Studies with Radioactive Tracers. XI. The Use of N-Ethylmaleimide-1-^{14}C in the Determination of Flour Sulfhydryls and Correlations between Masked Sulfhydryls and Loaf Volumes[1]

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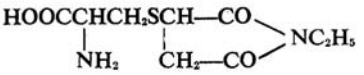
ABSTRACT

Further studies are reported on the radiochemical method for determining sulfhydryls of flour proteins, based on the reaction of the –SH group with N-ethylmaleimide-1-^{14}C, followed by hydrolysis to give ^{14}C-labeled S-succinyl-L-cysteine (III-^{14}C). This hydrolysis product, III-^{14}C, is separated by paper chromatography and its activity is converted to –SH concentration with the aid of a calibration with known concentrations of glutathione. By this method, the flour sulfhydryls can be separated into water-soluble and water-insoluble fractions and into accessible and masked types. Such –SH determinations were carried out on 21 flours and the results were analyzed statistically with loaf volume data obtained from baking with various levels of bromate. Highly significant positive correlations were obtained between masked sulfhydryls and loaf volumes. The highest correlation coefficients were found between masked sulfhydryls and maximum loaf volumes (+ 0.78**) and between protein contents and masked sulfhydryls (+ 0.77**).

From a study of the adducts formed between N-ethylmaleimide (NEMI) and L-cysteine or glutathione (adducts I and II, respectively), Lee and Samuels (1) have noted that under certain conditions some adduct I could be recovered after the peptide bonds of II were cleaved by hydrolysis. This observation led to the suggestion that the radioactivity of isotopically labeled adduct I, recovered after hydrolysis of NEMI-treated, ^{35}S-labeled peptides or of ordinary peptides treated with ^{14}C-labeled NEMI, can be used as a measure of the original sulfhydryl content. Preliminary studies based on these assumptions have indicated that, under appropriate conditions, the recovered adduct I could indeed be utilized for this purpose (2–4). Since adduct I can be further hydrolyzed to S-succinyl-L-cysteine (III) and ethylamine, the use of adduct I as a measure of the original sulfhydryl content will depend on the proper choice of hydrolysis conditions which will lead to a constant recovery of this adduct (4). It has also been pointed out (4) that a radiochemical determination based on adduct III should be feasible, provided the conditions of hydrolysis are such that labeled adduct I is completely converted to labeled adduct III. While this work was in progress, Tkachuk and Hlynka (5) reported the use of N-ethylmaleimide-1-^{14}C (NEMI-^{14}C) in the determination of flour sulfhydryls, based on the activity of the recovered adduct III-^{14}C. This paper reports further experimental data which confirm the applicability of such a radiochemical method for the assay of flour sulfhydryls, and describes the separation of these sulfhydryls into water-soluble and water-insoluble fractions as well as into accessible and masked types. Also presented are the results from studies made with 21 flours of different baking charac-

[1]Contribution from the Department of Chemistry and Chemical Engineering. For paper X, see Liau, Y. H., and Lee, C. C., Cereal Chem. 43: 706–715 (1966).



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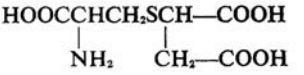

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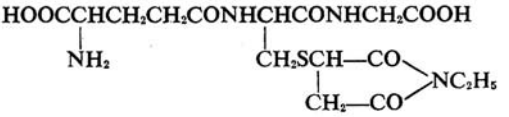

II

teristics to ascertain if any correlations exist between sulfhydryls and baking quality.

MATERIALS AND METHODS

N-Ethylmaleimide-1-^{14}C, thiolated gelatin (12 –SH groups per mol. wt. of 100,000), reduced glutathione, and L-cysteine hydrochloride were obtained from Schwarz BioResearch Inc., Orangeburg, N. Y. The adducts I-^{14}C and II-^{14}C, from reaction of NEMI-^{14}C with CySH or GSH, were prepared as previously described (1). For the preliminary studies, flour A was milled from Thatcher wheat and flour B was supplied by C. C. Tsen of the Grain Research Laboratory, Winnipeg, Man.

