SULFHYDRYL LOSSES DURING MIXING OF DOUGHS: COMPARISON OF FLOURS HAVING VARIOUS MIXING CHARACTERISTICS[1]

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ABSTRACT

Twelve flours of varied types were made into doughs in a farinograph and their sulfhydryl contents determined after 2, 5, 10, and 20 minutes of mixing. Sulfhydryl losses varied after 20 minutes of mixing from 38 to 64% of the original sulfhydryl content. An initial short period (2 to 5 minutes) of rapid decrease in sulfhydryl content was observed with most of the flours. With continued mixing, relatively large differences were found in the rate of decrease; treated by first-order kinetics, values for the rate constant for the 5-to-20-minute period ranged from 6 to 36 minutes^{-1}.

Doughs from three flours (including two durums) were mixed with added flour albumin. The initial decrease in sulfhydryl content then was more rapid, while the rate of loss from 5 to 20 minutes was decreased.

Stability values taken from farinograms correlated well with the sulfhydryl-loss rate constant for three hard red spring, three white, and two hard red winter wheat flours, and for the two durum flours supplemented with albumin. The nonsupplemented durums and two hard red winter wheat flours giving atypical farinograms did not show this relationship.

With two flours, the rate of loss of sulfhydryl groups in 10:1 water-flour suspensions decreased much more slowly than in the corresponding doughs.

The sulfhydryl content of straight-grade flour is about 1 μeq. per g. of flour (2,3,9). Despite this relatively low concentration, reagents

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that react specifically with sulfhydryl groups bring about marked changes in the physical properties of doughs during mixing (5). Such observations indicate that sulfhydryl groups may be an important factor in the wide natural variations found in the mixing characteristics of flours.

A considerable part of the sulfhydryl groups in some flours is lost during mixing of a dough in air and oxygen, presumably by oxidation (2,8). The available reported information, however, is limited to a few flours. If sulfhydryl groups do contribute to the mixing characteristics of different flours, it was thought that the rates of decrease of sulfhydryl content during dough mixing might be found to differ. The work reported here was undertaken to investigate this possibility.

Materials and Methods

The flours were unbleached, straight-grade, and experimentally milled, except for the durum and commercial hard red spring flours which were commercially milled. Protein and ash contents and the sources of the flours are given in Table I.

TABLE I
ASH AND PROTEIN CONTENTS OF FLOURS (DRY BASIS)

FLOUR	SOURCE	ASH	PROTEIN
		%	%
Durum No. 1	California	0.74	11.0
Durum No. 2	Dakotas	0.93	12.8
Mida, HRS	North Dakota	0.41	14.7
Lee, HRS	North Dakota	0.39	17.5
Commercial, HRS	Montana	0.45	15.6
Baart, SWS	Washington	0.62	13.0
Idaed, SWS	Washington	0.53	14.2
Elgin, Club	Washington	0.58	13.8
UN No. 1, HRW	Nebraska	0.49	12.8
UN No. 2, HRW	Nebraska	0.54	14.4
Cheyenne, HRW	Montana	0.45	16.5
Wasatch, HRW	Montana	0.44	13.6

Sulfhydryl determinations usually were carried out on lyophilized doughs. Flour and water were mixed for 2, 5, 10, and 20 minutes in a 50-g. farinograph bowl at 29°C. Immediately after mixing, each dough was compressed between blocks of dry ice, the frozen dough was lyophilized, and the dried dough ground (Wiley mill) through an 80-mesh screen. Previous work has shown that grinding itself does not cause a loss of sulfhydryl. After exposure to the atmosphere for one day, portions of the dried dough were dispersed in a buffer-urea medium and were titrated amperometrically with silver nitrate (9). Each individual sulfhydryl value reported below represents at least three titrations of differing sample sizes.

Results with suspensions (Elgin and Lee flours) were obtained as follows: Two grams of flour were added to 20 ml. of distilled water at room temperature (about 25°C.) during vigorous mixing with a magnetic mixer. This mixing lasted for about 30 seconds in every case. Then the mixer was set at a constant moderate speed and the mixing was timed for 5, 10, or 20 minutes at room temperature. A 2-g. sample was used for each of the three mixing times. Precautions were also taken to prevent heating of the suspension by the mixer. After the suspension was mixed for the proper length of time a 6-ml. aliquot was withdrawn and added immediately to a buffer-urea-salt solution; titration was then performed as usual (9).

