

EFFECTS OF THIOLATED GELATINS AND GLUTATHIONE ON RHEOLOGICAL PROPERTIES OF WHEAT DOUGHS¹

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ABSTRACT

Adding three thiolated gelatins varying in molecular weight and -SH content, and of glutathione, resulted in a slight decrease in farinograph absorption and in a pronounced reduction in dough development time and valorimeter value. Thiolated gelatin and glutathione increased the extensibility and decreased elasticity and extensigram areas of flours milled experimentally from four classes of wheat and of two commercially milled untreated flours. Glutathione consistently had the most detrimental effect; the low -SH-containing thiolated gelatin exerted the smallest effect. The modifications induced by the reducing agents could be reversed by adding an excess of oxidants or N-ethylmaleimide. The extent of reversibility depended on the extent of modification resulting from the action of the reducing agents. Excess of bromate or persulfate, but not of iodate or N-ethylmaleimide, was canceled on remixing. Glutathione and thiolated gelatin accelerated the rate of relaxation of strains introduced by mechanical treatment. Adding N-ethylmaleimide to doughs containing thiolated gelatin canceled the enhanced rate of relaxation; bromate reduced it less. Glutathione and high levels of thiolated gelatin of high thiol content decreased volume substantially and impaired loaf characteristics of bread baked from experimentally milled flour and a commercially milled flour.

The role and significance of reactive sulfhydryl groups in flour continue to intrigue cereal chemists in many research centers in the world (1,4,5,7,8,9). The study of the mechanism of action of reducing agents and oxidants on rheological properties has been concerned primarily with the action of the free thiol-containing cysteine or glutathione and the maturing agents bromate, persulfate, and iodate. A great amount of useful information has been obtained more recently by using specific sulfhydryl-blocking reagents, N-ethylmaleimide (NEMI) and *p*-chloromercuribenzoate (PCMB). The availability of thiol-containing compounds comparable in molecular weight to flour proteins such as thiolated gelatin prepared according to the procedure of Benesch and Benesch (2) or relatively simple techniques for the production of thiolated flour presents a new tool for study of the effect of -SH groups on dough properties (6). McDermott and Pace (7) found that adding thiolated gelatin modified load-extension curves of

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wheat flour doughs, whereas the effect of normal gelatin was insignificant. Bushuk and Hlynka (3), however, found that doughs containing as much as 2.5 μ moles -SH per g. of flour in the form of thiolated gelatin felt no different from control doughs.

In the present study the effects of thiolated gelatins varying in molecular weight and -SH content on the rheological properties and bread-baking potentialities of wheat doughs have been investigated. Additionally, the effects of oxidants on reversibility of Thiogel-induced changes in flour doughs were studied.

Materials and Methods

Two samples of untreated patent flour, commercially milled from hard red winter wheat, were used. The composition and characteristics of the flours are given in the table below: Additionally, four samples

Sample	Protein Content ($N \times 5.7$)	Ash	Farinograph Absorption
	%	%	%
A	11.9	0.43	64.9
B	14.4	0.39	62.4

of wheat were milled on a Miag Multomat³ mill; see table below for their characteristics. The thiolated gelatins were purchased from

	Extraction	Protein Content ($N \times 5.7$)	Ash	Farinograph Absorption
	%	%	%	%
Hard red winter	73.2	10.7	0.43	66.4
Soft red winter	70.9	9.1	0.36	56.0
Northern spring	72.7	13.1	0.44	64.6
Durum	67.0	11.9	0.56	63.6

Schwarz BioResearch, Inc., Mount Vernon, N.Y. Their properties are listed below. The gelatin used in this study was purchased from

	Average Molecular Weight	-SH Equivalents in 100,000 g.
Thiogel A	10,000	14.2
Thiogel B	100,000	13.3
Thiogel C	100,000	3.2

Difco Lab., Inc., Detroit, Mich. The oxidizing, -SH-blocking, and reducing agents employed were of analytical reagent grade and were purchased from Nutritional Biochemical Corp., Cleveland, Ohio, or from Fisher Scientific Co., St. Louis, Mo.