Of the 21 flours used in investigating possible correlations between sulfhydryls and baking quality, two were untreated, commercial flours obtained from the mill of the Saskatchewan Wheat Pool (designated Nos. 9 and 14); the remainder were derived from plant breeders' samples of hard red spring wheat originating from the Department of Crop Science of the University of Saskatchewan. Bread was baked from 100 g. flour, 3.0 g. yeast, 5.0 g. sucrose, 1.75 g. sodium chloride, 3.0 g. shortening, 4.0 g. nonfat dry milk, 0.1 g. ammonium dihydrogen phosphate, 0.3 g. nondiastatic malt, 0, 10, 20, or 30 p.p.m. potassium bromate, and absorption to suit the flour.

The procedures developed in the present work for the determination of sulfhydryls involve treatment of the peptide or flour with an excess of NEMI-^{14}C followed by complete hydrolysis to give III-^{14}C, the latter being separated by paper chromatography. The activity of the III-^{14}C spot, on comparison with suitable calibration data from a known sulfhydryl compound, serves as a measure of the original sulfhydryl content. The method differs from that of Tkachuk and Hlynka chiefly in that paper chromatography and an ordinary windowless Geiger counter were used instead of the more elaborate amino acid analyzer and scintillation detector (5). In studies with model sulfhydryl compounds, Tkachuk and Hlynka also buffered the reaction mixtures at pH 5.5; however, since it is known that sulfhydryls react rapidly with NEMI as long as the medium is not highly acidic (6), it was unnecessary to add buffer to the aqueous medium in the present work.

Determination of Sulfhydryls in Soluble Peptides

To a solution of the sulfhydryl sample (GSH or thiolated gelatin), a solution containing an excess of NEMI-^{14}C was added. In most of the

present studies, initially the reaction mixture contained about 0.005 to 0.10 μmole of sulfhydryl and about 0.50 μmole of NEMI-^{14}C. The mixture was allowed to stand at room temperature overnight. An appropriate amount of inactive GSH-NEMI adduct II as carrier and a sufficient quantity of 12*N* hydrochloric acid were then introduced, the volume being adjusted so that the final solution would be 6*N* in hydrochloric acid containing 2.0 mg. of adduct II per ml. Aliquots of each sample (½ ml.) were placed in Pyrex tubes, 7 mm. o.d., and degassed by several cycles of freezing (liquid nitrogen) and evacuating to below 10 μ of pressure. The tubes were sealed and the solutions hydrolyzed by heating in an oven at 120°C. for 22 hr. Aliquots (100λ) of each hydrolysate were subjected to descending paper chromatography for about 20 hr.; the solvent used was a mixture of 1-butanol, pyridine, acetic acid, and water at the volume ratio of 30:20:6:24 (2). The chromatograms were developed by spraying with 0.1% ninhydrin in acetone, and the distribution of radioactivity was recorded by a Nuclear-Chicago Actigraph chromatogram scanner. The radioactive and ninhydrin-positive spot corresponding to III-^{14}C was cut out and its activity measured in a windowless gas-flow Geiger counter. This activity, after correction for the background obtained from a control experiment, could then be related to the original sulfhydryl concentration with the aid of an appropriate calibration such as that shown in Fig. 1.

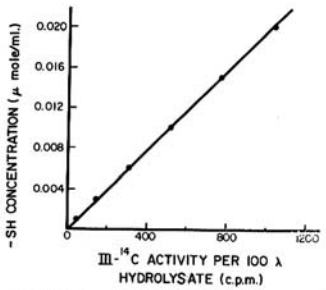

Fig. 1. Calibration relating –SH concentration with III-^{14}C activity using data from reaction of NEMI-^{14}C with GSH.

Determination of Flour Sulfhydryls

To 100 mg. of flour and 0, 36, or 50 mg. of urea was added a solution of 0.48 μmole of NEMI-^{14}C in 100λ of water. The materials were thoroughly mixed with a spatula and the resulting dough was allowed to stand overnight. The dough was then dispersed in an appropriate volume of 6*N* hydrochloric acid by magnetic stirring for about 10 min., or it was extracted several times with water before the residue was dispersed in 6*N* HCl. The aqueous extract was made 6*N* in hydrochloric acid by the addition of 12*N* HCl. After the introduction of inactive adduct II as carrier (2.0 mg./ml.), 0.5-ml. aliquots of the acidified extract or of the suspension from the entire dough or from the water-insoluble residue were degassed, hydrolyzed, and chromatographed for sulfhydryl assay as described for the water-soluble peptides.