The albumin sample was prepared from the commercial hard red spring wheat flour as directed by Pence and Elder (6); the yield was 0.37% of the flour. Albumin-supplemented doughs contained 0.37% (based on flour weight) of albumin added to the flour in the farinograph bowl before mixing. The sulfhydryl content of the albumin was 5.7 μeq. per g.; its contribution to the titer of a dough was negligible.

Previous work (10) has shown that a loss of about 5 to 10% of residual sulfhydryl groups occurred during the lyophilization process. The values presented in this paper therefore will be slightly lower than would be found with undried doughs. The error is consistent and in one direction, however, and should not affect the trends observed.

Farinograms were obtained on flour-water doughs, and the conventional technique was used (50-g. bowl).

Results and Discussion

Sulfhydryl losses during mixing are given in Table II for ten flours of widely varied type and source. Considerable differences in the rates of loss were found; this is more readily apparent in Fig. 1, in which data are plotted for four flours representative of different types.

In all of the doughs mixed in air the sulfhydryl content decreased most rapidly during the initial stages of mixing, although the actual losses in the first 2 minutes ranged from 0.10 to 0.35 μeq. of sulfhydryl per g. As mixing continued, a marked change in the rate of loss appeared in most cases beyond 2 minutes of mixing. Furthermore, it may be noted that the Pacific Northwest wheat flours (Idaed, Baart, Elgin) known typically to have poor mixing tolerance all showed low

TABLE II

SULFHYDRYL CONTENTS OF FLOURS AND DOUGH AFTER 2, 5, 10, AND 20 MINUTES OF MIXING IN AIR

FLOUR	–SH CONTENT MINUTES OF MIXING				
	0[a]	2	5	10	20
	μeq/g	μeq/g	μeq/g	μeq/g	μeq/g
Baart	0.96	0.61	0.60	0.56	0.54
Elgin	0.76	0.63	0.53	0.47	0.45
Idaed	0.82	0.63	0.60	0.55	0.48
Commercial	1.12	0.92	0.68	0.64	0.52
Mida	0.80	0.57	0.49	0.51	0.35
UN No. 2	0.82	0.71	0.67	0.59	0.51
UN No. 1	0.69	0.59	0.55	0.48	0.37
Lee	0.97	...	0.64	0.58	0.42
Wasatch	0.75	0.60	0.62	0.50	0.41
Cheyenne	0.98	0.69	0.62	0.60	0.37

[a] Whole flour.

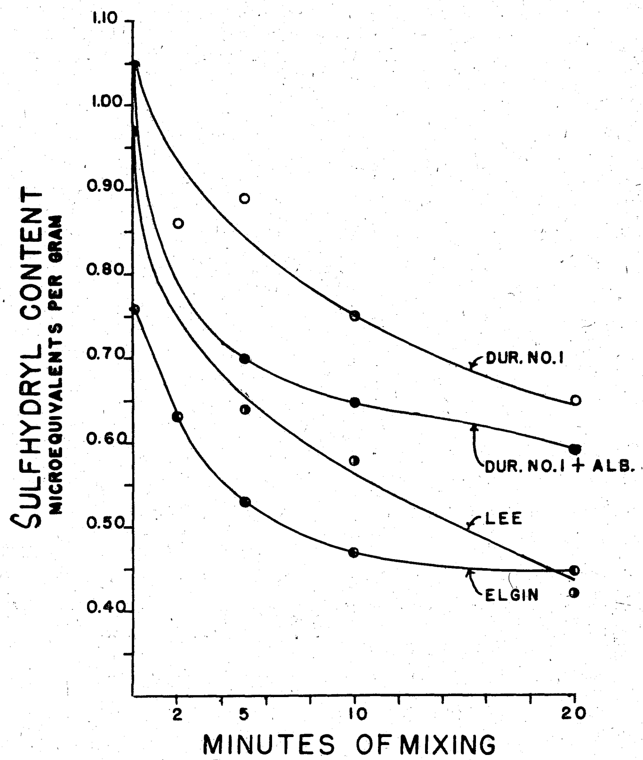

Fig. 1. Sulfhydryl contents of doughs after various mixing times.

rates of sulfhydryl loss beyond 5 minutes mixing.

