

CHANGES IN SULFHYDRYL AND DISULFIDE CONTENTS OF DOUGHS DURING MIXING UNDER VARIOUS CONDITIONS¹

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ABSTRACT

Changes in sulfhydryl (-SH), disulfide (S-S), and iodate and bromate where applicable, were measured simultaneously in doughs mixed for various lengths of time in nitrogen, in air, and in oxygen. Untreated doughs showed a definite loss of -SH and a slight loss of S-S during mixing in air or oxygen. Similar doughs mixed in nitrogen showed a small increase in -SH and no significant changes in S-S. In iodate-treated doughs mixed in nitrogen, -SH losses paralleled iodate losses, giving -SH/iodate molar ratios of about 6 for low iodate concentrations and 4 for iodate concentrations well in excess of -SH content of the flour. Losses of -SH and iodate (or bromate) are greater in continuously mixed doughs than in resting doughs. Bromate was found to be a slow -SH oxidizing agent for dough, as demonstrated that during mixing bromate oxidized -SH groups of dough more slowly than air. Doughs which show a marked decrease in resistance to extension as measured with the extensigraph (high iodate treatment and prolonged mixing in air or oxygen) show a definite loss of S-S as well as the expected loss of -SH. Accordingly, it is suggested that dough breakdown under certain conditions may involve oxidative cleavage of S-S bonds.

It is reasonably well established that sulfhydryl (-SH) groups of flour proteins are involved in the reactions with various improvers (3-9,11,12,15,17). In addition, the most recent mechanism postulated to explain the improver effect, that based on disulfide interchange reactions (7), also implicates the disulfide (S-S) groups. There have been a large number of studies of the changes in -SH groups produced by various chemical and physical treatments of doughs (2,4,14,15,16, and references therein). Analogous studies of the changes in S-S groups were nonexistent until the recent report of Axford, Campbell, and Elton (1) that under certain conditions of dough development by mixing, S-S bonds can undergo scission.

With the development of an accurate method for the determination of S-S groups in flour (18), it became possible to study the simultaneous changes in -SH, S-S, and iodate (or bromate) with essentially the same degree of accuracy. The changes in the first two chemical entities were measured in doughs mixed for various lengths of time in nitrogen, air, and oxygen and in all three entities in doughs similar-

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ly mixed but initially treated with known amounts of iodate (or bromate). The results are reported in this paper.

Materials and Methods

This study was made on an untreated straight-grade flour milled commercially from hard red spring wheat. The protein ($N \times 5.7$) and ash contents of the flour were 13.2 and 0.43% (on 14% m.b.). The -SH and S-S contents of the flour were 1.11 and 16.05 μ eq. per g. of flour, respectively. All chemicals used were reagent grade.

Preparation of Doughs. All doughs were mixed in the GRL mixer (10). These doughs were mixed to 55% absorption to give extensigrams of reasonable height, since the extensigraph was used in one experiment to give a measure of the physical state of the dough. 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 when doughs were mixed in these gases. All doughs contained 1% salt. The atmosphere and the mixing times used will be indicated in the section Results and Discussion.

Analytical Methods. Appropriate subsamples of dough were processed for extensigrams and for determination of residual iodate or bromate (where applicable) by amperometric titrations (3). Another portion of each dough (approx. 20 g.) was frozen in liquid nitrogen, freeze-dried, ground on a micro Wiley mill (60-mesh screen), and stored at -40°C . for subsequent analyses of -SH and S-S contents. -SH contents in flours or doughs were determined according to the modified method of Sokol, Mecham, and Pence (13) as described previously (17). The method for the determination of S-S bonds was also described previously (18). Unless stated otherwise, iodate (or bromate), -SH, and S-S are all expressed in μ eq. per g. of flour (dry basis).

Extensigrams. Doughs used for extensigrams were given a 10-min. reaction time (mix to shape) and a 20-min. rest period (shape to stretch). It was found that a rest period of 20 min. was sufficient time to eliminate the effect of work-hardening; accordingly, this time was adopted instead of the 45-min. rest period used in normal practice. During the reaction time and rest period, the dough was kept in a cabinet maintained at 30°C . and 95% r.h.

Results and Discussion

Effect of Mixing in Oxygen, Air, or Nitrogen. Table I summarizes data for doughs mixed in oxygen, air, or nitrogen in the GRL mixer

at normal speed (68 r.p.m.). It shows progressive decreases in -SH contents of doughs during continuous mixing in oxygen or air; in contrast, -SH contents of doughs mixed in nitrogen show a small but definite increase. These results confirm observations obtained previously in this (4,17) and other laboratories (15).