Farinograms were made by mixing 50 g. of flour in a small stainless-steel farinograph bowl (56-r.p.m. drive) with sufficient distilled water

³Mention in this publication of a trade product, equipment, or a commercial company does not imply its endorsement by the U.S. Department of Agriculture over similar products or companies not named.

to give a maximum dough consistency centered around the 500-B.U. line. From the results obtained, the absorption, dough development time, and valorimeter value were computed. Extensigrams were obtained by mixing 300 g. flour, 6 g. salt, and distilled water equal to the farinograph absorption (corrected for the salt and use of the large bowl). Thiolated gelatin, if added, was dissolved in part of the water. Doughs were mixed for 1 min., rested 5 min., and remixed until centered around 500 B.U. All doughs were nonyeasted. After the dough was removed from the farinograph bowl, two 150-g. portions were scaled, each rounded 20 times, moulded into dough cylinders, and placed in the extensigraph cabinets at 30°C. Curves were drawn for duplicate doughs at 45, 90, and 135 min. To test the effects of oxidants or NEMI on doughs previously reacted with reducing agents, the doughs were remixed for 3 min. at low speed in the farinograph to incorporate the oxidant or NEMI, moulded, rested for 135 min. in the extensimeter cabinet, and stretched. For relaxation studies, two batches of dough, each from 300 g. of flour, were prepared. Six dough pieces for following the relaxation process were scaled and moulded. One piece was stretched after a 45-min. rest. The other dough pieces were remoulded and stretched immediately or after time intervals of 5, 15, 30, or 45 min. after moulding.

The baking experiments were on a laboratory scale employing the straight-dough procedure. The basic formula was:

<i>Ingredients</i>	<i>Parts</i>
Flour	100
Water	variable
Yeast	2
Sucrose	6
Malted wheat flour	0.5
Shortening	3
Sodium chloride	2
Reducing agents	as indicated

After they were mixed to optimum consistency, the doughs were fermented at 30°C. After 110 min. the doughs were punched, and 50 min. later punched again, divided, rested for 20 min. and moulded. The doughs were proofed at 30°C. for 45 min. and baked for 25 min. at 210°C. After baking, the loaf volume was measured, and after cooling, the loaves were scored for crust color, symmetry, break and shred, texture, grain, and crumb color.

Results and Discussion

The effects of thiolated gelatin on farinograph characteristics of flours are shown in Table I. Comparison of the effects of the Thiogels

TABLE I
EFFECTS OF 0.5% THIOGEL ON FARINOGRAPH CHARACTERISTICS OF FLOURS

REAGENT AND LEVEL	COMMERCIAL H.R.W.			EXPERIMENTAL H.R.W.			EXPERIMENTAL S.R.W.		
	Absorp- tion	Dough Devel. Time	Valo- rimeter Value	Absorp- tion	Dough Devel. Time	Valo- rimeter Value	Absorp- tion	Dough Devel. Time	Valo- rimeter Value
	μmoles SH/g.	%	min.	%	min.	%	min.	%	min.
.....	67.4	13.0	88	66.4	8.0	76	56.0	9.5	81
Gelatin, 0	66.9	13.5	89	69.0	10.0	82	57.6	6.0	69
Thiogel A, 0.71	66.0	7.5	75	65.0	5.0	66	55.8	5.5	68
Thiogel B, 0.66	65.0	4.5	64	64.0	4.0	61	57.2	3.0	56
Thiogel C, 0.16	65.0	10.0	82	66.0	6.0	69	57.2	5.5	71
	EXPERIMENTAL H.R.S.			EXPERIMENTAL DURUM					
	Absorp- tion	Dough Devel. Time	Valo- rimeter Value	Absorp- tion	Dough Devel. Time	Valo- rimeter Value			
	%	min.	%	min.	%	min.			
.....	64.4	11.0	85	63.6	3.5	59			
Gelatin, 0	66.2	11.5	85	64.8	3.0	58			
Thiogel A, 0.71	64.4	7.0	73	62.4	3.0	55			
Thiogel B, 0.66	64.2	6.0	70	62.6	2.5	53			
Thiogel C, 0.16	65.0	9.5	81	63.0	3.0	56			

with either the control or the gelatin-containing flours shows a small decrease in water absorption. No consistent effect on water absorption has been recorded, however, among the three tested Thiogels themselves. It is significant, however, that the Thiogels caused a decrease in the dough development time, as might be expected if these agents act as typical dough-reducing agents. Thiogel B had the greatest and Thiogel C the least effect on decrease of either dough development time or valorimeter value. The slightly more pronounced effects of Thiogel B than of Thiogel A might be due to the higher stability of the high-molecular Thiogel B during prolonged mixing.

