reaction times with IAC up to 24 hours were necessary to get maximum sulfhydryl titers for ovalbumin; in addition, Olcott and Fraenkel-Conrat (11) indicated that IAC does not always react with sulfhydryl groups. They point out that tobacco mosaic virus can be inactivated by IAC with no decrease in sulfhydryl content. It may be that the sulfhydryl groups must be in a highly activated state to react with IAC. The number of such groups may be only a small fraction of the total number at any particular time. This would account for the inability of increased IAC concentrations to affect the sulfhydryl content of the dispersions (Table I).

In reactivity tests with flour similar to those made with NEMI and IAC, PCMBA as the specific reagent gave unsatisfactory results, apparently because of reversibility of the PCMBA-sulfhydryl reaction. This was indicated by rounded amperometric titration curves having no definite end point. They improved in shape only when large excesses of PCMBA were added. The results with NEMI provide evidence, in addition to that presented previously (13), that the specificity of the silver-tris [tris (hydroxymethyl) aminomethane] titration for sulfhydryl groups is, at least, very largely retained in the presence of nonprotein flour constituents, since the silver titer is reduced by 90%. It seems most likely that the few residual sulfhydryl groups are in such positions that they cannot react with NEMI. An inability of excessive amounts of NEMI to eliminate sulfhydryl titers completely at pH 10.4 has been reported by Bloksma (5) and, at a lower pH, by Matsumoto and Hlynka (9).

Effect of Potassium Bromate. Bloksma (4) found no changes in the sulfhydryl content of glutens separated from doughs to which bromate had been added. In contrast, Matsumoto and Hlynka (9) found a definite lowering of the sulfhydryl content of gluten proteins separated from doughs, mixed in the absence of oxygen, to which bromate or iodate was added. Observations made in the present study on whole doughs, rather than glutens, are given in Table II. The doughs (50 g. flour, 35 ml. distilled water, and the indicated amounts of yeast and potassium bromate) were mixed at 29°C. for 10 minutes in a farinograph and allowed to stand for 3 hours at room temperature before freezing and lyophilization. Titrations were made shortly thereafter. No effect of bromate was found on the sulfhydryl content of doughs from Lee and Idaed variety flours. However, with the fifth-break flour,

TABLE II

SULFHYDRYL CONTENT OF FERMENTED, NONFERMENTED, AND BROMATED DOUGHS

FLOUR	No YEAST		2.5% YEAST	
	Bromate	Sulfhydryl Content	Bromate	Sulfhydryl Content
	ppm	*μeq/g*	*ppm*	*μeq/g*
Lee[a]	0	0.68	0	1.17
	30	0.69	20	1.16
Idaed[a]	0	0.64	0	1.05
	50	0.64	40	1.02
Fifth-break[a]	0	0.96	0	1.34
	50	0.76	50	1.28

[a] The sulfhydryl contents of Lee, Idaed, and fifth-break flours were 1.04, 0.87 and 1.30 microequivalents per gram, respectively.

small decreases in sulfhydryl content as a result of potassium bromate additions were observed. The fifth-break flour has a very high protein content and a proportionately high sulfhydryl content; it cannot be considered a typical bread flour. However, this flour also has a very high baking response to bromate, in contrast to the Lee and Idaed flours, so that the decrease in sulfhydryl content is consistent with the correlation between sulfhydryl groups and bromate response first postulated by Sullivan (16).

Sulfhydryl Contents of Materials Extracted by Petroleum Ether. Sulfur-containing lipoproteins are known to be present in petroleum ether extracts of flours (2). Matsumoto and Hlynka (9) found that the sulfhydryl content of the petroleum ether extract of hard red spring flour was negligible in relation to the sulfhydryl content of other fractions. Similar results were obtained here. The averages of four to six individual determinations per sample were as follows:

Flour	*Sulfhydryl Content*	
	Untreated	*Extracted*
	μeq/g flour	*μeq/g flour*
Cookie flour (commercial)	0.66	0.66
Lee	0.89	0.86
Idaed	0.86	0.88

Effects of Added Detergent. An increase in the mixing stability of flours from addition of anionic detergents has been reported by Sullivan (15) and by Swanson *et al.* (17). This effect is opposite to that of NEMI and other sulfhydryl-blocking reagents (8) and suggests that the detergents act by protecting sulfhydryl groups during mixing. In addition, Klotz (7) and Van Scott and Flesch (18) reported high sulfhydryl titers for bovine serum albumin and fibrous proteins when

detergents were used as denaturing agents.