RESULTS AND DISCUSSION

Studies with Model Sulfhydryl Compounds

Much of the initial work was done with the known sulfhydryl compounds,

L-cysteine (CySH) and reduced glutathione (GSH). When the adduct I-^{14}C, prepared from CySH and NEMI-^{14}C (1), was subjected to hydrolysis in 6*N* HCl in a sealed tube, two radioactive spots appeared on the paper chromatogram, and these corresponded to unchanged I-^{14}C (R_f 0.49) and III-^{14}C (R_f 0.21), the chromatographic behavior of the latter being identical with that of authentic adduct III prepared according to the method of Morgan and Friedmann (7). Results from these studies also confirmed the earlier conclusion (4) that the extent of hydrolysis of I-^{14}C to give III-^{14}C was greatly influenced by both reaction temperature and reaction time. Some destruction of III-^{14}C was noted if air was present during the hydrolysis. In all subsequent work, following Tkachuk and Hlynka (5), the samples were degassed prior to hydrolysis in 6*N* hydrochloric acid at 120°C. for 22 hr.

When GSH was treated with an excess of NEMI-^{14}C to give adduct II-^{14}C, paper chromatography before and after hydrolysis gave a number of ninhydrin-positive and/or radioactive spots as summarized in Table I. Some

TABLE I

R_f VALUES, RADIOACTIVITY, AND NINHYDRIN SENSITIVITY OF COMPOUNDS INVOLVED IN THE HYDROLYSIS OF ADDUCT II-^{14}C

COMPOUND	R_f	RADIOACTIVITY	NINHYDRIN SENSITIVITY
Glycine	0.12	–	+
S-succinyl-L-cysteine (III-^{14}C)	.21	+	+
Glutamic acid	.24	–	+
GSH-NEMI-^{14}C adduct (II-^{14}C)	.27	+	+
Unknown[a]	.41	+	–
CySH-NEMI-^{14}C adduct (I-^{14}C)	.49	+	+
Unknown[a]	.54	+	–
Ethylamine	.57	–	+
NEMI-^{14}C	0.94	+	–

[a] Presumably from the decomposition of NEMI-^{14}C; comparisons of R_f values suggest that these unknowns are not maleic or fumaric acid.

differences in hydrolytic behaviors have been reported by Tkachuk and Hlynka (5). Apparently these workers found that reaction of NEMI-^{14}C and GSH gave an addition compound accompanied by a rapid release of ethylamine and that no I-^{14}C could be recovered in any of their hydrolysates. These observations might be explained if the analytical procedures used by Tkachuk and Hlynka were to lead to further hydrolysis of I-^{14}C and II-^{14}C. It may also be of interest to point out that in the preparation of II-^{14}C, the isolation of the product from aqueous solution involved the removal of much of the water under reduced pressure at about 50°C. (1). In a trial preparation with the water evaporated off at 70°–80°C. for about 5 hr., it was noted that the product contained some I-^{14}C besides the expected II-^{14}C; this again substantiated the conclusion that the peptide bonds of adduct II could be hydrolyzed with greater ease.

When solutions of GSH of various concentrations were treated with excess NEMI-^{14}C (of relatively low specific activity) and aliquots of the adduct II-^{14}C in 6*N* hydrochloric acid were degassed and hydrolyzed, the relative activities of the resulting III-^{14}C corresponded very well with the initial

sulfhydryl concentrations. Data from one of several such experiments are recorded in Table II. It can be noted from Table II that high recoveries of

TABLE II

DATA FROM HYDROLYSES OF REACTION PRODUCTS BETWEEN NEMI-^{14}C AND VARIOUS CONCENTRATIONS OF GSH AND THIOLATED GELATIN

COMPOUND	SH CONC.	ACTIVITY[a] II-^{14}C[b]	ACTIVITY[a] III-^{14}C Run 1[c]	ACTIVITY[a] III-^{14}C Run 2[d]	III-^{14}C RECOVERY[e] Run 1	III-^{14}C RECOVERY[e] Run 2	RELATIVE ACTIVITY[f] II-^{14}C	RELATIVE ACTIVITY[f] III-^{14}C[g]
	μmole/ml.	*c.p.m.*	*c.p.m.*	*c.p.m.*	%	%		
GSH	0.200	308	289	12	94	4	0.20	0.20
	0.600	925	875	608	95	66	0.60	0.61
	1.000	1,540	1,473	1,129	96	73	1.00	1.02
	1.200	1,813	1,745	1,279	96	71	1.18	1.21
Thiolated gelatin	0.300		419	153				0.29
	0.600		872	598				0.60

