THE OXIDATION OF WHEAT FLOUR

II. Effect of Sulfhydryl-Blocking Agents[1]

BETTY SULLIVAN, LELAND DAHLE, AND O. RUDOLPH NELSON

ABSTRACT

Certain sulfhydryl-blocking reagents not only shorten the mixing time of a flour, but also, when used in sufficient amounts to combine with –SH groups, eliminate the beneficial effect of maturing agents such as potassium bromate and potassium iodate. Extensigraph and baking data show that both N-ethylmaleimide and *p*-chloromecuribenzoate increase extensibility and decrease resistance to extension as compared to the control; these effects are not changed when bromate or iodate is added after the addition of the –SH-blocking agents. This gives further support to the involvement of –SH groups in flour maturing.

It is possible that intramolecular S–S bonds are changed to intermolecular S–S cross linkages by means of small amounts of –SH groups. Whereas the disulfide bonds may be primarily responsible for toughness and strength of a dough, there are undoubtedly weak linkages, such as thiolester, amide or hydrogen bonds, that require less energy to break and re-form.

The action of the optimum amount of a maturing agent on flour produces a dough with better machining characteristics and an improved baked product. This oxidation involves the sulfhydryl groups, but the relative importance of other groups and the manner of cross-linking of the protein chains are not known. The general mechanism of each of the improvers is apparently different, as reflected in the rheological properties of the doughs.

Mecham (4) and Mecham, Sokol, and Pence (5) have shown that certain of the –SH reagents, such as N-ethylmaleimide (NEMI) and *p*-chloromercuribenzoate (PCMB), affect the mixing time of a flour, shortening the time to maximum resistance and increasing the rate of breakdown. One might expect the opposite since a weakening of the curve is characteristic of the behavior of such compounds as glutathione, cysteine, and sulfite that act on the S–S bond.

Goldstein (3) showed, by means of extensigrams after a 135-minute rest period, that small amounts of ascorbic acid and potassium bromate improved the properties of dough considerably and that, when all available –SH groups were blocked with PCMB, addition of ascorbic acid and potassium bromate was without effect. This evidence supplies additional proof that improvers act on –SH groups. Goldstein pointed

---

[1]Manuscript received September 27, 1960. Contribution from the Research Laboratories of Russell Miller-King Midas Milling Company, Minneapolis, Minn.

out, however, that, because of the very small amounts of free –SH groups which gluten contains, it is unlikely that two –SH groups would be in close enough proximity to be oxidized to an S–S bond. He postulated that improvement can be more adequately explained by the action of the maturing agent in hindering an exchange between –SH and S–S groups. More recently, Frater, Hird, Moss, and Yates (2) presented some interesting data on the effect of iodate, N-ethylmaleimide, and cysteine on the rheological properties of dough. They think it likely that, at any given protein content of flour, the rheological properties of dough are directly related to the number of intermolecular disulfide bonds and the rate at which they can interchange with thiol groups. Since the S–S groups (as cystine) are present in five to ten times the amount of –SH groups, the interchange of –SH with S–S would seem to be one logical explanation. In order to investigate this subject further, some of Goldstein's experiments were repeated and extended as a basis for further elucidation of the reaction mechanism of flour oxidation.

Materials and Methods

indefinite

An untreated spring wheat, straight-grade flour of 0.43% ash, 12.50% protein, and 185 maltose value was used in these experiments.

The sodium salt of PCMB (Sigma Chemical Co., St. Louis, Mo.) was made up 0.3 g. to 100 ml. of water; 0.2 ml. of 6*N* sodium hydroxide was added to obtain complete solution. NEMI was obtained from Eastman Kodak Co. and, without further purification, made up at the same concentration as the PCMB.

Baking Tests with PCMB. A series of baking tests was performed to measure the effect of PCMB, bromate, and iodate. The flour was malted with 0.5% malt. A straight-dough formula was used, as follows: 300 g. flour at 14.0% moisture, 62.0% absorption, 2% salt, 4% sugar, 3% shortening, and 2% yeast. Doughs were mixed on a Hobart mixer to their optimum development and machined and baked in the conventional manner. When PCMB was used, the mixing was interrupted for 2 minutes to allow time to react before iodate or bromate was added. Since the dough quality and volume of the bread reflected sufficiently the action of the added reagents, these two factors are reported for breads made from the untreated flour and from the same flour to which PCMB was added in varying amounts. Iodate and/or bromate were also added in various amounts, and different levels of iodate or bromate were superimposed after the PCMB treatment.

