Evaluation of Sulfhydryl Oxidase as a Strengthening Agent for Wheat Flour Dough

S. P. KAUFMAN[1] and O. FENNEMA[2]

Cereal Chem. 64(3):172–176

ABSTRACT

Sulfhydryl oxidase (SO), an enzyme that catalyzes formation of disulfide bonds, was used in trials involving baking (two flour types), mixograph measurements (weak and strong flours), and flour-buffer suspensions to determine whether this enzyme would influence dough rheology. The enzyme was isolated from skim milk membranes using gel exclusion chromatography. SO was tested in the presence of two constituents known to enhance its activity, glutathione disulfide and horseradish peroxidase. 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB) was used to monitor SO activity during its purification and during subsequent trials with flour and buffer suspensions, with turbidity corrections employed in the latter case. SO had no detectable effect on loaf volume, mixograph measurements (mixing stability, extent of dough breakdown), and the number of free sulfhydryl groups in flour. The inability of SO to strengthen wheat dough is likely attributable to its inability to catalyze formation of disulfide bonds in the systems tested, or its known inability under any circumstances to catalyze thiol-disulfide interchange reactions.

Dough conditioners, or bread improvers as they are sometimes called, encompass a large group of dough additives that serve to alter the handling properties of dough or the sensory properties of bread or both. Various groups of conditioners are distinguishable by their respective effects and modes of action (Stauffer 1983). The groups of interest here are oxidants (improvers, or maturing agents) and reductants (dough weakeners).

The mechanistic action of dough strengthening agents (mainly nonspecific oxidizers) has never been clearly elucidated, although sulfhydryl groups and disulfide bonds appear to be involved (Bloksma 1978, Kaczkowski and Mieleszko 1980, Lasztity 1980).

This paper deals with the effects of sulfhydryl oxidase (SO) on wheat flour doughs. SO might be expected to have effects similar to those of chemical oxidizing agents, provided the formation of disulfide bonds is the primary mode by which chemical oxidizing agents function. Although sulfhydryl oxidase has a relatively low degree of substrate specificity when compared to other enzymes, it is far more specific than the chemical oxidizing agents now in use.

SO is a metalloglycoprotein found in the membrane fraction of bovine skim milk, and it catalyzes de novo formation of disulfide bonds in a variety of reduced thiol systems, but it does not catalyze sulfhydryl (SH)-disulfide interchange reactions (Swaisgood and Horton 1980). Cysteine, peptides (e.g., reduced glutathione), and proteins (reduced RNAse, Janolino and Swaisgood 1975; reduced chymotrypsinogen, Janolino et al 1978; reduced xanthine oxidase, Clare et al 1981) are able to serve as substrates for this enzyme (Swaisgood and Abraham 1980). The stoichiometry of the reaction is as follows (Janolino and Swaisgood 1975):

$$2\ RSH + O_2 \longrightarrow RSSR + H_2O_2$$

Swaisgood and Abraham (1980) presented evidence that direct interaction occurs between sulfhydryl oxidase and horseradish peroxidase, and that this interaction results in enhancement of thiol-oxidizing activity. They also noted that glutathione disulfide (0.8 m*M*) prevents the inhibition that high levels of reduced glutathione usually have on SO.

The results of this study should provide some insight as to the mechanisms by which oxidizing agents function in flour dough, and it is, of course, of interest to know whether indiscriminate chemical oxidizing agents can be replaced in part by a more selective enzyme-catalyzed system.

MATERIALS AND METHODS

Isolation of Crude SO

The method used was basically that of Janolino and Swaisgood (1975). The crude enzyme preparation was dialyzed against three changes of 0.047*M* phosphate buffer, pH 7.0, for a total time of not less than 15 hr. The greatest yield of SO was obtained by adjusting the dialysis retentate to 1.5% (w/v) protein and applying it directly to a column of Bio-Gel A-150m, with the sample volume not exceeding 22.5% of the total column volume (void volume +

[1]Present address: General Mills, Inc., 9000 Plymouth Ave., N. Minneapolis, MN 55427.