[a] Each value is the average of at least duplicate experiments. Chromatograms were developed with 100-λ aliquots of solution.
[b] From chromatograms of aqueous solutions of reaction mixtures without hydrolysis.
[c] Degassed at below 10 μ pressure prior to hydrolysis.
[d] Degassed at about 500 μ pressure prior to hydrolysis.
[e] The activities of II-^{14}C before hydrolysis were taken as 100%.
[f] Based on the data from run 1.
[g] Relative to the activity of the sample originally containing 0.2 μmole GSH/ml. as 0.20.

III-^{14}C with the expected relative activities were observed only in run 1, in which the samples were degassed at below 10 μ pressure prior to hydrolysis. With poor degassing (run 2), the low recoveries of III-^{14}C indicated its partial destruction during the hydrolysis reaction.

Included in Table II are the data from two samples of thiolated gelatin. For run 1 carried out with effective degassing, the relative activities of the resulting III-^{14}C agreed very well with expectation from the initial sulfhydryl concentrations. If the sulfhydryl content of the thiolated gelatin had not been known, it would have been possible to evaluate the sulfhydryl concentrations by interpolation from the GSH data. In other words, the activities of III-^{14}C derived from known concentrations of GSH could serve as a calibration for the assay of sulfhydryls of other peptides.

Calibration Relating III-^{14}C Activities and Low Concentrations of GSH Sulfhydryls

To extend the radiochemical determination to low concentrations of sulfhydryls, it was necessary to add inactive GSH-NEMI adduct II (2.0 mg./ml. of 6*N* HCl) as carrier prior to the hydrolysis to minimize any loss of the minute amounts of II-^{14}C or III-^{14}C. Also, it was noted that results were best if the activities of the chromatogram spots corresponding to II-^{14}C prior to hydrolysis and III-^{14}C after hydrolysis were corrected for the backgrounds of these spots obtained in a control experiment carried out in the presence of NEMI-^{14}C and inactive carrier II, but in the absence of the sulfhydryl-containing sample. A number of such experiments were tried with various concentrations of GSH, down to as low as 0.001 μmole/ml. As illustrations of the results, two sets of typical data are summarized in Table III. Results from experiment 1 (Table III) show that the relative activities

TABLE III

DATA FROM HYDROLYSES OF REACTION PRODUCTS BETWEEN NEMI-^{14}C AND LOW CONCENTRATIONS OF GSH

Exp. No.	–SH Concentration	Activity[a] II-^{14}C[b] Without Carrier	Activity[a] II-^{14}C[b] With Carrier[c]	Activity[a] III-^{14}C with Carrier[c]	III-^{14}C Recovery[d] with Carrier	Relative Activity[e] II-^{14}C Without Carrier	Relative Activity[e] II-^{14}C With Carrier	Relative Activity[e] III-^{14}C with Carrier
	μmole/ml.	*c.p.m.*	*c.p.m.*		%			
1	0.100	2,779	2,591	2,450	95	0.117	0.099	0.100
	0.070	1,879	1,812	1,714	95	.079	.069	.070
	0.050	1,292	1,314	1,252	95	.054	.050	.051
	0.018	414	473	445	94	.017	.018	.018
	0.010	238	263	246	94	0.010	.010	.010
2	0.020		1,140	1,044	92		.020	.020
	0.015		838	772	92		.015	.015
	0.010		558	519	93		.010	.010
	0.006		318	306	96		.006	.006
	0.003		162	146	92		.003	.003
	0.001		53	51	96		0.001	0.001

[a] Each value is the average of at least duplicate experiments. Chromatograms were developed with 100-λ aliquots of solution.

[b] From chromatograms of aqueous solutions of reaction mixtures without hydrolysis.

[c] Inactive adduct II, amounting to 2.0 mg./ml., was added as carrier before hydrolysis. The activities of II-^{14}C and III-^{14}C were corrected for the background activities obtained in control experiments with the carrier present.

[d] The activities of II-^{14}C before hydrolysis were taken as 100%.