TABLE I
-SH AND S-S CONTENTS OF DOUGHS MIXED IN OXYGEN, AIR, OR NITROGEN FOR VARIOUS PERIODS AT TWO MIXER SPEEDS, 68 AND 160 R.P.M.

MIXING TIME	SPEED — 68 R.P.M.		SPEED — 160 R.P.M.	
	-SH	S-S	-SH	S-S
min.	$\mu\text{eq./g.}$	$\mu\text{eq./g.}$	$\mu\text{eq./g.}$	$\mu\text{eq./g.}$
Oxygen				
5	0.65	15.80
10	0.55	15.86	0.45	15.90
20	0.47	15.54	0.34	15.72
40	0.40	15.56	0.34	15.68
Air				
5	0.73	15.65
10	0.60	15.79	0.45	15.95
20	0.50	15.84	0.34	15.51
40	0.47	15.52	0.34	15.63
Nitrogen				
5	0.90	15.50
10	0.91	15.63	0.98	15.84
20	0.96	15.86	1.03	15.74
40	1.01	15.80	1.05	15.72

Table I also presents analogous data for doughs mixed in the same mixer at much higher speed (160 r.p.m.). It shows that -SH contents of doughs mixed at the higher speed decrease faster than those mixed in oxygen or air at the normal speed. Also, at the higher mixing speed, the sulfhydryl contents of doughs mixed in air do not differ from those of doughs mixed in oxygen. Of the total -SH content in dough, only 69.4% -SH content is accessible to molecular oxygen. The remaining -SH groups appear to resist oxidation even with the higher-speed mixing for 40 min., which should minimize any physical barrier existing between -SH groups and oxygen.

The changes of S-S content in doughs subjected to various mixing conditions are not so significant (see Table I). A comparison of S-S contents between the original flour and doughs indicates decreases in S-S content by mixing; the decreases are slight and the values fluctuate to a certain extent. Nevertheless the results for doughs mixed in air or

oxygen at both speeds show a trend that the number of S-S groups decreases as mixing increases. In doughs mixed continuously in nitrogen, there is no decrease in the S-S contents (actually a slight increase) at the normal speed, but a slight decrease occurs at the higher speed of mixing.

Recently Axford, Campbell, and Elton (1) found that intense mechanical development of flour-water doughs gives a reduction in the total number of -SH and S-S (and other reducible sulfur-containing) groups. They reasoned that the scission of S-S bonds to produce thiyl radicals could take place in dough during the mechanical development. Although it is extremely difficult to determine whether or not thiyl radicals are produced in a complex system like dough, results presented above do not seem to support their claim. As shown in Table I, the total S-S content does not decrease when mixings in nitrogen are extended from 5 to 40 min. at slow speed; and only insignificant decreases, within the experimental error of the method used, are found for doughs mixed at the higher speed for 10 to 40 min. Thus it seems that the S-S bonds do not undergo the scission process during the mixings of the dough under experimental conditions used in this study.

Effect of Iodate in Doughs Mixed in Nitrogen and Oxygen. Results, comprising iodate and -SH losses in doughs treated with increasing concentrations of iodate and mixed for 5 min. in nitrogen or oxygen, are shown in Table II. Increasing amounts of iodate added cause progressive decreases in sulfhydryl content. At the low concentrations of iodate, lesser amounts of iodate are consumed but more -SH groups are lost when the mixings are done in oxygen instead of nitrogen. However, this effect of oxygen gradually decreases as doughs are treated with increasing amounts of iodate. No significant change in the S-S contents was observed for doughs treated with increasing amounts of iodate.

If the losses of -SH and iodate are expressed in μ moles per g. of flour or dough (both on a dry basis), the stoichiometric relation between moles of -SH groups oxidized and iodate consumed can be demonstrated. The molar ratios for the doughs mixed in nitrogen start at 7.0 and gradually approach 3.5 as the iodate concentration is increased well in excess of the -SH content. The ratios for the doughs mixed in oxygen start at 9.9 but seem to approach the value of 3.6 as the iodate concentration is increased. These values for oxygen doughs suggest that at low iodate concentrations oxygen competitively oxidizes a large number of -SH groups; the contribution of oxygen,

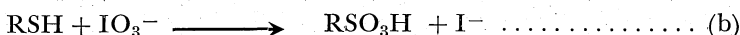
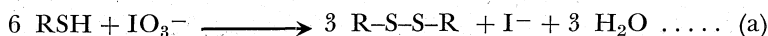
TABLE II

