THE OXIDATION OF WHEAT FLOUR

IV. Labile and Nonlabile Sulfhydryl Groups[1]

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ABSTRACT

The measurement of sulfhydryl groups in flour and in dough is discussed.

A fraction of the –SH groups appears to be reacted in a water slurry in the absence of oxygen.

When a dough is mixed, there is a rapid initial loss of –SH groups, but about half the measurable amount remains unreacted after prolonged mixing in air, varying with the flour used. Maximum improvement in the rheological properties of a dough and the resultant bread occurs upon addition of iodate reagents equivalent to approximately half the measurable –SH groups and somewhat less with specific –SH reagents such as N-ethylmaleimide. An explanation is offered which rationalizes the improver action of a blocking or oxidizing agent, based on the current hypothesis of sulfhydryl-disulfide interchange.

There is general agreement that the rheological behavior of flour dough is dependent on the sulfhydryl-disulfide groups of the proteins or, more specifically, on the intermolecular disulfide linkages of the gluten and the exchange reactions of sulfhydryl-disulfide groups. Recent work by Goldstein (10), Mecham (18), Mecham *et al.* (19), Bloksma (2), Frater *et al.* (8,9), Hird and Yates (11,12), Matsumoto and Hlynka (16), Matsumoto and Shimoda (17), Bushuk (3), and Bushuk and Hlynka (4,5,6), and several papers from this laboratory (24,25,26,27,28) have contributed to this field. One theory is that improvers and specific –SH reagents inhibit the exchange reaction between RSH and RS–SR by oxidizing or blocking some thiol groups which otherwise would cause too great extensibility. In addition, oxidizing agents may react on RSH to form new disulfide bonds, thus strengthening the dough. Reducing agents split the interchain S–S bonds, causing softness and extensibility. With our limited knowledge of protein structure, it has been difficult to prove these hypotheses unequivocally.

Goldstein (10), Mecham (18), Mecham *et al.* (19), Frater *et al.* (8,9), Hird and Yates (11,12), Bushuk and Hlynka (4,5,6), Meredith and Bushuk (20), Sullivan (24,25), and Sullivan *et al.* (26,27,28) have studied the effects of certain –SH-blocking agents and improvers on the mixing and extensigraph behavior of dough. When –SH-blocking

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agents are added to flour, they shorten the mixing time and, if added in amounts sufficient to combine with all the –SH groups, eliminate the effect of maturing agents (6,10,27). As Goldstein (10), Frater *et al.* (8,9), and Hird and Yates (11,12) originally suggested, the action of critical levels of –SH-blocking agents and improvers would appear to strengthen the dough, mainly through the inhibition of sulfhydryl-disulfide interchange, so that the equilibrium is shifted toward the maintenance of the optimum number and type of cross-links.

The work now reported extends these observations and forms the basis of a possible mechanism that seems to fit the experimental data. Some of the experiments are a repetition of work already in the literature; this was necessary in order to make a comparison with the –SH determinations and baking data.

Materials and Methods

Two flours were employed — an untreated, 100% straight-grade flour of 0.44% ash and 11.7% protein (at 14.0% moisture), milled from a hard winter wheat mix, and an untreated patent flour of 0.39% ash and 12.3% protein (at 14% moisture), milled from a winter-spring wheat mix.

Determinations of Sulfhydryl of Flour

The total –SH content of the flour was determined by a modification of the amperometric method of Kolthoff, Stricks, and Morren (13), using mercuric chloride as a titrant. Dahle and Sullivan (7) pointed out the advantages of quenching the –SH groups with *p*-chloromercuribenzoate (PCMB). N-ethylmaleimide (NEMI) or mercuric chloride can be used in the same manner to protect the –SH groups. Evidence indicates that a measurement of the absolute total of –SH groups in flour, a difficult assay, is dependent on devices of quenching and protein degradation that may not be completely achieved in urea slurries. For this reason, it is preferable to speak of total measurable quenched –SH values rather than total –SH content.

Total Measurable Quenched Sulfhydryl of Flour. The following procedure was found to be the most satisfactory for the measurement of total measurable quenched sulfhydryl of flour.

Flour (3.0 g.) was mixed into a solution made up of 96 ml. of deaerated 8*M* urea and 4 ml. 0.001*M* mercuric chloride. A 50-ml. aliquot was added to 50 ml. of a deaerated 0.1*M* borax-0.2*M* potassium chloride solution and a known excess (2 ml.) of glutathione (GSH) added. The GSH solution had been made up to contain approximately 1 μmole per ml. by using 30.8 mg. per 100 ml. A standard curve must be ob-

tained for the GSH solution. Three drops of n-octanol were added to defoam the solution and the excess GSH titrated amperometrically with a rotating platinum electrode and 0.001*M* mercuric chloride, using a constant e.m.f. of –0.23 volts vs. S.C.E. The calculations were as follows:

$$\frac{\text{Total } \mu\text{moles } HgCl_2 \text{ in solution} - \mu\text{moles GSH added}}{1.5} = \mu\text{moles sulfhydryl per g. flour}$$

(GSH as read on the standard curve.)