[e] Relative to the activity of the sample originally containing 0.010 μmole GSH/ml. as 0.010.

of II-^{14}C are in better agreement with the original –SH concentrations when inactive carrier is used. Figure 1 shows a straight-line plot of –SH concentration *vs.* III-^{14}C activity for the results from experiment 2 in Table III. This plot can serve as a calibration relating the observed activity of III-^{14}C to the original sulfhydryl concentration, provided the same batch of NEMI-^{14}C is used as reagent. Of course, if another batch of NEMI-^{14}C with a different specific activity were to be utilized, another calibration would be necessary.

Preliminary Tests with Two Flours

The applicability of the present method for determination of flour sulfhydryls was tested with two flours, designated A and B, whose protein contents were, respectively, 15.5 and 14.5%, on 14% moisture basis. The sulfhydryls were determined, with and without the presence of urea (8), for the total flour and after separation into water-soluble and water-insoluble fractions. As illustrations, the activity distributions on the chromatograms of the hydrolysates of the water-soluble and water-insoluble fractions of NEMI-^{14}C-treated flour A, without urea, are shown in Fig. 2. It is seen that radioactive spots besides that of III-^{14}C are present. These likely resulted from decomposition and/or reaction of the excess NEMI-^{14}C with protein components other than sulfhydryl, probably similar to those studied by Smyth and coworkers (9). The III-^{14}C spot, however, is distinctly separate and its activity can be converted to –SH concentration with the calibration shown in Fig. 1.

The results from the studies with flours A and B are summarized in Table IV. As expected, higher –SH contents were found in the presence of urea than in the absence of this denaturing agent. This difference could thus

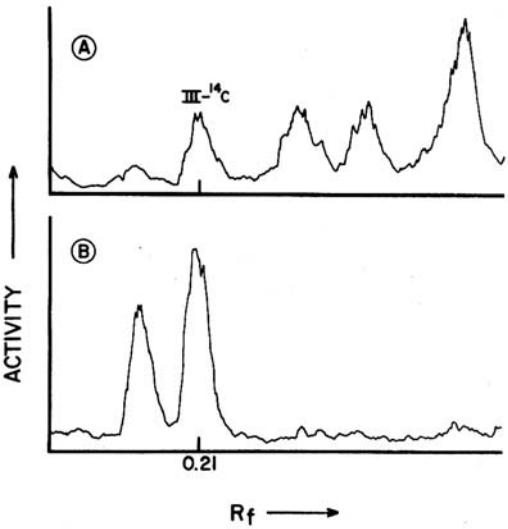

Fig. 2. Activity distributions on paper chromatograms. A, hydrolysate of water-soluble fraction from NEMI-^{14}C-treated flour; B, hydrolysate of water-insoluble fraction from NEMI-^{14}C-treated flour.

serve as a measure of the amount of the less accessible or masked sulfhydryls of the flour. From flour A it is seen that the sum of the water-soluble and water-insoluble sulfhydryls, measured with added urea, agreed very well with the total sulfhydryls for the whole flour. The total sulfhydryl content of flour B, measured by amperometric titration in the presence of urea (8,10), was

TABLE IV

DATA FROM SULFHYDRYL DETERMINATIONS FROM 100-MG. SAMPLES OF FLOURS A AND B

FLOUR	UREA ADDED	FRACTION	TOTAL VOLUME[a]	III-^{14}C ACTIVITY[b]	–SH CONC.[c]	–SH CONTENT[d]
	mg.		*ml.*	*c.p.m.*	*μmole/ml.*	*μmole/g. dry flour*
A	50	Total flour	20	316	0.0062	1.42
	50	Water-soluble	10	112	0.0023	0.27
	50	Water-insoluble	10	515	0.0100	1.14
	0	Water-soluble	10	97	0.0020	0.23
	0	Water-insoluble	10	308	0.0060	0.69
B	50	Total flour	20	284	0.0056	1.31
	50	Total flour	10	585	0.0114	1.33
	36	Total flour	10	570	0.0112	1.31
	50	Water-insoluble	5	947	0.0184	1.08
	36	Water-insoluble	5	940	0.0182	1.06
	0	Water-soluble	3	397	0.0078	0.27
	0	Water-soluble	5	223	0.0044	0.26
	0	Water-insoluble	5	776	0.0150	0.88
	0	Water-insoluble	5	759	0.0148	0.87

[a] The final volumes, in *6N* HCl, of the suspensions or aqueous extracts; only 0.5-ml. aliquots were subjected to hydrolysis.