LOSS OF IODATE AND -SH DURING 5 MINUTES OF MIXING IN NITROGEN AND IN OXYGEN, OF DOUGHS TREATED WITH INCREASING AMOUNTS OF IODATE

ATMOS- PHERE	IODATE			-SH		MOLAR RATIO OF -SH Loss
	Added	Loss		Residual	Loss	Iodate Loss
	$\mu\text{eq./g.}$	$\mu\text{eq./g.}$	$\mu\text{moles/g.}$	$\mu\text{eq./g.}$	$\mu\text{eq./g.}$	
Nitrogen	0.29	0.29	0.0483	0.77	0.34	7.0
	0.58	0.58	0.097	0.60	0.51	5.9
	1.16	1.05	0.175	0.47	0.64	3.7
	2.32	1.32	0.220	0.33	0.78	3.5
	4.65	1.53	0.255	0.22	0.89	3.5
Oxygen	0.29	0.29	0.0483	0.63	0.48	9.9
	0.58	0.58	0.097	0.52	0.59	6.1
	1.16	0.94	0.157	0.41	0.70	4.5
	2.32	1.20	0.200	0.30	0.81	4.0
	4.65	1.52	0.253	0.20	0.91	3.6

inssofar as the oxidation of -SH groups is concerned, decreases and eventually disappears as the amount of iodate added increases.

The oxidation of sulfhydryl compounds by iodate may proceed between the two extremes, as proposed by Hird and Yates (8,9).



The ratio RSH to IO_3^- could thus vary between 6 and 1. For cysteine and glutathione the ratios were found to be 4; for thiolated gelatin and reduced proteins of flour, they were between 3.7 and 4.8 (8,9).

If it is assumed that the molar ratio of 7.0 obtained for the lowest iodate concentration (see Table II) is high because of the presence of a trace amount of oxygen (which would be important competitively because of the low concentration of iodate used), the data show that for two lower iodate concentrations used, the ratio is probably 6 whereas for highest concentration it could be 4. Accordingly it appears that the use of the stoichiometry of equation (a) at technological concentrations of iodate is justified. For higher levels of iodate such as are sometimes used in the continuous process, the more accurate stoichiometric equation might be of the type



which gives a molar ratio of 4. These results and considerations are in general agreement with those published by Hird and co-workers (8,9).

Effect of Continuous Mixing and Prolonged Resting on the Iodate Reaction. Further experiments were made to evaluate the effect of physical barriers on the iodate reaction by continuous mixing in nitrogen and resting for 5, 15, 25, and 35 min. after mixing in nitrogen for 5 min. Results, presented in Fig. 1, show that iodate is a fast -SH-

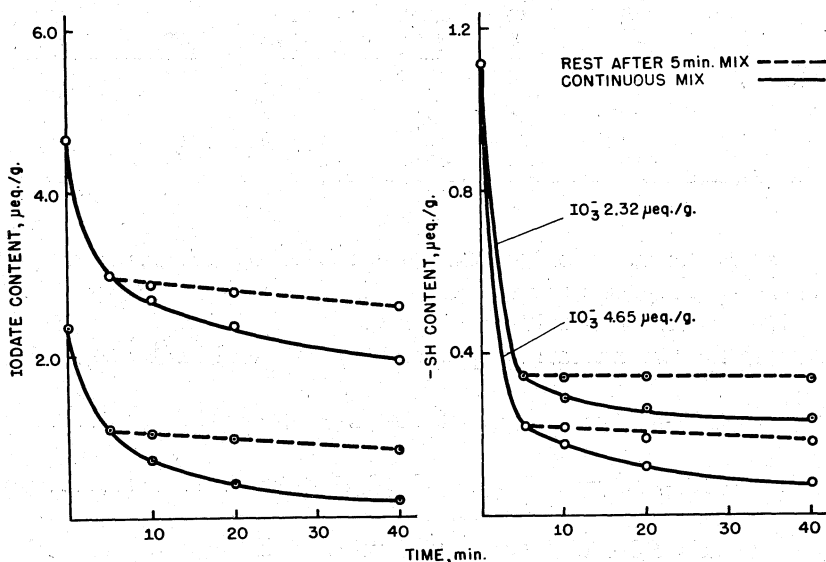


Fig. 1. Effect of continuous mixing and prolonged resting on the iodate reaction.