Total Measurable Unquenched Sulfhydryl of Flour. This measurement differed from the previous one only by the absence of mercuric chloride as a quenching agent and by the use of 1.0*M* potassium chloride in place of 0.2*M* potassium chloride as recommended by Kolthoff *et al.* (13). Nitrogen protection was provided throughout the determination. The slurry was titrated directly by mercuric chloride.

Water-Soluble Unquenched Sulfhydryl of Flour. Flour (20 g.) was added to 100 ml. of thoroughly deaerated water and mixed 3 min. with constant stirring. The suspension was centrifuged 5 min. at 2,000 r.p.m. under nitrogen. A 50-ml. aliquot was transferred to a 250-ml. beaker containing 50 ml. of deaerated 0.1*M* borax-1*M* potassium chloride solution. A few drops of octanol were added and the solution titrated amperometrically with 0.001*M* mercuric chloride. One milliliter of mercuric chloride used equals 1 μmole of sulfhydryl per 10 g. of flour.

Water-Soluble Quenched Sulfhydryl of Flour. If no precipitation of water-solubles by mercuric chloride is assumed, the measurement differed from that of the unquenched water-soluble sulfhydryl by the presence of mercuric chloride as a quenching agent and the addition of a known excess of GSH prior to titration by mercuric chloride. Here 0.2*M* potassium chloride was employed as the supporting electrolyte.

Time Studies. For time studies on flour slurries, aliquots were removed at designated time intervals, quenched, and measured as indicated above. Urea was added to the water-flour slurries before measurement. Results are expressed as the amount of sulfhydryl reacted or as the increment change from the initial total measurable quenched –SH value.

Determination of Sulfhydryl in Dough

Reagents. 1. Titrant, 0.001*M* mercuric chloride.
2. Electrolyte solution, 0.1*M* borax-1.0*M* potassium chloride.
3. Glutathione, 2 μmoles per ml. (30.7 mg. per 50 ml.).

4. Mercuric chloride (0.0005*M*) -ethanol solution, 10 ml. 0.01*M* mercuric chloride diluted to 200 ml. with absolute ethanol.
5. Urea, 8*M*.

Procedure. A 1.5- to 2.5-g. portion of dough obtained from the mixer was placed on weighing paper, weighed, and transferred to a mortar containing 10 ml. of the mercuric chloride-ethanol solution. The paper was reweighed immediately and this value subtracted from the dough-plus-paper figure. Then the dough was carefully crushed in ethanol into fine flakes and 15 ml. of 8*M* urea added, the pestle being washed in the process. This mixture was stirred intermittently for 1 hr. with a glass rod, the rod was washed with a small amount of water, and the solution allowed to stand for 24 hr. ±1 hr. Speed is critical until after the urea is added. In the doughs measured from the mixer, a maximum time of 5 min. elapsed before the quenching reagent was added.

A mixture of 50 ml. of the electrolyte solution plus 40 ml. of water was deaerated; the sample was transferred to the electrolyte solution, with double distilled water used for washing. Three drops of n-octanol were added to defoam the solution which then was deaerated for 10 min. and 3 ml. of GSH solution (6 micromoles) added. The solution was titrated according to the procedure previously given for quenched sulfhydryl of flour.

Standard Curve. Ten milliliters of the mercuric chloride-ethanol solution were added to 100 ml. of a deaerated electrolyte solution made up of 50 ml. of water and 50 ml. of 0.1*M* borax-0.2*M* potassium chloride solution. This was deaerated for 3 min., 3 ml. of GSH were added, and the solution was titrated with 0.001*M* mercuric chloride. Five minutes were allowed for the mercuric chloride-GSH reaction.

Calculations:

$$\frac{\text{Dough end-point (ml.)} - \text{standard GSH end-point (ml.)}}{\text{wt. of dough} \times \text{flour ratio}^*} = \frac{\mu\text{moles sulfhydryl per g. flour at 14\% moisture}}{}$$

$$^*\text{Flour ratio} = \frac{\text{wt. of flour at 14\% moisture}}{\text{wt. of flour} + \text{water}^{**} + \text{other additives}}$$

**Absorption obtained from farinograph curve at 500 units.

The results can be expressed as sulfhydryl reacted or as increment change from –SH value of the flour obtained under conditions employed in dough measurement.

Farinograph and Extensigraph Measurements. Farinograms were made by the constant-dough (480-g.) method (1). Extensigrams were

obtained using 2% salt and absorption as measured by the farinograph for 500 B.U. consistency. All the absorption water, including the small amount needed to dissolve the –SH reagent, was added to the flour in a 300-g. mixing bowl; the dough was mixed 1 min., followed by a 5-min. rest, and then remixed 5 min., a total of 6 min. This was considered the optimum mixing time for the flour as judged by the farinogram. After the dough was taken from the farinograph bowl, three 150-g. lots were weighed, rounded twenty times, and moulded; one lot was stretched immediately and the other two were placed in the fermentation cabinet at 30°C. The duplicate doughs were given a relaxation period of either 60 or 180 min. and stretched. The control flour was measured similarly. The results were averaged.

Gassing Power. Gassing power was determined according to the usual AACC procedure (1).