[b] Each value is the average of at least duplicate hydrolyses. Chromatograms were developed using 100-λ aliquots of each hydrolysate.

[c] From the calibration shown in Fig. 1.

[d] Corrected for the original moisture contents of 12.6 and 14.5%, respectively, for flours A and B.

found to be 1.32 μmoles per g. of dry flour,[2] a value which is in excellent agreement with the results obtained by the radiochemical method.

The present method of analysis of flour sulfhydryls may be considered to have certain advantages over the more widely used amperometric titrations. Because of the lability of some of the sulfhydryls in flour, conditions for sample preparation and for the titration have to be controlled strictly (8). One great advantage of the present method results from the fact that NEMI-^{14}C reacts with sulfhydryls to form stable adducts which could be subjected to further manipulations, such as fractionation, without the possibility of losses of unprotected sulfhydryls. Another advantage of the radiochemical assay lies in its inherent high sensitivity. In the present work, determinations were made on 100-mg. samples of flour. Theoretically, it should be feasible to use samples of still smaller size, especially if NEMI-^{14}C of higher specific activity were employed. It might also be suggested that although the present work was directed at the determination of flour sulfhydryls, most likely the same method should be applicable for the assay of sulfhydryls in other proteins.

Sulfhydryl Analyses and Baking Studies on Twenty-One Flours

The sulfhydryls of flour have been studied extensively because of the possible roles these groups may play in baking. Recent work, together with discussions of the pertinent literature, has been presented by Sullivan and coworkers (11) and by Mecham and Knapp (12). In the present studies, baking and sulfhydryl analyses were carried out on 21 flours. Attempts were then made to ascertain if there are any correlations between loaf volumes and the various types of sulfhydryls as delineated by the radiochemical method. The experimental results from the 21 flours are summarized in Tables V and VI. Statistical analyses gave the correlation coefficients between protein contents and loaf volumes, between protein contents and the various types of sulfhydryls, and between the various sulfhydryls and loaf volumes. These correlation coefficients are shown in Table VII and the regression equations for the more highly significant correlations are given in Table VIII.

From Table VII, it is seen that of the various types of sulfhydryls, the masked sulfhydryls are most significantly correlated with loaf volumes. The best correlation coefficient (+0.78**) is obtained between masked sulfhydryls and maximum loaf volumes. This relationship is illustrated graphically in Fig. 3. Essentially as good a correlation also exists between protein contents and masked sulfhydryls (+ 0.77**). It may also be noted that the correlation between masked sulfhydryls and maximum loaf volumes is better than any of the correlations between protein contents and the various loaf volumes. Protein contents of flours are routinely determined and used as a criterion of quality. The present findings, if confirmed by more extensive experiments, suggest that the measurement of masked sulfhydryls in flour may have considerable significance in evaluations of baking quality, such as in

[2]The amperometric determination was made by C. C. Tsen of the Grain Research Laboratory, Winnipeg, Man. Since the sulfhydryl content of only one flour was measured by the radiochemical method and by amperometric titration, further studies on more flours by both methods will be needed in order to ascertain any correlations between the two methods.

TABLE V

PROTEIN CONTENTS AND LOAF VOLUME DATA FROM THE 21 FLOURS STUDIED

FLOUR NO.	PROTEIN [a]	LOAF VOLUME [b]				
		$KBrO_3$ LEVEL P.P.M.				
		0	10	20	30	Maximum [c]
	%	cc.	cc.	cc.	cc.	cc.
1	12.9	700	810	830	800	830
2	13.3	750	770	830	880	880
3	13.3	760	850	930	920	930
4	13.5	690	840	850	900	900
5	13.6	780	870	980	930	980
6	13.8	770	890	950	920	950
7	13.8	820	880	900	850	900
8	13.8	745	880	930	800	930
9	13.9	770	825	830	770	830
10	13.9	780	820	890	870	890
11	13.9	830	920	920	880	920
12	14.2	760	900	960	930	960
13	14.2	820	910	920	870	920
14	14.5	880	900	890	840	900
15	14.6	815	920	970	910	970
16	14.7	680	830	910	890	910
17	14.7	830	930	980	920	980
18	15.2	845	910	950	840	950
19	15.4	880	980	900	850	980
20	15.6	830	930	1000	980	1,000
21	15.8	780	930	970	1,020	1,020

[a] N × 5.7, on 14% moisture basis.
[b] Average of at least duplicate loaves.
[c] The highest volume obtained on treatment with 0, 10, 20, or 30 p.p.m. $KBrO_3$.