To determine whether the sulfhydryl content of doughs would be altered by inclusion of a detergent, doughs were prepared from Lee variety flour in a farinograph and sampled after 35 minutes of mixing. Farinograms showing the effect of detergent are pictured in Fig. 1. It

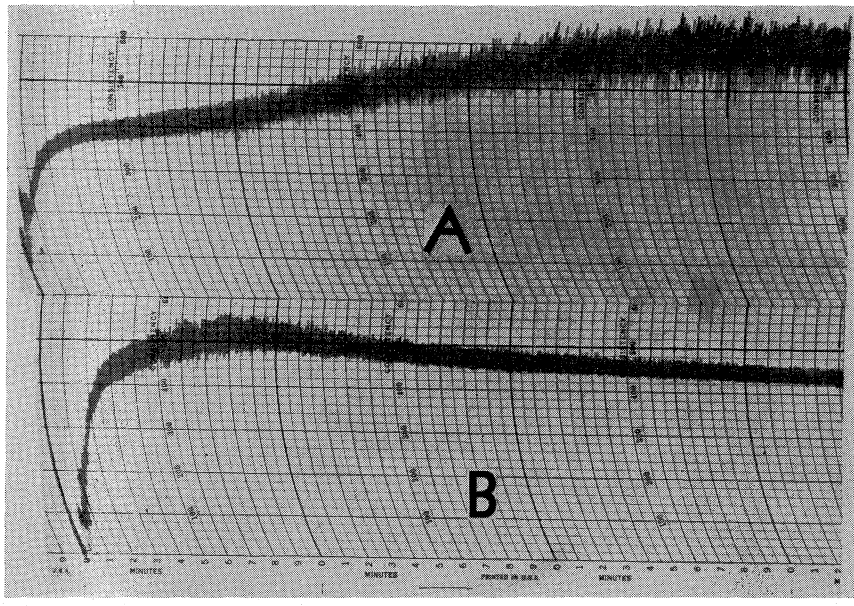

Fig. 1. Farinograms showing the effect of detergent on mixing characteristics of Lee flour. A, 50 g. Lee flour, 35 ml. water, and 0.5 g. Duponol "R." B, 50 g. Lee flour, 35 ml. water.

is apparent that the control dough had broken down well before 35 minutes, while the dough containing detergent maintained nearly maximum resistance to mixing. However, no significant difference in sulfhydryl content of the two doughs was found; values were 0.44 μeq. per g. with detergent and 0.47 without.

General Discussion

The reactivity of the sulfhydryl groups in flour seems to cover a wide range. Some sulfhydryl groups appear to be quite labile, as judged by the substantial loss on mixing in air and by the effect of temperature of the titration mixture, reported earlier. On the other hand, the slowness with which some sulfhydryls are blocked by NEMI and the presence of sulfhydryls in titration slurries after long mixing in air show that some are relatively unreactive or inaccessible. The

differences in reactivity do not appear to be confined either to gluten or nongluten proteins, although those in the soluble proteins have been reported to react more rapidly (9).

The comparisons of sulfhydryl-blocking reagents reported here were made under pH conditions more favorable to reaction than are present in normal doughs. Nevertheless, the greater effectiveness of NEMI, as compared to IAC, in blocking sulfhydryl groups parallels the more marked effect of NEMI on mixing curves (10).

Changes in sulfhydryl content caused by potassium bromate were noticeable only with the fifth-break flour. This flour gives a marked bromate response in baking, and its high protein and sulfhydryl content also provide more favorable circumstances for accurate analyses than are found in the typical flours used in baking. In relating these observations to those of Matsumoto and Hlynka (9), it should be pointed out again that their observations on the effects of bromate and iodate were made on doughs mixed in nitrogen. Our data on fifth-break flour were obtained from doughs mixed in air, so that the decrease in sulfhydryl may be attributed to a bromate effect beyond that caused by air oxidation.[3] The decreases observed are so small, however, that their significance with respect to dough improvement cannot be evaluated without further investigation.

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[3] Cunningham and Hlynka (6) have shown that oxygen appears to reduce the rate of reaction of bromate in dough. In doughs made from straight-grade and patent flours mixed in air, the small number of sulfhydryl groups that are reactive to bromate may have been decreased further by air oxidation.

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