Baking Procedures: Sponge Dough. Commercial 1-lb. loaves were made by the sponge-dough process. Ingredient percentages were: 64% absorption (over-all), 2.5% yeast, 3.0% shortening, 2.0% salt, 8.0% sugar, and 5.0% nonfat dry milk. The yeast, shortening, and 65% of the flour were used in the sponge, which was mixed 1 min. at first speed and 1.5 min. at second speed on a Hobart A-120 Mixer. The temperature of the sponge from the mixer was 78°F. and the sponge time was 4.25 hr. The sponge was divided equally into two parts at the dough stage, the balance of the flour (35%) and other ingredients added, and the two doughs mixed 6 and 8 min. The dough temperature was 80°F., with a floor time of 30 min. The doughs were scaled at 18 oz. and given a 15-min. intermediate proof before mechanical moulding; proofing time was 1 hr. at 105°F. The loaves were baked at 435°F. for 23 min.

Baking Procedures: Straight Dough. Commercial 1-lb. loaves were made by the straight-dough process. Ingredient percentages, based on flour as 100, were: 58% absorption, 2.0% salt, 2.0% yeast, 3.0% shortening, and 5.0% sugar. The doughs were mixed 7 min. in a Hobart A-120 Mixer; the temperature of the dough from the mixer was 80°F. Fermentation time was 1.5 hr. to the first punch and then 0.5 hr., after which the doughs were scaled at 18 oz. and rounded. An intermediate proof of 20 min. was given before moulding; proofing time was 1 hr. at 105°F. The loaves were baked for 30 min. at 435°F.

Results

Table I shows the loss of the unprotected –SH groups of the patent flour when it was slurried in an oxygen-free medium. Percentagewise, the loss is greatest in the water-soluble fraction. This and other data indicate that a true value of the –SH content of flour or its fractions

cannot be assessed without the expedient of quenching, and that interpretation from the unquenched values can be misleading.

TABLE I
LOSS IN SULFHYDRYL CONTENT WITHOUT QUENCHING

	–SH VALUES OF FLOUR		Loss
	Quenched	Unquenched	
	μmole/g. flour	*μmole/g. flour*	%
Total	1.00	0.64	36
Water-soluble	0.36	0.11	70
Remainder (by difference)	0.64	0.53	17

There is always a rapid initial loss of sulfhydryl on mixing a dough even for a few minutes, as many results in the literature already have shown (21,23,29). Dough does not completely disperse in 8*M* urea, and the slope of the titration curve is not sufficiently steep to obtain adequate duplication. This may be due to oxidation by air and/or reaction with endogenous material present in flour. When ethanol was added to the mercuric chloride solution used for quenching, the dough was more easily dispersed and the duplication improved to within 5% error.

When a dough is allowed to stand in 8*M* urea-mercuric chloride or 8*M* urea-ethanol-mercuric chloride, there is a slight increase of sulfhydryl (0.1 to 0.2 μmole) up to about 24 hr. when the values level off or decrease slightly. When allowance is made for a labile fraction of sulfhydryl lost very quickly on mixing and before quenching, the –SH values of the doughs calculated to flour used and of the flour, itself, are very close when the same medium and time are used for dispersion. Flour-water-salt doughs give slightly lower results than flour-water doughs.

Figure 1 shows the change in the –SH content of the straight-grade flour-water-salt doughs mixed for varying times. In one series (curve A), the sulfhydryl was measured in doughs taken directly from the farinograph mixer; in the other series (curve B), the doughs were mixed for the same time intervals and then given a 1-hr. rest period at constant temperature (30°C.) before measurement. The –SH content is expressed as μmoles of sulfhydryl reacted per g. of flour, the sulfhydryl found at each mixing time being subtracted from the quenched –SH content of the flour measured in the same medium (1.1 μmoles sulfhydryl per g.).

There is a rapid initial loss of sulfhydryl on mixing in air and then, during the interval from 5 to 10 min. (the range of optimum mixing

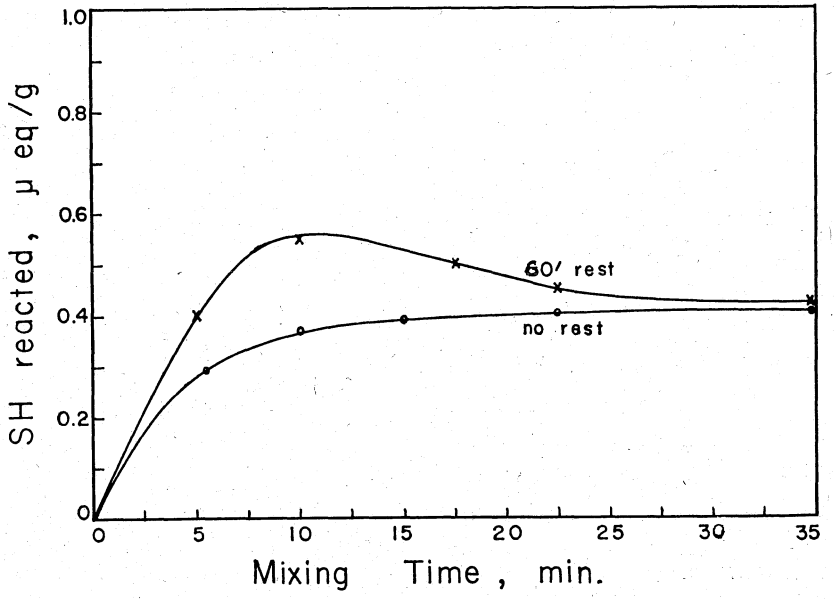

Fig. 1. Change in sulfhydryl content of doughs on mixing and resting.

time), a significant spread in –SH values between the doughs taken immediately from the mixer and those allowed a 60-min. rest. Then the –SH values of both "no-rest" and resting doughs become the same and remain quite constant on prolonged mixing, in spite of radical changes in dough properties.