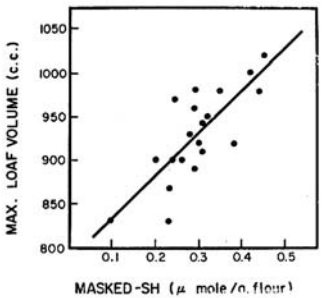

Fig. 3. Correlation between masked sulfhydryls and maximum loaf volumes.

plant-breeding programs. The statistical results in Table VII also indicate that of the various types of sulfhydryls delineated in the present work, masked sulfhydryls are the best index of protein quantity and quality.

As an attempt to differentiate the effects of protein quantity and protein quality, it would be of interest to analyze for possible correlations between masked sulfhydryls and loaf volumes on a basis of constant protein content. When the masked sulfhydryl contents (Table VI) were divided by the corresponding protein contents (Table V), the ratios obtained (masked –SH/percent protein) are measures of masked sulfhydryls on a unit protein

TABLE VI

DATA FROM SULFHYDRYL ANALYSES ON THE 21 FLOURS STUDIED

FLOUR NO.	SULFHYDRYL CONTENT				
	TOTAL –SH[b]	ACCESSIBLE –SH[c]			MASKED –SH[d]
		Water-Soluble	Water-Insoluble	Sum	
	μmole/g. flour[a]	μmole/g. flour[a]	μmole/g. flour[a]	μmole/g. flour[a]	μmole/g. flour[a]
1	0.93	0.17	0.66	0.83	0.10
2	1.01	0.17	0.61	0.78	0.23
3	0.94	0.18	0.48	0.66	0.28
4	1.05	0.19	0.60	0.79	0.26
5	1.04	0.15	0.60	0.75	0.29
6	1.27	0.23	0.72	0.95	0.32
7	0.89	0.17	0.52	0.69	0.20
8	1.10	0.15	0.64	0.79	0.31
9	0.99	0.14	0.62	0.76	0.23
10	1.15	0.18	0.68	0.86	0.29
11	1.19	0.21	0.68	0.89	0.30
12	0.89	0.13	0.47	0.60	0.29
13	0.98	0.13	0.47	0.60	0.38
14	1.06	0.14	0.68	0.82	0.24
15	0.93	0.17	0.52	0.69	0.24
16	1.27	0.22	0.74	0.96	0.31
17	1.03	0.18	0.50	0.68	0.35
18	1.15	0.17	0.66	0.83	0.32
19	1.20	0.24	0.53	0.77	0.43
20	1.32	0.22	0.68	0.90	0.42
21	1.47	0.27	0.76	1.03	0.44

[a] On dry basis.
[b] Determined in the presence of urea.
[c] Determined in the absence of urea (urea has practically no effect on the amount of water-soluble –SH).
[d] Difference between total –SH and sum of accessible sulfhydryls.

TABLE VII

CORRELATION COEFFICIENTS BETWEEN PROTEIN CONTENTS AND LOAF VOLUMES, PROTEIN CONTENTS AND SULFHYDRYLS, AND SULFHYDRYLS AND LOAF VOLUMES

	PROTEIN	LOAF VOLUME				
		$KBrO_3$ LEVEL				
		0 p.p.m.	10 p.p.m.	20 p.p.m.	30 p.p.m.	Maximum
Protein		+0.53*	+0.75**	+0.56**	+0.42	+0.70**
Total –SH	+0.64**	+0.09	+0.26	+0.33	+0.46*	+0.48*
Water-soluble –SH	+0.49*	+0.00	+0.25	+0.22	+0.50*	+0.45*
Water-insoluble, accessible –SH	+0.20	–0.23	–0.23	–0.08	+0.07	–0.07
Sum of accessible –SH	+0.32	–0.18	–0.10	+0.01	+0.22	+0.10
Masked –SH	+0.77**	+0.43*	+0.66**	+0.61**	+0.56**	+0.78**

basis. Statistical analysis with the various loaf volumes gave positive correlations. The correlation coefficients between masked –SH/percent protein and loaf volumes at 0, 10, 20, and 30 p.p.m. $KBrO_3$ and maximum loaf volume are, respectively, + 0.35, + 0.62**, + 0.59**, + 0.52**, and + 0.73**. These values are only slightly lower than the corresponding ones