The effects of thiolated gelatin on extensigraph characteristics of hard red spring wheat flour are summarized and compared with the action of reduced glutathione, in Table II. Similar results were obtained for the flours milled from the other three wheat samples. At the levels employed, the three Thiogels increased the extensibility of the tested doughs to an extent comparable with the action of glutathione. Glutathione had, however, a much more pronounced effect similar to that normally associated with reducing agents. Under the experimental conditions employed, there was no consistent difference between the actions of thiolated gelatins with molecular weights of 10,000 or 100,000 provided they did not differ in thiol content. Adding excess bromate to doughs previously treated with reducing agents showed that the extent of modification and potential reversal of this modifi-

TABLE II
EFFECTS OF 0.5% THIOLATED GELATINS AND OF GLUTATHIONE ON RHEOLOGICAL PROPERTIES OF HARD RED SPRING WHEAT FLOUR DOUGHS

REAGENT AND LEVEL	EXTENSIGRAPH DATA ^a							
	After 45 min.		After 90 min.		After 135 min.		135 min. after Addition of KBrO ₃ ^b	
	A	B	A	B	A	B	A	B
<i>μmoles</i> <i>SH/g.</i>								
.....	20	143	12	123	12	141	15	145
Gelatin, 0	22	151	13	141	13	139	16	153
Thiogel A, 0.71	23	73	23	77	26	120	26	192
Thiogel B, 0.66	21	104	22	130	22	133	21	140
Thiogel C, 0.16	25	226	22	254	18	217	16	177
Glutathione, 0.66	25	31	26	41	25	87	24	38

^a A = length, cm.; B = area, cm.²

^b KBrO₃ equivalent to 300% of added -SH groups.

cation by oxidants varied with the reducing agent used. Whereas adding glutathione resulted in a significant, irreversible change in rheological characteristics as assessed by the extensigraph, the changes introduced by the thiolated gelatins could be partially reversed. It is feasible that the difference in action between the thiolated gelatins and glutathione is a result of the association of the former with the wheat gluten, as observed by McDermott and Pace (7). Such an association would tend to confine the action of thiolated gelatins to a limited number of sites on the wheat protein molecule. The justification for employing an excess of oxidant is shown in the data summarized in Table III. Levels of bromate as high as equivalent to 100% of added -SH groups (in the form of thiolated gelatin) resulted in no

TABLE III
EFFECTS OF DIFFERENT LEVELS OF KBrO₃ ON UNTREATED COMMERCIALY MILLED FLOUR (SAMPLE A) CONTAINING 0.5% THIOGEL B

REAGENT AND LEVEL	KBrO ₃ ^b	EXTENSIGRAPH DATA ^a							
		After 45 min.		After 90 min.		After 135 min.		135 min. after Addition of KBrO ₃	
		A	B	A	B	A	B	A	B
<i>μmoles</i> <i>SH/g.</i>									
.....		20	160	13	140	11	120	15	137
Gelatin, 0		20	172	14	157	12	134	14	116
Thiogel B, 0.66		20	147	19	147	19	161	21	144
Thiogel B, 0.66	25	20	122	19	133	21	140	21	144
Thiogel B, 0.66	50	20	128	19	140	20	134	22	146
Thiogel B, 0.66	100	19	124	19	133	21	133	21	143

^a A = length, cm.; B = area, cm.²

^b Equivalent to % of the thiol groups added.

measurable reversal of the action of the reducing agent. A more detailed and extensive study of the effect of oxidants on Thiogel-treated doughs is given in Table IV. The reaction time of the reducing agents

TABLE IV
EFFECTS OF OXIDANTS AND NEMI ON RHEOLOGICAL PROPERTIES OF A COMMERCIALY MILLED FLOUR (SAMPLE A), TREATED WITH REDUCING AGENTS

REAGENT	LEVEL	OXIDANT OR NEMI	OXIDANT LEVEL	FARINOGRAPH DATA		EXTENSIGRAPH DATA ^a	
				Absorp- tion	Dough Devel. Time	A	B
	$\mu\text{moles SH/g.}$		mg./g.	%	min.		
0.5% gelatin		66.4	14.5	19	125
		66.0	11.5	21	145
		KBrO ₃	0.333	66.4	15.5	18	121
		(NH ₄) ₂ S ₂ O ₈	0.455	66.4	13.0	15	119
		KIO ₃	0.427	66.4	14.5	12	88
	NEMI	0.250	66.4	14.5	12	74	
Thiogel B	0.66	62.8	2.5	22	106
		KBrO ₃	0.333	62.8		22	155
		(NH ₄) ₂ S ₂ O ₈	0.455	62.8		14	109
		KIO ₃	0.427	62.8		9	90
		NEMI	0.250	62.8		10	82
Glutathione	0.66	65.2	4.0	24	66
		KBrO ₃	0.333	65.2		24	78
		(NH ₄) ₂ S ₂ O ₈	0.455	65.2		25	128
		KIO ₃	0.427	65.2		19	83
		NEMI	0.250	65.2		21	48

^a A = length, cm.; B = area, cm.²

with the flours was 45 min., and stretching with the extensigraph was carried out after an additional reaction time of 135 min. The large excess of either bromate or persulfate (in the absence of reducing agents) had no deleterious effect on the doughs, probably because of remixing after the first rest period. In the case of iodate and NEMI, the remix did not reverse the deleterious effect of excess oxidant or -SH-blocking reagent. Again, adding oxidants to Thiogel-treated flours reversed the effect of reducing agent on rheological properties of the dough. No measurable effect has been observed when any of the oxidants or NEMI was added to glutathione-treated flour.