[2]Person to whom requests for reprints should be directed. Department of Food Science, University of Wisconsin-Madison 53706.

internal volume). The column was then developed with 0.047*M* phosphate, pH 7.0, using the maximum flow rate (1.0–3.0 ml/min). All fractions (10 ml) were collected with a Gilson model FL-100 fraction collector (Gilson Medical Electronics Inc., Middleton, WI). The desired excluded material (void volume fraction) was usually concentrated to a protein concentration of 0.8 mg/ml using an Amicon model 8400 ultrafiltration cell equipped with a PM 30 membrane (Amicon Corporation, Danvers, MA) and operating at 52 psi with nitrogen gas (prepurified).

The final SO preparations used in this study had a mean activity of 0.081 units/mg protein (1.0 unit of SO is an amount sufficient to oxidize 1 μmole of glutathione per minute at 35° C and pH 7.0) and a mean protein concentration of 0.86 mg/ml. A mean yield of 5.4 units of SO was obtained per liter of milk. This SO preparation was used in tests involving flour-buffer suspensions, mixograph measurements, and baking.

Assays for SO Activity

Assays of the SO preparations were performed as described by Janolino and Swaisgood (1975). Assays for SO activity in flour and buffer suspensions were more involved and require a more detailed description. In the following procedure, "treatment" replicates refer to replicates of samples containing active SO, inactive SO, or KIO_3, each used at a particular concentration in a flour-buffer (0.047*M* phosphate, pH 7.0) suspension. "Analytical" replicates refer to replicates taken for analysis from a single sample treatment (entire sampling sequence shown in Fig. 1).

Each treatment replicate containing active or inactive crude SO (0, 3.0, or 6.0 units) was sampled five times. Two 2-ml analytical replicates were reacted with 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) in a dissociating buffer, whereas the three other samples (2.0, 1.0, and 0.5 ml) were simply placed in a dissociating buffer (to determine absorbance caused by light scattering alone). Treatments were normally replicated three times. Typically, enough crude SO preparation to give 3.0 or 6.0 units (assayed just before addition) of activity was combined with 19.5 or 39 units, respectively, of horseradish peroxidase (type I, Sigma) in 0.047*M* phosphate buffer, pH 7.0 to enhance SO activity (Swaisgood and Abraham 1980). GSSG (glutathione, oxidized form, disodium salt; grade IV, Sigma) was then added to achieve a final concentration of 0.08 m*M*. This was added to prevent substrate inhibition of SO (Swaisgood and Abraham 1980). Inactive enzyme controls were autoclaved for 30 min prior to addition of the GSSG. The resulting solutions were then equilibrated at 35 ± 0.5° C. Variable amounts of flour were weighed into beakers so that upon addition of the enzyme/GSSG solutions, a final concentration of 2.00 g of flour per 10 ml of mixture was obtained. The point at which the solutions were mixed with flour was regarded as zero time. The resulting mixture was then placed in a jacketed glass beaker mounted on a magnetic stirrer, and water was circulated through the jacket to maintain a constant reaction temperature of 35 ± 0.5° C. During the first 2 min, the mixture was stirred by hand to disperse flour clumps. After 2.0 min, the magnetic stirrer was started and operated at a speed sufficient to produce a small vortex in the suspension.

After 30 min had elapsed, two 2.0-ml analytical replicates of the mixture were withdrawn from the beaker and each was injected into a 7.0-ml portion of dissociating solution consisting of 5.0*M* Gu-HCl (guanidine hydrochloride; grade I, water soluble, Sigma), 10 m*M* ethylenediaminetetraacetic acid (Sigma), and 8.0*M* urea (A. R. Mallinckrodt) in 0.1*M* phosphate buffer, pH 9.0 (Beveridge et al 1974). Next, 1.0 ml of a solution containing 1.0 m*M* DTNB in 0.047*M* phosphate buffer, pH 7.0, was added, and the mixture was agitated for 30 sec (vortex mixer). The assay mixture was then centrifuged at approximately 1,000 × *g* for 10 min. Three milliliters of the resulting supernatant fluid was removed with a syringe, and the absorbance was determined once at 540 nm, and three times at 412 nm, at 1-min intervals. The elapsed time between addition of the DTNB solution and the readings at 412 nm was also recorded to assure that chromatic instability of the thiol (DTNB) anion did not contribute to experimental error. While the first two 2.0-ml analytical replicates were being centrifuged, the second series (2.0, 1.0, and 0.5 ml) of samples, used to determine the correction for turbidity, was withdrawn, and each was injected into 7.0 ml of the dissociating solution and mixed for 30 sec. The final volume of each of these samples was adjusted to 10.0 ml with 0.047*M* phosphate buffer, pH 7.0. These samples were treated in a manner identical to that given to the previous DTNB analytical replicates, except that the absorbance of each sample was measured only once at 412 nm.