oxidizing agent during mixing, but the rates of -SH oxidation and iodate consumption slow down sharply during rest after 5 min. of mixing. Furthermore, -SH groups of dough appear to resist being oxidized completely. For example, with the concentration of iodate added of 4.65 $\mu\text{eq.}$ per g. of flour and with continuous mixing for 5 and 40 min., the residual -SH contents equal about 20 and 8% respectively of the original -SH content in flour. It may be assumed, as most protein chemists believe, that a portion of the total -SH content is masked or buried in some protein structures. Once all of the accessible -SH groups have been oxidized, without unfolding of the structure, the remaining -SH groups become increasingly resistant to further oxidation by iodate or any oxidizing agent such as oxygen. In addition, the rate of diffusion of iodate ions to the site of reaction and other

stereochemical factors might also affect the rate of oxidation in a resting dough. No significant change in S-S contents was observed during continuous mixing or resting.

Changes in Iodate, -SH and S-S, and Extensigraph Properties. Table III gives the results for doughs containing 0 and 1.4 $\mu\text{eq.}$ iodate per g. of flour mixed in nitrogen and in oxygen for 5 and 15 min. This experiment was designed to examine the changes in -SH and S-S contents in doughs which show marked breakdown as indicated by the extensigram, e.g. that produced by prolonged mixing in oxygen of doughs treated with relatively high concentrations of iodate (6). The extensigrams for the same doughs are shown in Fig. 2.

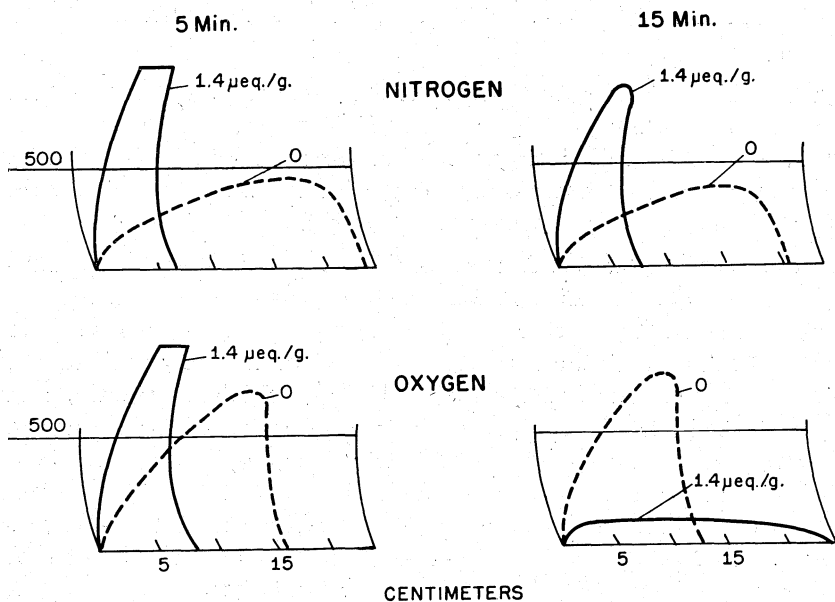


Fig. 2. Extensigrams for doughs containing 0 and 1.4 $\mu\text{eq.}$ iodate per g. of flour and mixed in nitrogen and oxygen for 5 and 15 min.

As shown in Table III, the anticipated decreases in -SH and iodate were obtained in this experiment. However, it is most interesting to find a definite decrease of S-S content in the iodate-treated dough when the mixing is done in oxygen for 15 min. Neither iodate nor oxygen alone can lower the S-S content appreciably (see above). It appears that, with prolonged mixing in oxygen, either iodate in the presence of oxygen can further oxidize the S-S bonds in dough, or oxygen can attack the S-S bonds after most of the -SH groups have

TABLE III

LOSS OF IODATE, -SH, AND S-S DURING 5 AND 15 MINUTES OF MIXING IN NITROGEN AND IN OXYGEN, OF DOUGHS TREATED WITH 1.4 μ EQ. IODATE PER G. FLOUR

MIXING TIME	IODATE LOSS	-SH		S-S	
		Residual	Loss	Residual	Loss
min.	μ eq./g.	μ eq./g.		μ eq./g.	
Nitrogen					
5-Control	...	0.90	0.21	15.50	0.55
5	1.15	.43	.68	15.63	0.42
15-Control92	.19	15.64	0.41
15	1.39	.42	.69	15.66	0.39
Oxygen					
5-Control65	.46	15.80	0.25
5	1.05	.34	.77	15.50	0.55
15-Control51	.60	15.59	0.46
15	1.15	0.32	0.79	15.03	1.02

been oxidized. The reduction of the S-S bonds parallels the marked breakdown of the same dough (see Fig. 2). Further studies are required to confirm this preliminary observation that decrease in S-S content of dough is directly responsible for the breakdown process produced by prolonged mixing of overoxidized doughs.