Figure 2 illustrates some extensigraph data on the doughs from the straight-grade flour at varying mixing times. The H values measured on doughs taken immediately from the mixer showed figures off the graph at the 5-, 10-, and 15-min. mixing times and a linear decrease with the increase in mixing time. The H values on the resting (1 hr.) dough were the highest between 20 and 30 min. and then decreased. Area measurements on the 1-hr.-resting dough showed the highest figures at 10 min., coinciding with the maximum sulfhydryl reacted, followed by a progressive decrease in area on increased mixing, as would be expected.

Smith, Van Buren, and Andrews (22) found that the –SH values of patent flour doughs were small and changed very little from 2 to 30 min. of mixing. Second clear flour doughs had much higher –SH values and showed greater changes during dough rest. There appeared to be no relationship either between changes in sulfhydryl or between final –SH values and change in extensigrams. These authors also remarked on the extreme lability of the –SH groups. Sokol, Mecham,

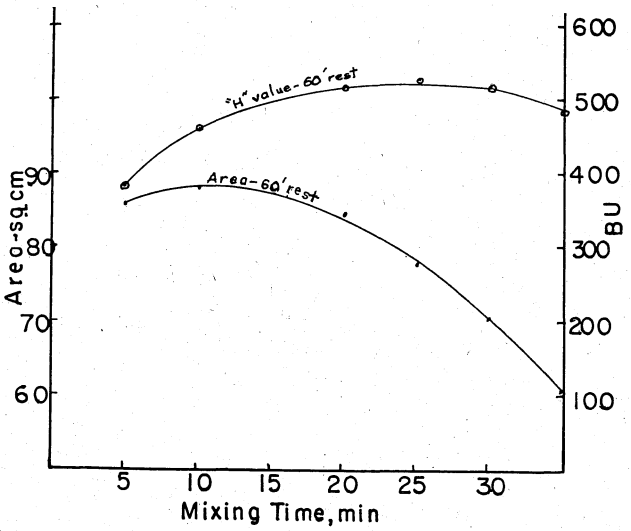

Fig. 2. Change in extensigram values with increasing mixing time on doughs with 1-hr. rest.

and Pence (23) studied –SH losses during the mixing of doughs from twelve different flours. They observed –SH losses from 38 to 64% after 20 min. of mixing and also found a rapid decrease in –SH content during the 2 to 5-min. mixing times. With continued mixing, relatively large differences in the rate of decrease occurred. The rate constant for the 5 to 20-min. interval ranged from 6 to 36 min. for flour of varied types. Stability values on the farinogram correlated well with the –SH loss rate constant for all flours except the durums and two hard red winter wheat flours. All except one or two of the flours showed a continuous loss of sulfhydryl with mixing. In a recent paper by Tsen and Hlynka (29), the original and defatted flour doughs were mixed in air and oxygen for 2.5, 5, 10, and 20 min. and the –SH content measured. The original flour dough lost sulfhydryl more rapidly than the defatted flour dough because, according to these authors, –SH groups of the original flour are oxidized by two reactions: direct reaction with oxygen, and by lipid peroxides formed during mixing. In these experiments too, no leveling-off of the –SH values was found on extended mixing. In our laboratory, experiments on several Bakers' Patent and straight-grade flours have shown a leveling-off of –SH values after 10 to 15 min. of mixing in air, as illustrated in Fig. 1.

Effect of NEMI and Iodate on –SH Measurements and Dough Properties. The patent flour was employed for further –SH determinations and extensigraph and baking tests, using different levels of NEMI

and iodate. The patent flour measured from 1.0 to 1.1 μeq. sulfhydryl per g. in replicate tests.

The –SH values of doughs at various levels of NEMI and iodate are illustrated in Figs. 3 and 4.

Extensigraph data for NEMI and iodate are given in Table II. Baking data are shown in Tables III-A and III-B.