In the positive-control replicates, 0, 3.0, and 10.0 ppm of KIO_3 (reagent grade; Allied Chemicals, New York, NY) were added to

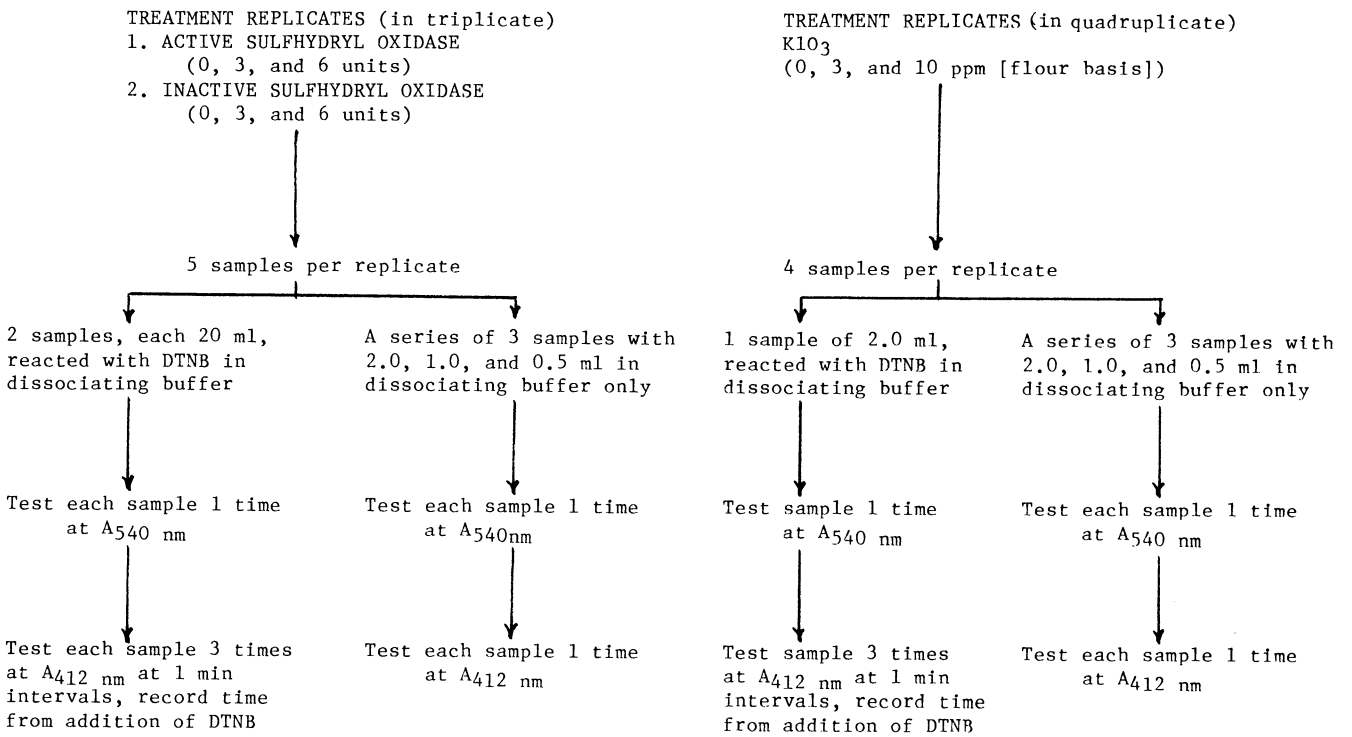

Fig. 1. Diagram of sampling sequence used to determine sulfhydryl oxidase activity in flour and buffer suspensions.

the flour, and the trials were replicated four times at each concentration of KIO_3. The assay for measuring the oxidative action of KIO_3 was identical to that used for the active and autoclaved enzyme preparations, except that only one sample from each treatment replicate was tested for reaction with DTNB.