The effects of adding oxidants and NEMI to a commercial flour are given in Table V. The flours were mixed in the farinograph bowl with the reducing agents, sodium chloride, and water required to center the consistency around the 500-B.U. line. After a reaction time of 45 min. in bulk in the extensigraph, the doughs were remixed for 3 min. at low speed with the oxidizing agents or NEMI. After scaling, the doughs were moulded and shaped, rested for 135 min., and stretched.

TABLE V
EFFECTS OF OXIDANTS AND NEMI ON RHEOLOGICAL PROPERTIES OF A COMMERCIALY MILLED FLOUR (SAMPLE B), TREATED WITH REDUCING AGENTS

REAGENT AND LEVEL	EXTENSIGRAPH DATA ^a							
	Controls		KBrO ₃ ^b		KIO ₃ ^b		NEMI ^b	
	A	B	A	B	A	B	A	B
<i>μmoles SH/g.</i>	19	182	18	145	13	128	12	76
Gelatin	21	176	21	168	20	112	19	115
Thiogel A, 0.71	27	132	28	218	12	99	15	107
Thiogel B, 0.66	28	146	26	191	12	114	16	133
Thiogel C, 0.16	19	161	16	127	11	97	11	84
Glutathione, 0.66	23	39	25	75	21	102

^aA = length, cm.; B = area, cm.²

^bEquivalent to 300% of -SH content of Thiogel B.

Doughs to which NEMI was added after previous reaction with glutathione were difficult to handle. Results of that series are, therefore, omitted. The over-all picture is similar to that recorded with the experimentally milled flours.

The rate of relaxation in doughs following the introduction of strains was studied in a flour treated with the three Thiogels and glutathione and compared with either the control or the gelatin-containing flours. Figure 1 shows that balling and shaping introduced

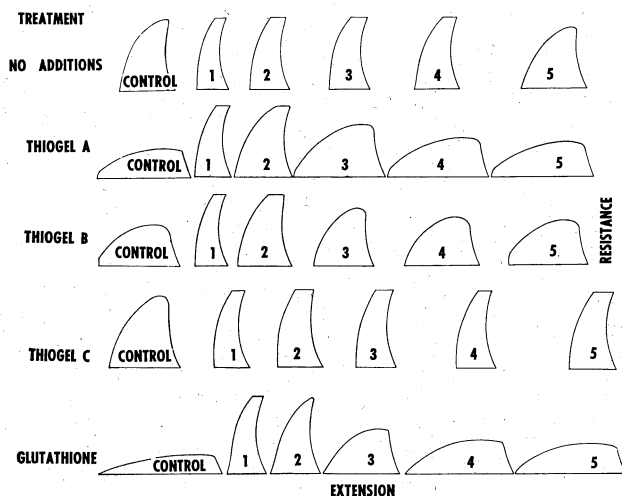


Fig. 1. Extensigrams of doughs tested at various time intervals after moulding. The reagents were added at levels of 0.71, 0.66, 0.16, and 0.66 μ eq. -SH per g. of flour, for Thiogel A, B, C, and glutathione, respectively. Control was stretched 45 min. after moulding; doughs 1, 2, 3, 4, and 5 were stretched immediately, 5, 15, 30, and 45 min., respectively, after moulding at the end of the initial 45-min. rest period.

strains that resulted in a dough markedly toughened and shortened. These strains relaxed only slowly in the control doughs. Adding reducing agents did not prevent the buildup of strains in the dough after balling and shaping but allowed these strains to relax more and at a faster rate than in the control.