Farinograph and Extensigraph Data. Farinograms were made by the constant-dough (480 g.) method (1). Extensigrams were obtained as follows: a farinogram of the flour, reagent, and water was first obtained to determine the correct absorption. The extensigraph doughs were all given a 5.5-minute total mixing time, as found optimum from the farinogram of the control flour. All doughs were nonyeasted and contained 2% salt based on the flour weight. All the absorption water, including the small amount (20 to 40 ml.) used to dissolve the –SH reagent, was added to the flour in a 300-g. mixing bowl. The dough was mixed 1 minute, followed by a 12-minute rest period. The remaining 5.0 ml. of water or solution containing the improver, if any, were added and the dough remixed for 4.5 minutes.

After the dough was removed from the farinograph bowl, two 150-g. portions were scaled, each rounded 20 times, molded into dough cylinders, and immediately placed in the fermentation cabinets at 30°C. The doughs were allowed to relax 45 minutes and stretched. This procedure was repeated at 90, 135, and 180 minutes.

The control flour was measured in a similar manner and the results averaged. Results are expressed as resistance, extensibility, and area at 45, 90, 135, and 180 minutes.

Results

Mixing Curves. Farinograms of the flour with no treatment and with NEMI (15 mg.%), PCMB (30 mg.%), and iodate (3 mg.%) are shown in Fig. 1.

Small amounts of NEMI and iodoacetamide (about equivalent amounts to combine with sulfhydryl) weaken a mixing curve (4). PCMB and iodate behave in a similar manner, as Fig. 1 shows, but, as is well known, bromate does not affect the curve.

Response of Iodate and Bromate to PCMB-Treated Flours. The baking results on flour/to which PCMB, iodate, and bromate were added are summarized in Table I.

Table I shows that PCMB improves the volume of an untreated flour below the critical level of binding of –SH groups. Iodate and bromate give the usual beneficial effect. When most, or all, of the sulfhydryl groups are bound (with this particular flour, this occurs at slightly over 0.025% PCMB), the dough becomes stringy and extensible, the volume decreases, and iodate or bromate superimposed on the PCMB-treated flour shows no beneficial effect.

Extensigraph Data. Figure 2 and Table II give results for the control flour and the same flour to which was added 5 mg.% of potassium bromate and 1 mg.% of potassium iodate.

The usual increase in resistance and decrease in extensibility compared to the control are observed. The effect of bromate and iodate,