Determination of Free Sulfhydryl Groups

In those samples containing active or inactive enzyme, two corrections had to be performed to obtain accurate information on flour SH-values. First, the absorbance at 412 nm (absorbance maximum of the thiol [DTNB] anion) had to be corrected for turbidity. This was accomplished in accord with the procedure of Sliwkowski et al (1980), earlier work having indicated the appropriateness of this approach to flour and gluten systems (Beveridge et al 1974). Second, the number of free SH-groups introduced by the enzyme preparation (approximately 10% of the enzyme-flour reaction mixture) had to be determined and corrected for. This was done as described under the subheading Assays for SO Activity.

Determination of Protein Concentration in Crude Enzyme Preparations

Protein determinations were carried out using the dye-binding protein assay of Bio-Rad Laboratories (Richmond, CA). Absorbances were determined at 595 nm using blanks consisting of dye reagent plus 100 μl of 0.047M phosphate buffer, pH 7.0.

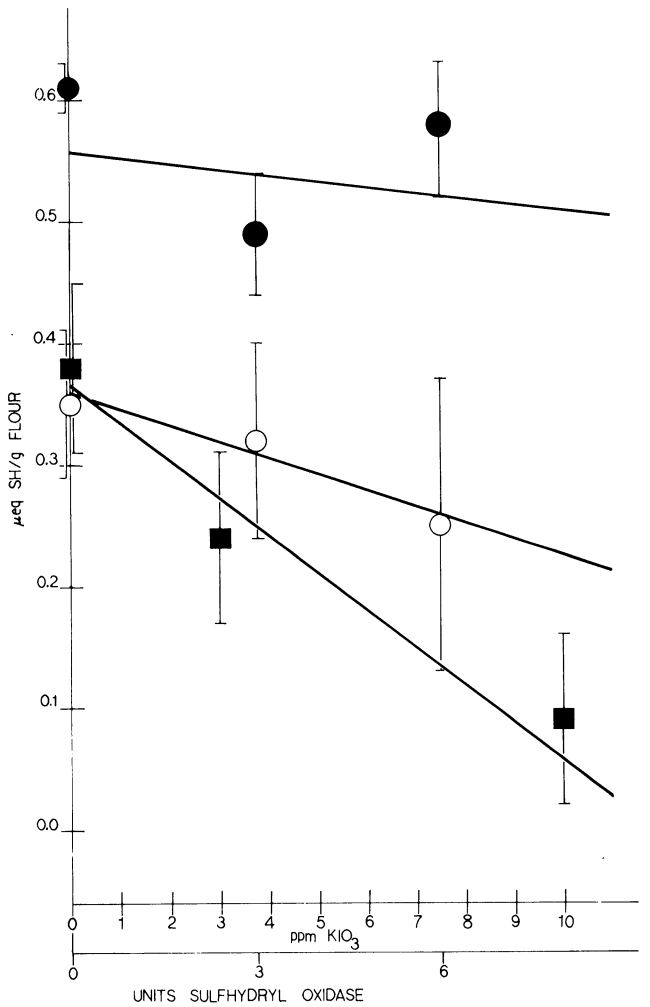

Fig. 2. The effect of sulfhydryl oxidase (active and autoclaved control) and KIO on free sulfhydryl groups in flour-buffer suspensions. Final pH was approximately 6.8. ● = active sulfhydryl oxidase (slope = −0.00548; regression coefficient = −0.2682; O = autoclaved preparation of sulfhydryl oxidase (slope = −0.0167; regression coefficient = −0.9738); ■ = samples treated with KIO_3 (slope = −0.0282; regression coefficient = −0.9822). Error bars represent one standard deviation. Both active and autoclaved preparations of sulfhydryl oxidase contained horseradish peroxidase (active and inactive, respectively), and glutathione disulfide.

Determination of pH Values

During the course of enzyme purification and during studies of flour-buffer suspensions, all pH measurements were conducted with an Orion research model 701A Digital Ionalyzer (Orion Research Inc., Cambridge, MA) equipped with a Corning no. 476050 electrode (Corning Glass Works, Corning, NY).