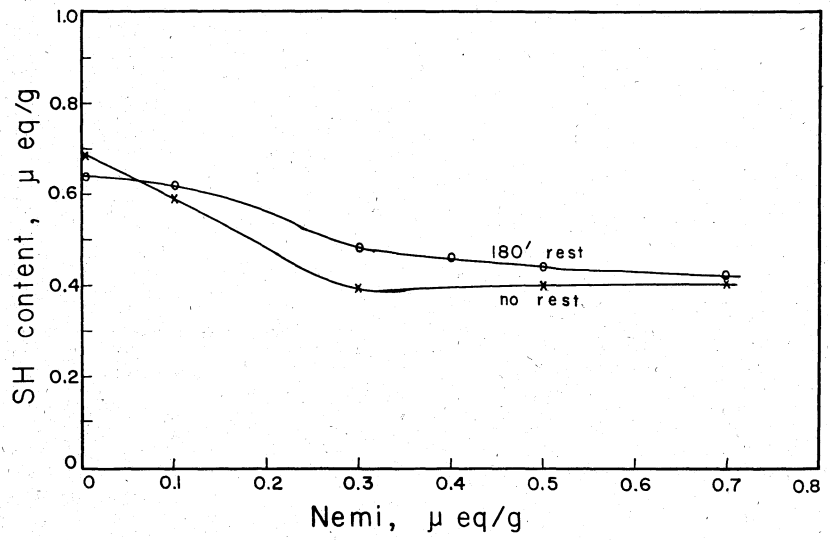

Fig. 3. Effect of NEMI on –SH content of dough.

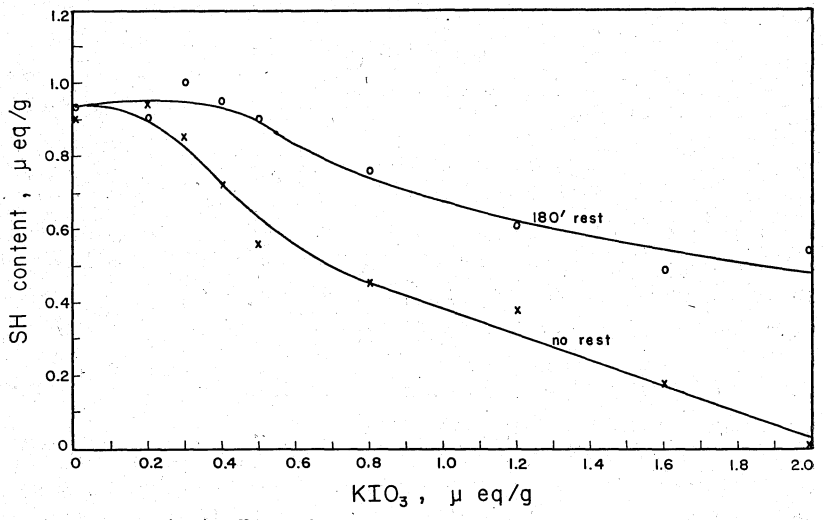

Fig. 4. Effect of iodate on the –SH content of dough.

TABLE II

EFFECT OF N-ETHYLMALEIMIDE AND IODATE ON EXTENSIGRAMS OF PATENT FLOUR

Additive	No Rest		180-Minute Rest	
	E	H	E	H
μeq./g. flour				
Untreated control	78	1,000++	195	280
0.1 N-ethylmaleimide	68	1,000++	192	290
0.3	74	940	180	330
0.4	72	770	160	340
0.5	78	600	165	300
0.7	93	330	170	210
0.2 Iodate	68	1,000++	195	360
0.3	67	1,000++	190	375
0.4	62	1,000++	180	410
0.5	61	1,000++	185	475
0.7	65	1,000++	140	710
1.0	65	1,000++	83	880

TABLE III-A

EFFECT OF N-ETHYLMALEIMIDE ON BAKING PROPERTIES

N-Ethylmaleimide	Mixing Time	Dough Quality	Volume[a]	Grain
μeq./g. flour	*min.*			
	6	Good	100 (2,350 cc.)	96
Untreated control	8	Good	100 (2,425 cc.)	96
	6	Very good	101	96
0.1	8	Very good	94	95
	6	Very good	100	96
0.2	8	Slightly soft	94	95
	6	Slightly sticky	93	95
0.3	8	Slightly sticky	88	93
	6	Slightly sticky	85	93
0.4	8	Slightly sticky	81	93

[a] Volumes compared with control on each mixing time.

NEMI. There is a rapid loss of sulfhydryl on the addition of increments of NEMI to a level of 0.3 μeq. With further additions of NEMI, the –SH values of the "no-rest" doughs remained quite constant at about 0.4 μeq. of the –SH content. The doughs with 3 hr. rest showed very little variation in –SH values compared to the doughs taken immediately from the mixer, unlike the control or the iodated doughs.

Dough properties as revealed by areas of the extensigrams were optimum at about 0.3 to 0.4 μeq. NEMI.

Baking data, as shown in Table III-A, indicates that the best dough properties and baking results were achieved at 0.2 μeq. NEMI and the shorter mixing time. The optimum level of NEMI as shown by the

extensigrams was too much for optimum baking results, as reflected in stickier doughs and poorer volume. NEMI did not markedly affect the gassing power of the doughs until a level of 0.4 μeq. was employed. In the sixth hour, gassing power of the control was 450 mm. mercury; with 0.2 to 0.3 μeq. NEMI, 440 mm.; and with 0.4 μeq., 410 mm. At over 0.5 μeq., the gassing power dropped to nearly one-half, as has been shown by Lee and Reynolds (14).