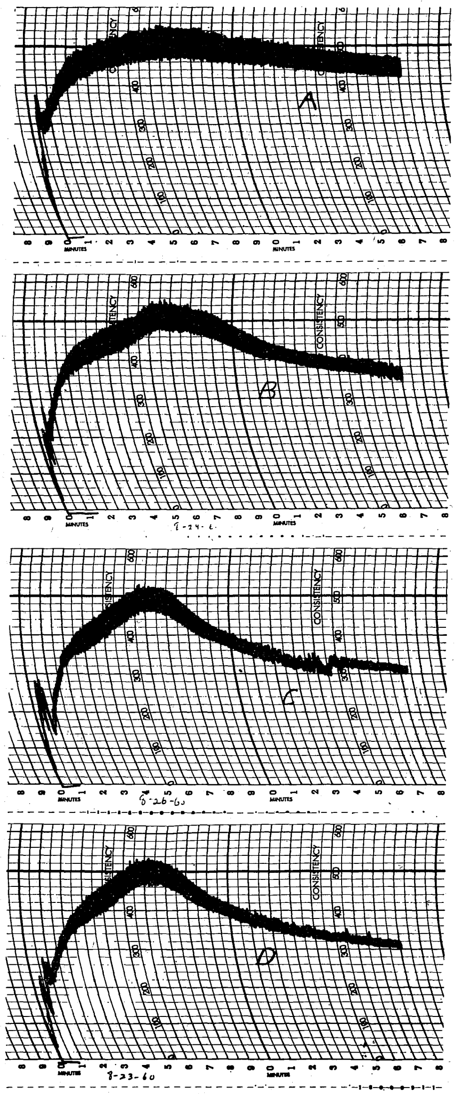

Fig. 1. Farinograms showing effect of iodate, NEMI, and PCMB on mixing characteristics. All curves based on 480-g. dough at 500 B.u. A, control (untreated flour), 61.8% absorption at 14.0% moisture; B, control plus 3 mg.% potassium iodate, 63% absorption at 14.0% moisture; C, control plus 15 mg.% N-ethylmaleimide, 63.3% absorption at 14.0% moisture; D, control plus 30 mg.% *p*-chloromercuribenzoate, 64.9% absorption.

as measured on the extensigraph, is not as pronounced in a flour-water-salt dough as in a dough containing yeast. No yeast was used in

TABLE I

EFFECT OF PCMB AND SUBSEQUENT OXIDIZING TREATMENT ON A PATENT FLOUR

	PCMB[a]	IODATE[b]	BROMATE[c]	DOUGH QUALITY	VOLUME
	%	*ppm*	*ppm*		
Control	0.0	0.0	0.0	Elastic	100
	0.0	5.0	0.0	Slightly tough	104
	0.0	7.5	0.0	Slightly tough	107
	0.0	10.0	0.0	Elastic, slightly tough	112
	0.0	12.5	0.0	Tough	114
	0.0	15.0	0.0	Tough	115
	0.0	25.0	0.0	Very tough	116
	0.0	0.0	10.0	Slightly tough	113
	0.0	0.0	15.0	Slightly tough	121
	0.0	0.0	20.0	Tough, slightly short	119
	0.0	0.0	25.0	Tough, short	120
	0.0	0.0	50.0	Very tough	115
	0.01	0.0	0.0	Slightly short, slightly soft	108
	0.02	0.0	0.0	Slightly soft, extensible	113
	0.03	0.0	0.0	Soft, extensible	89
	0.04	0.0	0.0	Stringy, very soft	78
	0.0175	0.0	20.0	Slightly soft, extensible	116
	0.0200	0.0	20.0	Slightly soft, extensible	116
	0.0250	0.0	20.0	Slightly soft, extensible	100
	0.0275	0.0	20.0	Soft, extensible	88
	0.0300	0.0	20.0	Short, stringy, soft	81
	0.0300	0.0	50.0	Short, stringy, soft	92
	0.0330	0.0	20.0	Short, stringy, very soft	70
	0.0450	0.0	20.0	Short, stringy, very soft	53
	0.02	10.0	0.0	Slightly tough	101
	0.03	25.0	0.0	Soft, extensible, stringy	83

[a] A level above 0.025% PCMB was needed to exceed the optimum level as reflected in loaf volume. All PCMB doughs were exceedingly extensible; those with the high amounts of PCMB (over 0.03%) were stringy.

[b] This flour had a higher-than-normal tolerance to iodate. Iodated doughs were all on the tough side until they came to the molder; then they started to mellow out. With this flour, 10 p.p.m. iodate gave the best loaf.

[c] The optimum bromate response was given at 10 p.p.m. Amounts of 20 and 25 p.p.m. gave doughs that were tough. Dough characteristics and loaves below standard were produced by 25 p.p.m. iodate and 50 p.p.m. bromate.

any of the extensigraph experiments in order to avoid any further complications in interpretation.

The same control flour was tested on the extensigraph with 10 and 15 mg.% of N-ethylmaleimide (NEMI). There is a sharp decrease in R and increase in E values; the higher amount of NEMI caused the greater change, as shown in Table II. This –SH reagent, like PCMB, increases extensibility rather than decreasing it as many maturing agents do. Three samples of the control flour were each treated with 15 mg.% of NEMI. One sample was then treated, after a rest period, with 5 mg.% potassium bromate and the other with 1 mg.% potassium iodate. Results are shown in Table II and Fig. 2.