TABLE VIII

REGRESSION EQUATIONS BETWEEN DEPENDENT VARIABLE Y AND INDEPENDENT VARIABLE X

X[a]	Y[a]	CORRELATION COEFFICIENT	REGRESSION EQUATION	STANDARD ERROR
Protein	Total –SH	0.64**	Y = -0.67 + 0.13X	0.12
Protein	Masked –SH	0.77**	Y = -0.83 + 0.079X	0.05
Protein	Loaf vol., no $KBrO_3$	0.53*	Y = 254 + 37.5X	49
Protein	Loaf vol., 10 p.p.m. $KBrO_3$	0.75**	Y = 189 + 48.6X	35
Protein	Max. loaf vol.	0.70**	Y = 301 + 44.3X	37
Masked –SH	Loaf vol., no $KBrO_3$	0.43*	Y = 703 + 276X	48
Masked –SH	Loaf vol., 10 p.p.m. $KBrO_3$	0.66**	Y = 752 + 421X	40
Masked –SH	Max. loaf vol.	0.78**	Y = 783 + 489X	33

[a] Protein in %, –SH in μmole/g. flour, loaf volume in cc.

given in Table VII between total masked –SH and the various loaf volumes without any correction for variations in protein contents. It is seen that high and positive correlations still exist, especially with maximum loaf volume, even after variations in protein contents are taken care of. The data thus indicate that at least for the 21 flours studied, masked sulfhydryl contents are positively related to baking quality.

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Literature Cited

1. LEE, C. C., and SAMUELS, E. R. Adducts from the reaction of N-ethylmaleimide with L-cysteine and with glutathione. Can. J. Chem. 39: 1152–1154 (1961).
2. LEE, C. C., and SAMUELS, E. R. A radiochemical method for the estimation of the sulfhydryl to disulphide ratio in wheat gluten. Can. J. Chem. 40: 1040–1042 (1962).
3. LEE, C. C., and SAMUELS, E. R. (Communication to the Editor). Studies with radioactive tracers. VI. The oxidation of sulfhydryl groups of gluten by bromate and iodate. Cereal Chem. 39: 482–484 (1962).
4. LEE, C. C., and SAMUELS, E. R. The possible use of ^{14}C-labeled N-ethylmaleimide for the estimation of sulfhydryl groups. Can. J. Chem. 42: 164–167 (1964).
5. TKACHUK, R., and HLYNKA, I. Reactions of flour protein sulfhydryl with N-ethylmaleimide and iodate. Cereal Chem. 40: 704–716 (1963).
6. LEE, C. C., and SAMUELS, E. R. The kinetics of reaction between L-cysteine hydrochloride and some maleimides. Can. J. Chem. 42: 168–170 (1964).
7. MORGAN, E. J., and FRIEDMANN, E. Interaction of maleic acid with thiol compounds. Biochem. J. 32: 275–287 (1953).
8. SOKOL, H. A., MECHAM, D. K., and PENCE, J. W. Further studies on the determination of sulfhydryl groups in wheat flours. Cereal Chem. 36: 127–133 (1959).
9. SMYTH, D. G., NAGAMATSU, A., and FRUTON, J. S. Some reactions of N-ethylmaleimide. J. Am. Chem. Soc. 82: 4600–4604 (1960).
10. TSEN, C. C., and HLYNKA, I. Flour lipids and oxidation of sulfhydryl groups in dough. Cereal Chem. 40: 145–153 (1963).
11. SULLIVAN, BETTY, DAHLE, L. K., and SCHIPKE, J. H. The oxidation of wheat flour. IV. Labile and nonlabile sulfhydryl groups. Cereal Chem. 40: 515–531 (1963).
12. MECHAM, D. K., and KNAPP, CHERYL. The sulfhydryl content of doughs mixed under nitrogen. Cereal Chem. 43: 226–236 (1966).

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