Measurements during the mixograph studies were performed with a Beckman Expandomatic SS-2 pH meter (Beckman Instruments Inc., Palo Alto, CA). Following completion of selected mixogram traces, the pH electrode was pushed into the resulting dough ball, and the pH was recorded after a stable reading was attained.

Flour History, Protein, and Moisture Data

The flour used in the enzyme/buffer suspension was a commercially available, white, unbleached and unenriched type (Bay State Milling Co., North Quincey, MA). It had a protein content of 10.8% and a moisture content of 12.3%. Between trials, this flour was stored under nitrogen at approximately −10° C.

Two different types of flours were used in the mixograph studies. One, a "weak" flour (relatively short time to mixing peak), had the following properties: variety, Tam 105; growing location, Garden City, KS; protein, 13.0% (Infraalyzer); ash, 0.48%; moisture, 13.0% (Infraalyzer); flour bake absorption, 59.5 ml of H_2O/100 g of flour (NaCl, sugar, yeast, ascorbate and $KBrO_3$); mixograph absorption, 6.25 ml of H_2O/9.90 g of flour (flour and H_2O only).

The second, classified as a "strong" flour (relatively long time to mixing peak), had the following properties: variety, a blend from Kansas State University (Manhattan, KS); protein, 12.4% (Infraalyzer); moisture 13.9% (Infraalyzer); flour bake absorption, 60.0 ml of H_2O/100 g of flour; mixograph absorption, 6.30 ml of H_2O/9.99 g of flour.

Two standard flours different from the above (U.S. Department of Agriculture; unbleached, unenriched), one strong and the other weak, were used in the actual bake tests.

Mixograph Studies Involving Enzyme Preparations

The procedure used was that of Finney and Shogren (1972), and the studies were conducted at the USDA Grain Marketing Research Center (Manhattan, KS). For the most part, the same proportions of constituents as used in the samples containing flour-buffer suspensions were used in the mixograph trials (0.3 units of active or autoclaved SO, 0.8 mM GSSG and 2.0 units of horseradish peroxidase). However, when KIO_3 was used, it was added at much higher levels (1,000 ppm, flour basis) in the mixograph trials than in the flour suspension treatments (10 ppm, flour basis). Active and inactive enzyme preparations (various concentrations), as well as KIO_3 and *o*-phenanthroline (2,100 ppm, flour basis [Eastman Kodak Co.]; in combination with enzyme preparations) were tested on both weak and strong flours. Routinely, active and autoclaved SO, horseradish peroxidase, and GSSG were combined and held for 24 hr prior to mixograph trials.

Bake Tests Using Enzyme Preparations

Bake tests were performed at the USDA Grain Marketing Research Center (Manhattan, KS) using the method of Finney (1984). The breadmaking laboratory was maintained at 25 ± 0.5° C.

Active or inactived SO enzyme preparations (0.3 units) were combined with horseradish peroxidase (189 units) and GSSG (100 ppm, flour basis) 5 min before their addition to the flour.

RESULTS AND DISCUSSION

Activity of SO in Flour and Buffer Suspensions

Data in Figure 2 illustrate the effect of active SO (plus horseradish peroxidase and glutathione disulfide) on the free sulfhydryl content of flour-buffer suspensions. Comparison of the slopes (derived by linear regression) indicates that active SO had

essentially no effect on the free SH-groups of flour, whereas KIO_3 had a substantial effect. Autoclaved samples tended to lower the sulfhydryl content of the samples as the concentration of inactive SO increased. The ability of the autoclaved samples to behave in this fashion (data in triplicate) may be attributable to the release of iron from the enzymes's active center, thus allowing it to catalyze either directly or indirectly (e.g., via lipid hydroperoxides) autoxidation of free SH-groups. Alternatively, freed iron might form complexes with SH-groups, thus accounting in a nonoxidative manner for the observed decline in free SH-groups. The original flour, as it was not enriched, contained little iron, and this iron may not have been free to exert a catalytic role. The marked differences in the y-intercept values for the lines in Figure 2 result from the procedure used to correct for turbidity of the samples. It is apparent from the turbidity correction data in Table I that the slope value for the active enzyme/flour sample was smaller than that of the other samples. This caused the y-intercept of the line for the active enzyme (Fig. 2, upper line) to be positioned above the y-intercepts for the lines for the autoclaved SO and KIO_3-treated samples. This small slope value for turbidity correction indicates that the active enzyme preparation had some property other than turbidity that either reduced the A_{412} nm value or increased the A_{540} nm value relative to the values obtained for the autoclaved and KIO_3 samples. What property might cause this behavior is not clear, but one can speculate that an interaction between SO and horseradish peroxidase may have been involved (Swaisgood and Abraham 1980). Interpretation of data in Figure 2 should rely on the slopes of the curves, not on the y-intercept values.