TABLE II

DEPRESSION OF RESPONSE TO IODATE AND BROMATE IN DOUGHS CONTAINING N-ETHYLMALEIMIDE (NEMI)

	Treatment																				
	No Treatment			5 mg.% $KBrO_3$			1 mg.% KIO_3			10 mg.% NEMI			15 mg.% NEMI			15 mg.% NEMI +5 mg.% $KBrO_3$			15 mg.% NEMI +1 mg.% KIO_3		
TIME	R[a]	E[a]	A[a]	R	E	A	R	E	A	R	E	A	R	E	A	R	E	A	R	E	A
minutes																					
45	450	220	124	495	203	127	540	202	137	215	209	58	125	257	39	160	230	48	150	208	40
90	710	200	177	780	183	181	810	150	150	275	187	68	180	215	47	200	210	56	170	225	46
135	830	151	155	885	135	146	940	133	151	290	194	73	160	250	45	200	205	51	155	265	49
180	930	125	144	970	105	120	1000	114	136	290	185	67	170	235	44	200	230	55	165	252	46

[a] R = resistance; E = extensibility; A = area.

TABLE III

EFFECT OF PARA-CHLOROMERCURIBENZOATE (PCMB) ON DOUGH MOBILITY AS MEASURED BY THE EXTENSIGRAPH

	Increments of PCMB (in mg. %)																				
	0			10			15			20			25			30			35		
TIME	R[a]	E[a]	A[a]	R	E	A	R	E	A	R	E	A	R	E	A	R	E	A	R	E	A
minutes																					
45	450	220	124	430	218	119	380	203	100	310	190	79	190	225	62	100	235	28	110	200	25
90	710	200	177	690	172	150	650	165	139	590	150	114	430	140	83	150	230	40	130	176	27
135	830	151	155	870	145	156	740	138	128	800	115	112	625	115	94	195	205	49	130	178	25
180	930	125	144	990	120	139	890	122	134	845	108	690	690	106	92	230	185	54	150	168	31

[a] R = resistance; E = extensibility; A = area.

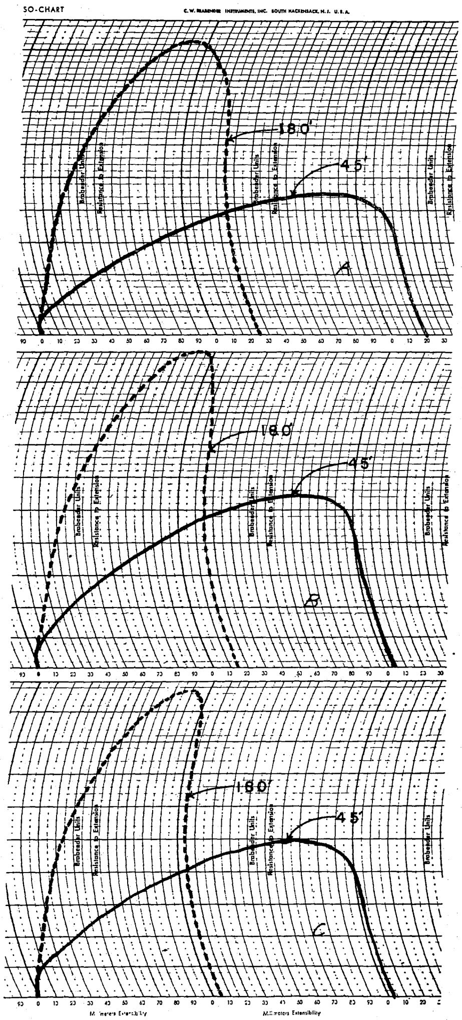

Fig. 2, A–C. Extensigraph data on the effect of iodate and bromate after blocking of –SH groups. A, untreated flour; B, 1 mg.% potassium iodate; C, 5 mg.% potassium bromate.

The maturing effect of both bromate and iodate was eliminated when those treatments were superimposed on NEMI-treated flour, although the bromate showed a slight increase in R values as compared to the NEMI control.

PCMB is another reagent considered specific for the sulfhydryl groups. Table III illustrates the effect of increments of the sodium salt of PCMB, from 10 to 35 mg.%, on extensigraph curves.