Iodate. There is the usual rapid initial loss of sulfhydryl, as has been frequently observed, then a continuous loss of sulfhydryl with increasing iodate concentration. Doughs with 3 hr. rest showed parallel, but higher, –SH values. This behavior is quite different from the NEMI doughs where values for the doughs taken directly from the mixer and for the resting doughs were more closely alike and corresponded to approximately half the total measurable –SH groups, probably the labile fraction. The other, nonlabile, half appeared to be oxidized immediately by high levels of iodate, but apparently was restored on rest, presumably as a result of some reductive mechanism. This corresponds to the apparently unreacted gluten sulfhydryl observed by Lee and Samuels (15) when they employed high levels of iodate as an oxidant.

Extensigrams, as shown in Table II, gave best results at a level of approximately 0.5 μeq. iodate.

The best performance in baking tests also occurred at a level of about 0.4 to 0.5 μeq. iodate, as reflected in machining characteristics,

TABLE III-B

EFFECT OF IODATE ON BAKING PROPERTIES

IODATE	MIXING TIME	DOUGH QUALITY	VOLUME[a]	GRAIN
μeq./g. flour	*min.*			
	6	Good	100 (2,350 cc.)	96
Untreated control	8	Good	100 (2,425 cc.)	96
	6	Good	101	98
0.1	8	Good	95	98
	6	Very good	109	100
0.2	8	Very good	100	99
	6	Excellent	113	100
0.3	8	Excellent	105	99
	6	Excellent	110	100
0.4	8	Excellent	105	100
	6	Excellent	110	100
0.5	8	Excellent	107	100
	6	Soft, sticky	107	98
1.0	8	Very soft	103	95

[a] Volumes compared with control on each mixing time.

volume, and grain. The doughs exhibited much more tolerance to iodate than to blocking agents. At 1.0 μeq. iodate, the dough became soft and sticky. Although the volume was satisfactory, the grain was poor.

Effect on Baking Properties of –SH Groups of Bovine Serum Albumin. In an effort to simulate more closely the effect of the –SH groups of the water-soluble proteins, some baking experiments with bovine serum albumin (BSA) were conducted. The sample of BSA used contained 9.5 μmoles sulfhydryl per g. Two levels of BSA (1.6 and 3.2%) were added to untreated patent flour, equivalent to an addition of 0.16 and 0.32 μmole of sulfhydryl per g. of flour, respectively. Table IV illustrates results of straight doughs made from the control, the control plus the lower level of BSA, and both flours with iodate treatment optimum for the control. The higher level of BSA was used as is and with the sulfhydryl of the additive exactly blocked with NEMI by prior reaction.

TABLE IV

EFFECT OF BOVINE SERUM ALBUMIN ON BAKING PROPERTIES[a]

ADDITIVE	VOLUME	GRAIN
Control	100	100
BSA, 1.6%	97	99+
Iodate, 0.5 μeq.	106	101
BSA, 1.6% + 0.5 μeq. iodate	103	101
BSA, 3.2%	96	97
BSA, 3.2% + 96 μeq. NEMI	101	101

[a] The patent flour was used with additions of 1.6 and 3.2% BSA, the BSA providing 0.16 and 0.32 micromoles –SH per g. flour. NEMI was added to the BSA in solution in amount to exactly block the –SH of the BSA.

Results were as expected for the levels of sulfhydryl provided by the amount of BSA used. The bread with the BSA showed a younger exterior, rounder cell structure, and lower volume. Iodate improved both the control and the BSA loaf, the latter showing a lower volume. Experiments with the higher level of BSA showed the usual effect of greenness. Blocking the –SH groups of the BSA with NEMI showed the expected improvement – giving, in fact, a better loaf than the control in both volume and grain.

Discussion

Lee and Samuels (15) observed 10 to 15% loss of gluten sulfhydryl in a dough after a 3-hr. reaction time in the presence of potassium bromate at a level corresponding to the stoichiometric equivalent of 1.8 μmoles sulfhydryl per g. flour. Under the same conditions, 40 to 80% loss was observed in the presence of potassium iodate correspond-

ing to 1.4 μmoles sulfhydryl. It is considered that these levels of iodate and bromate are in excess of the total –SH content of flour; yet only a partial loss of gluten sulfhydryl was observed in a 3-hr. reaction time. This is further confirmation that gluten sulfhydryl has a comparatively low reactivity to the typical improver agents.

Measurement of the –SH content of doughs mixed in air for different times shows a rapid initial loss of sulfhydryl and then a relatively constant level of, roughly, 50% of the initial quenched value, even though the extensigraph and baking data show wide variations in dough quality, from typical underdevelopment to marked overdevelopment as produced by prolonged mixing to 40 min. It would seem that the –SH groups in the more reactive form (which, presumably, would be the more soluble –SH groups of the albumins and globulins) are rapidly oxidized. The more soluble –SH groups amount to, roughly, half of the total –SH content.

The fact that, after the initial loss, the –SH content of a dough remains fairly constant on prolonged mixing would seem to indicate that the remaining –SH groups are not reactive under conditions of mixing or that new –SH groups are being formed as rapidly as the others are oxidized, the former explanation being more plausible. The short, sticky dough obtained on long mixing may be due to ruptured S–S bonds without formation of –SH groups.