Albumin Supplementation of Durum Doughs. Sulfhydryl contents of doughs mixed from two durum flours also were determined. The decreases in sulfhydryl content showed a less pronounced change of rate in the 2-to-5-minute range than occurred with other flours (Fig. 1). Pence *et al.* (7) have shown that durum flours characteristically contain less of the beta-albumin protein components than common or club wheats. An albumin preparation from a common wheat flour therefore was added to the durum flours (and to the Montana Cheyenne) to determine whether the rate of loss of sulfhydryl groups would be affected. The results are given in Table III, and those for Durum No. 1 are shown in Fig. 1. With all three flours, the albumin addition increased the rate of loss of sulfhydryl groups in the early stages of mixing; the change in the curve was then more abrupt and the decrease during the remaining mixing was slower than in the control.

TABLE III
SULFHYDRYL CONTENTS OF DURUM FLOURS, MONTANA CHEYENNE FLOUR, AND THEIR DOUGHS BEFORE AND FOLLOWING ALBUMIN SUPPLEMENTATION AFTER 2, 5, 10, AND 20 MINUTES OF MIXING IN AIR

FLOUR	–SH CONTENT MINUTES OF MIXING				
	0[a]	2	5	10	20
	μeq/g	*μeq/g*	*μeq/g*	*μeq/g*	*μeq/g*
Durum No. 1	1.05	0.86	0.89	0.75	0.65
Durum No. 1 + alb.	1.05	...	0.70	0.65	0.59
Durum No. 2	0.95	0.84	0.72	0.62	0.46
Durum No. 2 + alb.	0.95	...	0.65	0.58	0.55
Cheyenne	0.98	0.69	0.62	0.60	0.37
Cheyenne + alb.	0.98	...	0.51	0.39	0.35

[a] Whole flour.

Reaction Rate Constants. In order to provide a numerical comparison of the rates of sulfhydryl loss for possible correlation with mixing curve characteristics of the flours, reaction rate constants were determined for the 5-to-20-minute period of mixing. First-order kinetics were used. This approach appeared preferable to the calculation of percent loss of sulfhydryl groups after a specified time of mixing, because the latter value would be affected by the original sulfhydryl contents which varied by a factor of 1.6 (0.69 to 1.12 μeq. per g. flour).

Treatment of the rates of loss of sulfhydryl groups as first-order undoubtedly represents an oversimplification. Even in a logarithmic plot, the original sulfhydryl contents of the flours usually do not fall into line, and the 2-minute values do not in several cases. On the other hand, when only the 5, 10, and 20-minute values are plotted, a zero-order plot (rectangular coordinates) is nearly as satisfactory as a first-order plot. For purposes of comparison and correlation, however, first-order treatment seemed more nearly suitable, since a wider range of first-order rate constant values were obtained.

Examples of the first-order plots, chosen to show cases of relatively large and small deviations of experimental points from the calculated line, are shown in Fig. 2. Reaction rate constants for all the flours are given in Table IV. The k values are distributed throughout the range from 6.5 to 36.4 $\times 10^{-3}$ minutes $^{-1}$.

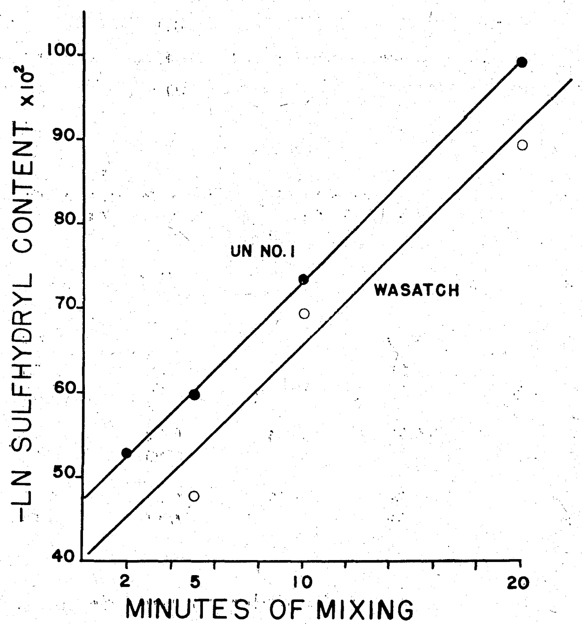

Fig. 2. Time of mixing vs. natural logarithm of the sulfhydryl content for UN No. 1 and Wasatch flours.