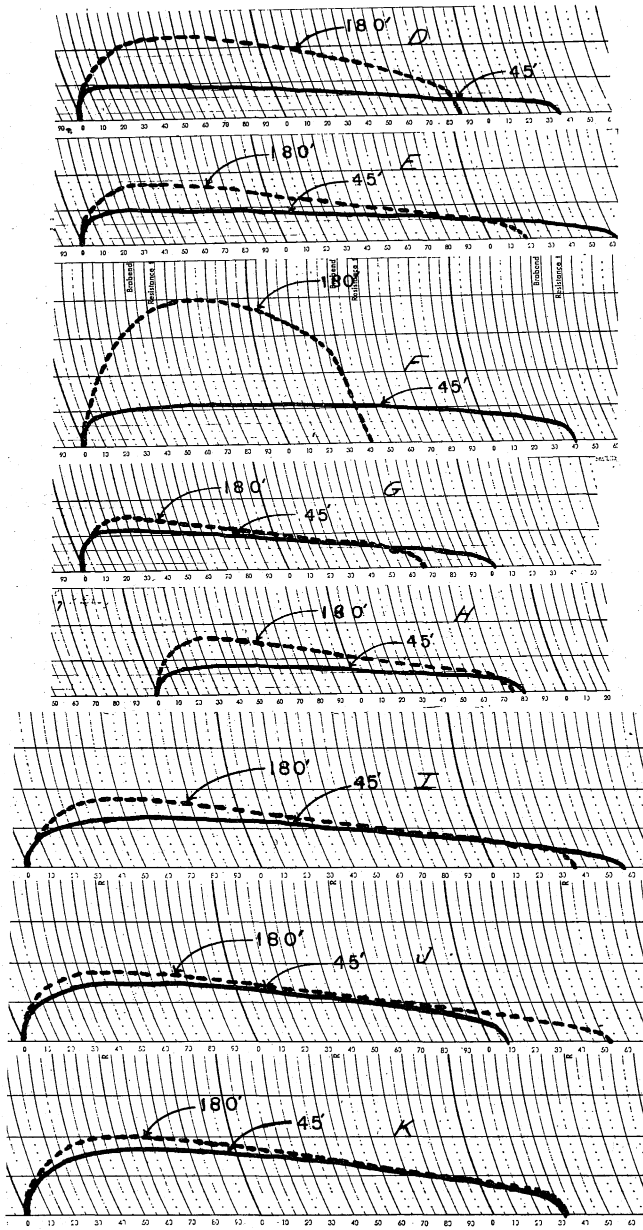

Fig. 2, D–K. Extensigraph data on the effect of iodate and bromate after blocking of –SH groups. D, 30 mg.% PCMB; E, 30 mg.% PCMB plus 1 mg.% potassium iodate; F, 30 mg.% PCMB plus 5 mg.% potassium bromate; G, 35 mg.% PCMB; H, 35 mg.% PCMB plus 5 mg.% potassium bromate; I, 15 mg.% NEMI; J, 15 mg.% NEMI plus 1 mg.% potassium iodate; K, 15 mg.% NEMI plus 5 mg.% potassium bromate.

TABLE IV

DECREASE IN BROMATE AND IODATE RESPONSE IN DOUGHS WITH INCREASING AMOUNTS OF PARA-CHLOROMERCURIBENZOATE (PCMB)

PCMB	TIME	Treatment[a] 0			3 mg.% $KBrO_3$			1 mg.% KIO_3		
		R	E	A	R	E	A	R	E	A
mg.%	*minutes*									
0	45	450	220	124	495	203	127	540	202	137
0	90	710	200	177	780	183	181	810	150	150
0	135	830	151	155	885	135	146	940	133	151
0	180	930	125	144	970	105	120	1000	114	136
25	45	190	225	62	275	200	77	190	219	58
25	90	430	140	83	525	135	95	333	177	78
25	135	625	115	94	730	103	91	380	137	70
25	180	690	106	92	790	97	96	380	140	74
30	45	100	235	28	115	240	39	95	262	30
30	90	150	230	40	265	175	65	150	245	42
30	135	195	205	49	330	142	66	160	220	43
30	180	230	185	53	400	140	73	175	218	44
35	45	110	200	25	80	180	17			
35	90	130	176	27	130	163	26			
35	135	130	178	25	160	176	33			
35	180	150	168	31	145	165	30			

[a] R = resistance; E = extensibility; A = area.