The optimum level of NEMI and iodate, as judged by both the extensigraph and baking tests, is shown when about half, roughly, of the measurable –SH groups of flour are reacted. Baking tests with iodate show an optimum amount at about half the sulfhydryl, whereas optimum shown by NEMI is somewhat less. NEMI is highly specific, reacting rapidly and irreversibly with the –SH groups, and, when it is used on a mole-to-mole basis with sulfhydryl, its action must be interpreted as practically complete removal of free sulfhydryl from the field of action. Since a blocking agent acts only partially as an improver, mainly through irreversible combination with the most labile sulfhydryl, its optimum reaction cannot be compared directly with oxidant improvers where the S–S groups formed can still exchange. Since the –SH content of flour appears to be distributed about equally between the labile and the nonlabile forms (although this varies with the particular flour), it seems highly probable that the more labile, presumably water- and salt-soluble, –SH groups are preferentially reacted. Glutens were washed from the patent flour alone and with the addition of 0.5, 1.0, and 10.0 μmoles of NEMI. While the glutens with NEMI were somewhat more difficult to recover than the

control, gluten of good quality, stronger and tougher than the control, was obtained on working. At the 10-μmole level, the gluten was more difficult to recover and, on working, was extremely extensible. It can be fairly assumed that the NEMI acted on the sulfhydryl of the soluble protein, since the latter was removed by washing and, hence, did not harm the gluten quality. The pronounced effect of NEMI on mixing curves would indicate that the soluble protein is most important in determining a farinogram pattern.

Glutathione, cysteine, and other –SH compounds have a marked weakening effect on dough. It is believed that this effect is due to an interchange of protein disulfide and, for instance, glutathione:

$$GSH + PrSSPr \rightleftharpoons PrSSG + PrSH$$

Since flour contains low-molecular-weight proteins with –SH groups, their effect is considered quite similar.

The weakening effect observed on the addition of bovine serum albumin to flour, as evidenced by the baking data, can be explained by the above. Accordingly, a hypothesis is presented which, if validated by further experiments, offers a rational explanation of the action of blocking agents and improvers. It is postulated that, since a partial removal of the free –SH groups strengthens the dough matrix, a dynamic equilibrium exists between the –SH groups of the nongluten proteins and the –SH and S–S groups of the gluten proteins, as follows:

$$Pr^1SH + Pr^2S\text{–}SPr^2 \rightleftharpoons Pr^2SH + Pr^1SSPr^2$$

contributes more to dough strength — contributes less to dough strength

Pr^1 = nongluten protein;
Pr^2 = gluten protein.

The above reaction would satisfy observations on the –SH and S–S interchange, if such exists, and explain the changes in the rheological properties of the dough. The Pr^1SH may be preferentially reacted because of greater accessibility or solubility, thus shifting equilibrium to the left. Removal of the free sulfhydryl of the nongluten proteins of flour would lessen the weakening effect of sulfhydryl on the disulfide bonds of the gluten. If one assumes that Pr^2SH is less reactive and mobile than nongluten sulfhydryl and that Pr^1SSPr^2 does not contribute as much to the strength and toughness of the dough as does

Pr^2SSPr^2, this reversible reaction would occur during mixing and fermentation, varying with time, temperature, manipulation of the dough (mixing, moulding), and the amount of blocking agent or improver. With oxidizing improvers such as iodate, bromate, and water-soluble peroxides, some of the nongluten –SH groups could be removed also by the formation of disulfide linkages of the albumins. At higher levels of iodate, some of the –SH groups could be eliminated by oxidation and the formation of sulfinic, sulfenic, and sulfonic acids. The optimum amount of improver is considered that which provides the proper balance of free sulfhydryl and gluten S–S. It would appear that an excess of the labile –SH groups of the nongluten proteins is the most damaging to the protein matrix of the dough, and that proper maturing requires the elimination of most of these groups. Some –SH groups are necessary to maintain the integrity of dough structures. The remaining –SH groups of the gluten are sufficient for the interchange reactions that provide the precise disulfide bridges necessary for optimum rheological properties and stabilization of the structure of the gluten. The configuration of the gluten proteins always will tend toward a random state of lowest free energy.

The similarity of improving action observed with critical amounts of iodoacetamide, PCMB, and NEMI, as well as with oxidizing agents such as iodate, bromate, and persulfate, has been demonstrated in several laboratories and suggests that both blocking and oxidizing agents may produce beneficial effects by removing most of the water-soluble –SH groups from the –SH–S–S equilibrium existing in flour, by the mechanisms outlined. The fact that a fairly constant amount of sulfhydryl remains on prolonged mixing or after treatment with high levels of NEMI shows that certain –SH groups are unreactive and total sulfhydryl may remain constant even though dough quality becomes worse.

The foregoing discussion has been based on interpretation of indirect evidence. So far it has been difficult to design experiments that give unequivocal proof of the oxidation mechanism. Perhaps the further use of –S-labeled cysteine or albumins would give more direct evidence. As more is learned about protein structure, a clearer picture will emerge.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.), p. 137. The Association: St. Paul, Minnesota (1957).
2. Bloksma, A. H. Die Bedeutung von Thiol und Disulfid-gruppen im Kleber für die Backfähigkeit. Getreide Mehl **9**: 65–69 (1958).