Correlation with Mixing Curve Characteristics. By examination of the rate constants, it was apparent that in general the soft wheat flours gave low values of k, while the hard red spring wheat flours gave markedly higher values. Farinograph curves for these flours showed in most cases the differences in mixing characteristics typical of the various classes of flour. When peak time, drop-off and stability were measured (1), the reaction rate constants seemed related most consistently to the stability values. The stability values are given in Table IV, together with the rate constants.

In general, as rate constants increase, stability values increase. The two durum and the two Montana hard red winter wheat flours are exceptions; these flours gave high rate constants but had poor stability

TABLE IV

DOUGH STABILITIES AND SULFHYDRYL-LOSS RATE CONSTANTS FOR VARIOUS FLOURS AFTER 20 MINUTES OF MIXING IN AIR

Flour	Stability Time	Rate Constant
	minutes	*minutes*$^{-1}$ $\times 10^3$
Durum No. 1	0.9	20.0
Durum No. 1 + alb.	1.1	11.2
Elgin	1.2	10.0
Baart	1.4	6.5
Wasatch	2.2	26.5
Cheyenne	2.5	36.4
Cheyenne + alb.	2.5	23.0
Durum No. 2 + alb.	4.0	10.3
Durum No. 2	4.9	29.8
Idaed	4.9	14.7
Commercial	8.0	18.3
Mida	9.7	24.6
UN No. 2	12.8	17.7
UN No. 1	13.9	25.3
Lee	13.3	28.7

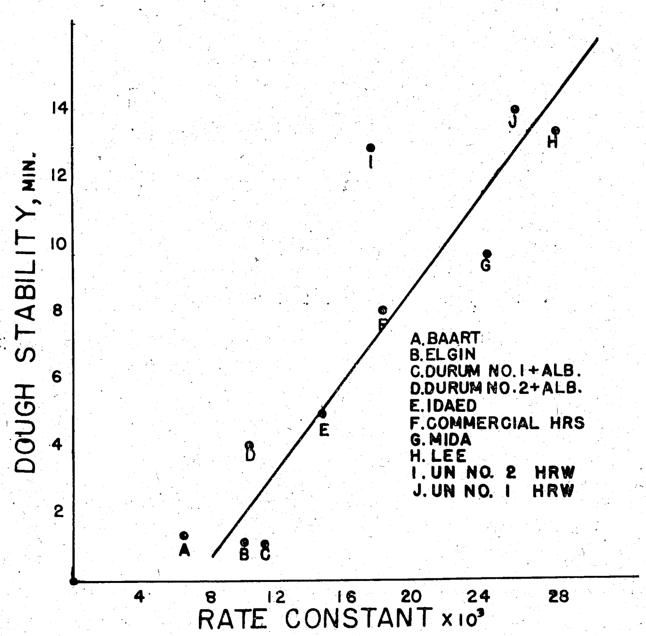

Fig. 3. Correlation of dough stabilities with apparent first-order rate constants.

to mixing in the farinograph. Supplementations of the durum doughs with the hard red spring albumin had little effect on their stability but brought the rate constants into line with the general trend. In fact, the correlation coefficient for rate constant vs. mixing stability is +0.92 ** if the supplemented durum values are used and the two Montana flours are excluded. This relationship is illustrated in Fig. 3. The very low stability value of the Cheyenne flour is not typical for this variety of wheat. Farinograph curves to illustrate the nature of the Cheyenne and Wasatch flours are shown in Fig. 4. No reason for their failure to fit into the rate constant vs. mixing stability relationship can be given, however.