With higher levels of PCMB, the resistance decreases and the extensibility increases. The dough becomes soft and stringy. When bromate or iodate was added to the dough after addition of PCMB presumably sufficient to combine with all the –SH groups, there was no maturing effect. It is noteworthy, however, that, whereas 30 mg.% PCMB was sufficient to block the iodate reaction, 35 mg.% PCMB was required to block the bromate effect completely. Results are shown in Table IV and Fig. 2.

Discussion

Specific –SH reagents, such as NEMI and PCMB, not only affect the mixing time of flour but also increase the extensibility and decrease the resistance to extension; whereas common maturing agents produce a reverse effect. At first thought, it is puzzling that certain sulfhydryl-blocking agents weaken the mixing curve in a manner analogous to that of –SH-containing compounds such as glutathione and cysteine. The rate to maximum and the drop after maximum sharply increase with both types of compounds. The –SH-containing compounds in sufficient amount break existing disulfide cross-links, resulting in a soft, sticky dough and a weak curve. Blocking agents, such as NEMI and mercury compounds, on the other hand, combine with the free –SH groups of the flour proteins, preventing the interchange with the S–S groups and the formation of new cross-links. This possibility, as well as others, has been discussed by Mecham (4) in relation to mixing behavior.

Specific –SH reagents increase extensibility and decrease resistance to extension according to the state of oxidation of dough during mixing and the consequent availability of –SH groups. Extensigraph and baking data confirm and extend the experiments of Goldstein (3), which showed that when –SH groups are blocked, maturing agents do not exert their beneficial effect. It was observed, however, that the amounts of NEMI and PCMB that stop the iodate reaction are not sufficient to inhibit completely the bromate effect. A level of NEMI and PCMB somewhat higher than the amount calculated to react with the total sulfhydryl is required to suppress completely the bromate response of the flour. This may mean that the –SH determination is too low or that the stoichiometric equivalents of NEMI or PCMB added would not react with all the –SH groups of a given flour within the duration of the experiment, even though complete reaction might be achieved under different conditions or over a longer period of time. It is also probable that the number of –SH groups available for titration after denaturation with urea is not the same as available in an undenatured dough.

The total sulfhydryl of the flour used in these experiments measured 0.41 micromoles per g. This amount would be equivalent to 5 mg.% NEMI or 16 mg.% PCMB. The total sulfhydryl of the flour was determined by amperometric titration with mercuric chloride. An equivalent amount of NEMI did not titrate all the sulfhydryl under the same conditions, although it appeared that about 75% of the total sulfhydryl could be titrated. Thus, it would appear that the amounts of some blocking agents required to bind the –SH groups of a dough may not reflect an accurate measure of sulfhydryl, since accessibility and reactivity of these groups to various blocking agents vary. Moreover, the extent of the response to blocking agents and improvers depends on the mixing and mechanical treatment given a dough.

When the usual maturing agents are employed, it would seem probable that some intramolecular S–S bonds are changed to intermolecular S–S cross-linkages through the mediation of very small amounts of thiol groups. It is presumed that the intermolecular disulfide bridges confer toughness and greater resistance to extension to the dough. When a dough stands, its flow properties increase with time. And, when energy is applied through work such as mixing, rounding, or molding, more opportunity is given for exchange of –SH and S–S groups. It is highly probable that many of the cross-links conferring beneficial elastic properties on dough are not covalent disulfide bonds, but weaker linkages such as thiolester or hydrogen bonds. The large number of amide side chains of the flour proteins would be expected to form hydrogen bonds that could be easily broken and re-formed. More intensive study is needed to determine the nature and energy of these bonds.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (6th ed.), p. 137. The Association: St. Paul, Minnesota (1957).
2. Frater, R., Hird, F. J. R., Moss, H. J., and Yates, J. R. A role for thiol and disulfide groups in determining the rheological properties of dough made from a wheaten flour. Nature **186:** 451–454 (1960).
3. Goldstein, S. Sulfhydryl- und Disulfidgruppen der Klebereiweisse und ihre Beziehung zur Backfahigkeit der Brotmehle. Mitt. Lebensm. Hyg. Bern **48:** 87–93 (1957).
4. Mecham, D. K. Effects of sulfhydryl-blocking reagents on the mixing characteristics of doughs. Cereal Chem. **36:** 134–145 (1959).
5. 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).