3. BUSHUK, W. Accessible sulfhydryl groups in dough. Cereal Chem. **38:** 438–448 (1961).
4. BUSHUK, W., and HLYNKA, I. The bromate reaction in dough. IV. Effect of reducing agents. Cereal Chem. **38:** 309–316 (1961).
5. BUSHUK, W., and HLYNKA, I. The bromate reaction in dough. V. Effect of flour components and some related compounds. Cereal Chem. **38:** 316–325 (1961).
6. BUSHUK, W., and HLYNKA, I. The effect of iodate and N-ethylmaleimide on extensigraph properties of dough. Cereal Chem. **39:** 189–195 (1962).
7. DAHLE, L., and SULLIVAN, BETTY. Presence and probable role of thioctic acid in wheat flour. Cereal Chem. **37:** 679–682 (1960).
8. FRATER, R., HIRD, F. J. R., and Moss, H. J. Role of disulphide exchange reactions in the relaxation of strains introduced in dough. J. Sci. Food Agr. **12:** 269–273 (1961).
9. FRATER, R., HIRD, F. J. R., Moss, H. J., and YATES, J. R. A role for thiol and disulphide groups in determining the rheological properties of dough made from wheaten flour. Nature **186:** 451–454 (1960).
10. GOLDSTEIN, S. Sulfhydryl und Disulfidgruppen der Klebereiweisse und ihre Beziehung zur Backfähigkeit der Brotmehle. Mitt. Gebiete Lebensm. Hyg. Bern **48:** 87–93 (1957).
11. HIRD, F. J. R., and YATES, J. R. Oxidation of cysteine, glutathione and thioglycollate by iodate, bromate, persulphate and air. J. Sci. Food Agr. **12:** 89–95 (1961).
12. HIRD, F. J. R., and YATES, J. R. The oxidation of protein thiol groups by iodate, bromate, and persulphate. Biochem. J. **80:** 612–615 (1961).
13. KOLTHOFF, I. M., STRICKS, W., and MORREN, L. Amperometric mercurimetric titration of sulfhydryl groups in biologically important substances. Anal. Chem. **26:** 366–372 (1954).
14. LEE, C. C., and REYNOLDS, L. M. Some studies with maleimide-type sulfhydryl-blocking agents. Cereal Chem. **39:** 427–436 (1962).
15. LEE, C. C., and SAMUELS, E. R. Studies with radioactive tracers. VI. The oxidation of sulfhydryl groups of gluten by bromate and iodate. Cereal Chem. **39:** 482–484 (1962).
16. MATSUMOTO, H., and HLYNKA, I. Some aspects of the sulfhydryl-disulfide system in flour and dough. Cereal Chem. **36:** 513–521 (1959).
17. MATSUMOTO, H., and SHIMODA, M. Studies on breadmaking. XV. On the mechanism of dough softening caused by thioglycollic acid. J. Ferm. Technol. (Japan) **34:** 410–414 (1956).
18. MECHAM, D. K. Effects of sulfhydryl-blocking reagents on the mixing characteristics of doughs. Cereal Chem. **36:** 134–145 (1959).
19. MECHAM, D. K., SOKOL, H. A., and PENCE, J. W. The dough-mixing behavior of gluten and other flour fractions treated with N-ethylmaleimide. Cereal Chem. **37:** 129–141 (1960).
20. MEREDITH, P., and BUSHUK, W. The effects of iodate, N-ethylmaleimide, and oxygen on the mixing tolerance of doughs. Cereal Chem. **39:** 411–426 (1962).
21. SMITH, D. E., and ANDREWS, J. S. The uptake of oxygen by flour dough. Cereal Chem. **34:** 323–336 (1957).
22. SMITH, D. E., VAN BUREN, J. P., and ANDREWS, J. S. Some effects of oxygen and fat upon the physical and chemical properties of flour doughs. Cereal Chem. **34:** 337–349 (1957).
23. SOKOL, H. A., MECHAM, D. K., and PENCE, J. W. Sulfhydryl losses during mixing of doughs: Comparison of flours having various mixing characteristics. Cereal Chem. **37:** 739–748 (1960).
24. SULLIVAN, BETTY. The mechanism of the oxidation and reduction of flour. Cereal Chem. (6). Suppl: 16–31 (1948).
25. SULLIVAN, BETTY. Review of physical characteristics of gluten and reactive groups involved in change in oxidation. J. Agr. Food Chem. **2:** 1231–1234 (1954).
26. SULLIVAN, BETTY, DAHLE, L., and LARSON, E. The oxidation of wheat flour. I. measurement of sulfhydryl groups. Cereal Chem. **38:** 272–280 (1961).
27. SULLIVAN, BETTY, DAHLE, L., and NELSON, O. R. The oxidation of wheat flour. II. Effect of sulfhydryl-blocking agents. Cereal Chem. **38:** 281–291 (1961).

28. SULLIVAN, BETTY, HOWE, MARJORIE, SCHMALZ, F. D., and ASTLEFORD, G. R. The action of oxidizing and reducing agents on flour. Cereal Chem. **17:** 507–524 (1940).
29. TSEN, C. C., and HLYNKA, I. Flour lipids and oxidation of sulfhydryl groups in dough. Cereal Chem. **40:** 145–153 (1963).