Effect of Continuous Mixing and Prolonged Resting on the Bromate Reaction. Extensive studies on the bromate reaction have been carried out in this Laboratory (3-5). Previous work points out that the -SH groups are involved in this reaction. Recently with simple -SH compounds, reduced proteins of flour, thiolated gelatin, and flour suspensions, Hird and Yates (8) found that -SH groups were oxidized slowly by bromate. However, with doughs prepared from straight-grade flours milled from wheat varieties Lee and Idaed, Sokol, Mecham, and Pence (15) found no effect of bromate on the -SH content, and only small decrease in -SH content was observed for doughs from a fifth-break flour. This experiment was therefore undertaken to measure the -SH oxidation and bromate consumption in doughs treated with various concentrations of bromate and mixed or rested for different periods. All the doughs were mixed in nitrogen to eliminate the effect of oxygen. When bromate was added at a rate of 1.25 μ eq. per g. (30 p.p.m.) of flour (not shown), no significant change in -SH contents was observed for doughs mixed for 5, 10, 20, or 40 min., although small measurable losses in bromate were obtained. This finding with respect to -SH groups confirms that of Sokol *et al.* for the straight-grade flours (15). Therefore, higher concentrations of bromate, as indicated in Tables IV and V, were tried. Results for continuous mixing at two concentrations of bromate are shown in Table IV,

whereas Table V gives the results for the same bromate concentrations with the doughs mixed for 5 min. and rested for increasing periods. No significant change in the S-S contents was found during continuous mixing or prolonged mixing.

TABLE IV
BROMATE AND -SH LOSSES IN DOUGHS CONTAINING 4.65 AND 18.56 μ EQ. BROMATE PER G. OF FLOUR DURING CONTINUOUS MIXING FOR VARIOUS TIMES IN NITROGEN

CONTINUOUS MIXING	BROMATE		-SH	
	Added	Loss	Residual	Loss
<i>min.</i>	μ eq./g.		μ eq./g.	
5	4.64	0.05	0.91	0.20
10	4.64	0.10	.84	.27
20	4.64	0.15	.81	.30
40	4.64	0.32	.76	.35
5	18.56	0.95	.84	.27
10	18.56	1.21	.81	.40
20	18.56	1.45	.66	.45
40	18.56	1.72	0.56	0.55

TABLE V
BROMATE AND -SH LOSSES IN DOUGHS CONTAINING 4.64 AND 18.56 μ EQ. BROMATE PER G. OF FLOUR AFTER DOUGHS WERE MIXED IN NITROGEN FOR 5 MINUTES AND RESTED FOR VARIOUS TIMES

TOTAL TIME 5 MIN. MIX + REST	BROMATE		-SH	
	Added	Loss	Residual	Loss
<i>min.</i>	μ eq./g.		μ eq./g.	
5	0	0	0.97	0.14
60	0	0	.90	.21
120	0	0	.91	.20
180	0	0	.89	.22
240	0	0	.86	.25
5	4.64	0.05	.91	.20
60	4.64	0.26	.85	.26
120	4.64	0.41	.75	.36
180	4.64	0.43	.69	.42
240	4.64	0.61	.66	.45
5	18.56	0.95	.84	.27
60	18.56	1.46	.71	.40
120	18.56	1.50	.62	.49
180	18.56	1.61	.56	.55
240	18.56	1.76	0.48	0.63

Tables IV and V show slow but definite decreases in bromate and in -SH contents of doughs during continuous mixings in nitrogen and prolonged restings when relatively high bromate concentrations are used. The small decreases confirm previous observations (3-5) that in dough the bromate reaction is extremely slow. Because of the small and slow changes in -SH and bromate contents, the molar ratios of -SH

groups oxidized to bromate consumed are not as reliable as those for iodate-treated doughs, accordingly these ratios are not reported here.

During the continuous mixing, the extent of -SH groups oxidized by bromate (Table IV) is less than those of -SH groups oxidized by air or oxygen (Table I) under the same mixing conditions. This indicates that air is more effective as the -SH oxidizing agent than bromate (18.56 μ eq. per g.) during mixing. On the other hand, in a resting dough air is less reactive than bromate, probably because of the limited diffusion of air into the dough.

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