In Table VI are summarized the data of the effects of bromate and

TABLE VI

EFFECTS OF KBrO_3 AND NEMI ON RATE OF RELAXATION OF A COMMERCIALY MILLED FLOUR (SAMPLE A), TREATED WITH 0.5% THIOGEL B (0.66 $\mu\text{MOLES -SH/G. FLOUR}$)

REAGENT	LEVEL OF REAGENT ^a	CONTROL ^b	DOUGHS					
			1	2	3	4	5	
KBrO_3	12.5	A ^c	18	8	10	10	11	15
		B	173	94	119	116	134	175
KBrO_3	100	A	18	8	9	12	12	13
		B	189	90	109	142	142	165
NEMI	100	A	12	7	9	8	9	10
		B	140	80	105	92	100	104

^a Equivalent to % of the thiol groups added.

^b Control was stretched 45 min. after moulding; doughs 1, 2, 3, 4, and 5 were stretched immediately, 5, 15, 30, and 45 min. rest after moulding at the end of the initial 45 min. rest period.

^c A, length, cm.; B, area, cm.²

TABLE VII

EFFECTS OF REDUCING AGENTS ON BAKING CHARACTERISTICS OF A COMMERCIALY MILLED FLOUR (SAMPLE B)

REAGENT	LEVEL	LOAF VOLUME	TOTAL SCORE
<i>g./100 g. flour</i>	<i>$\mu\text{moles SH/g.}$</i>	<i>ml.</i>	
.....	..	945	89
Gelatin			
0.10	0	1005	91
0.25	0	1015	89
0.50	0	1005	89
1.00	0	965	89
Thiogel A			
0.10	0.14	975	88
0.25	0.36	915	86
0.50	0.71	810	82
1.00	1.42	710	68
Thiogel B			
0.10	0.13	915	84
0.25	0.33	970	86
0.50	0.66	875	85
1.00	1.32	800	84
Thiogel C			
0.10	0.03	995	88
0.25	0.08	970	87
0.50	0.16	1005	87
1.00	0.32	985	88
Glutathione			
0.004	0.13	910	86
0.010	0.33	785	81
0.020	0.66	650	69
0.040	1.32	560	53

NEMI on relaxation studies of doughs treated with Thiogel B. NEMI decreased the rate of relaxation to a larger extent than did bromate, a slow oxidizing agent. Frater *et al.* (4) suggested that the major effect of NEMI or of iodate was to stabilize the strained and/or oriented structure normally introduced into the dough during balling and shaping. The rapid action of NEMI in blocking -SH groups does not permit the dough to relax as does bromate that has a slow action.

The baking test was performed with a commercially milled flour (sample B) treated with gelatin, the three Thiogels, and glutathione at different levels. The results are presented in Table VII. Thiogel A and B at the 0.5% and 1% levels decreased the loaf volume and affected the other bread characteristics somewhat. Thiogel C seemed to have under certain conditions a slight improving effect. Glutathione added at a comparable sulfhydryl level had a detrimental effect throughout the whole course of the baking process; the loaf volume was considerably decreased and the bread characteristics were affected adversely.

Baking tests of the four experimentally milled flours containing the reducing agents are summarized in Table VIII. The effects of the

TABLE VIII
EFFECTS OF REDUCING AGENTS ON BAKING CHARACTERISTICS OF
EXPERIMENTALLY MILLED FLOURS

FLOUR	REAGENT	LOAF	TOTAL
		VOLUME	
	$\mu\text{moles SH/g.}$	ml.	
Hard red winter	...	780	81
Gelatin, 0.25%	0	850	83
Thiogel A	0.35	775	82
Thiogel B	0.33	790	83
Thiogel C	0.08	795	80
Glutathione	0.33	705	75
Soft red winter	...	710	72
Gelatin, 0.25%	0	750	77
Thiogel A	0.35	700	72
Thiogel B	0.33	690	72
Thiogel C	0.08	725	76
Glutathione	0.33	695	75
Northern spring	...	900	84
Gelatin, 0.25%	0	905	87
Thiogel A	0.35	875	88
Thiogel B	0.33	835	86
Thiogel C	0.06	965	92
Glutathione	0.33	810	86
Durum	...	675	73
Gelatin, 0.25%	0	660	72
Thiogel A	0.35	650	68
Thiogel B	0.33	550	59
Thiogel C	0.06	665	69
Glutathione	0.33	550	54

reducing agents depended on the kind of flour used. Whereas glutathione had a consistently high detrimental effect, the thiolated gelatins varied in their effect on the different flours. High levels of Thiogel A or B decreased loaf volume and bread quality appreciably. Thiogel C, low in $-SH$ content, had little or no effect on flours milled from hard red winter, soft red winter, or durum wheat. Both the commercially milled strong flour and the experimentally milled flour from northern spring wheat seem to be improved slightly by the addition of Thiogel C. This effect is comparable to that observed on addition of very low levels of proteases and seems to be a mellowing effect of the "bucky" high-protein flour.

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