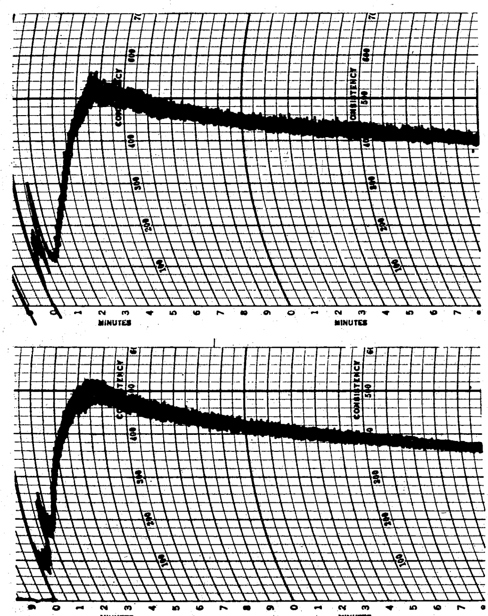

Fig. 4. Top curve, farinogram of Cheyenne hard red winter flour, 72.2% absorption at 14% moisture. Bottom curve, farinogram of Wasatch hard red winter flour, 70.4% absorption at 14% moisture.

Flour Suspensions. In previous work (10), the reactivity of the sulfhydryl groups of flour as determined in suspensions in urea solution covered a considerable range; some groups reacted fairly rapidly while others appeared to be inaccessible to sulfhydryl-blocking reagents. Iodoacetamide, for example, blocked fewer sulfhydryl groups than were lost during mixing of doughs in air in the present study. This suggested that the action of dough mixing renders sulfhydryl groups more accessible than does the stirring of a flour suspension without dough development. Possibly a related suggestion is that made by Bushuk and Hlynka (4), i. e., that the reaction of bromate in doughs is controlled by the physical process of mixing a dough.

Therefore the rate of loss of sulfhydryl groups in a flour-water (1:10, w/v) suspension was determined with the Elgin and Lee flours. Losses were much slower in suspensions than in doughs; the data are given in Table V. In view of the very low rate of loss beyond 10 minutes' mixing, it appears that sulfhydryl groups fail to become available for reaction rather than that the decreased rate results from lack of oxygen or from dilution of some other flour constituent reacting with sulfhydryl groups.

TABLE V

SULFHYDRYL CONTENTS AFTER 5, 10, AND 20 MINUTES OF MIXING AND RATE CONSTANTS OF SULFHYDRYL LOSS FOR FLOUR-WATER SUSPENSIONS

	–SH CONTENT MINUTES OF MIXING			RATE CONSTANT
	5	10	20	
	μeq/g	*μeq/g*	*μeq/g*	*minutes*$^{-1}$ $\times 10^{3}$
Elgin	0.66	0.62	0.62	3.5
Lee	0.84	0.77	0.72	9.8

General Discussion

The suggestion that sulfhydryl groups participate in some way in determining the mixing characteristics of flours was based on the effects of sulfhydryl-blocking reagents on dough properties (3). The observations presented in this paper support such a suggestion with a different kind of evidence. Doughs prepared from flours of varied types were found to differ with respect to the rate of loss of sulfhydryl groups during the course of mixing, demonstrating a natural variation among flours. The rate can be altered by addition of albumin components, suggesting one possible basis for the natural variation. Finally, a tendency for mixing stability and rate of loss of sulfhydryl content to vary together was observed with a limited number of flours, although the exceptions emphasize the need for more investigation. Nevertheless, some relationship between sulfhydryl groups and dough development and stability to mixing seems fairly certain.

It was suggested earlier (5) that the development and stable portions of dough mixing curves may represent the periods when sulfhydryl groups are present in quantities large enough to promote appreciable sulfhydryl-disulfide interchange; with adequate interchange the building-up of strains in the dough is reduced and its breakdown deferred.

The results in the present paper indicate that sulfhydryl groups are still present in reasonable amount (50 to 60% of the original flour sulfhydryl content) in those doughs of poor mixing stability even after they have been severely overmixed. Consequently it appears that the sulfhydryl groups must not only be present in the dough but that they must also be *accessible* for reaction. The flours of good mixing stability may illustrate this condition; they continue to decrease in sulfhydryl content over a long period of time. In fact, the observations on the flour-water suspensions together with those on doughs suggest that the loss (presumably by oxidation) of sulfhydryl groups *follows* their participation in the changes involved in dough formation and development. If these changes include sulfhydryl-disulfide interchange, the sulfhydryl groups produced by the interchange would appear to be more susceptible to oxidation than those originally present.

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