Mixograph Studies with Sulfhydryl Oxidase

Mixograph trials, always replicated, were conducted using 10-g samples of weak and strong flour. The following variables were tested in the initial trials: 1) flour + 6.3 ml of H_2O; 2) flour + 6.3 ml of 0.047*M* phosphate buffer, pH 7.0; 3) flour + 0.3 units of SO + 2 units of horseradish peroxidase and 0.8 m*M* GSSG in 6.3 ml of 0.047*M* phosphate buffer, pH 7.0; 4) Same as 3 except SO was inactivated in an autoclave prior to use; and 5) flour + 2.3 ml H_2O + 4 ml of 0.047*M* phosphate buffer, pH 7.0, containing sufficient KIO_3 to yield 1,000 ppm on a flour basis.

SO had no noticeable effect on the time to attain the mixing peak (mixing stability) or on the extent of dough breakdown (mixogram tail height) in either weak or strong flours (data not shown). In fact, the flour samples containing phosphate buffer as the only additive exhibited the strongest mixing properties. This is most likely attributable to the well noted "salt effect" (increased mixing time and dough stability; Galal et al 1978). A behavior that was observed with both flours, but especially with the strong flour, was the rapid decrease in dough stability (slender tail and decreased tail height) that occurred in the presence of the autoclaved enzyme preparations (these data, which are not shown, were derived in a manner similar to that described for sample 8 in Fig. 3). This behavior was also observed to a lesser degree with the KIO_3-treated samples (data also not shown) and may have been caused by an oxidative reaction, as a decrease in dough stability by either oxidizing or reducing agents (small molecular weight thiols and disulfides) has been observed by various researchers in mixograph tests (Schroeder and Hoseney 1978, Hoseney et al 1980, Faubion and Hoseney 1981). To test the possibility that some of the dough additives had oxidizing properties, *o*-phenanthroline (iron chelator; *o*-phenanthroline-ferrous complex has no SH-oxidizing capacity; Kobashi 1968) was added to flour samples containing active or autoclaved enzyme preparations. This was done at the beginning of the mixograph trial just after the addition of buffer. One would have expected less dough breakdown in the presence of *o*-phenanthroline if the breakdown had been caused by Fe (free or in protein complexes) catalyzing autoxidation reactions, but this result was not obtained (data not shown). This still left the possibility that dough breakdown may have been caused by SH/disulfide (S-S) interchange between glutathione disulfide (GSSG), present in the active and autoclaved enzyme preparations, and the flour proteins. Data in Figure 3 and Table II reveal that the apparent ability of GSSG to catalyze this reaction diminished as the incubation time of GSSG with active SO and horseradish peroxidase (incubated without flour) was increased. Samples containing autoclaved enzymes did not exhibit this behavior. This result with the active enzyme samples is most likely attributable to the reported interaction of undenatured SO with GSSG (Swaisgood and Abraham 1980). In the samples containing autoclaved enzymes, the observed dough instability is likely caused by SH/S-S interchange of GSSG with the SH/S-S groups of flour proteins, resulting in disaggregation of some large molecular weight flour proteins.

TABLE I
Values Used for Turbidity Correction

Treatment/System	Slope (A_{412} nm/A_{540} nm)	Y Intercept (A_{412} nm)	References
KIO_3/flour	2.36	−0.00385	This study
Autoclaved enzyme/flour	2.32	−0.00214	This study
Active enzyme/flour	1.49	0.0572	This study
None/whey protein concentrate	2.25	0.007	Hardham 1981
None/whole milk	2.94	0.007	Sliwkowski et al 1980
Theoretical	2.95	0.000	Sliwkowski et al 1980

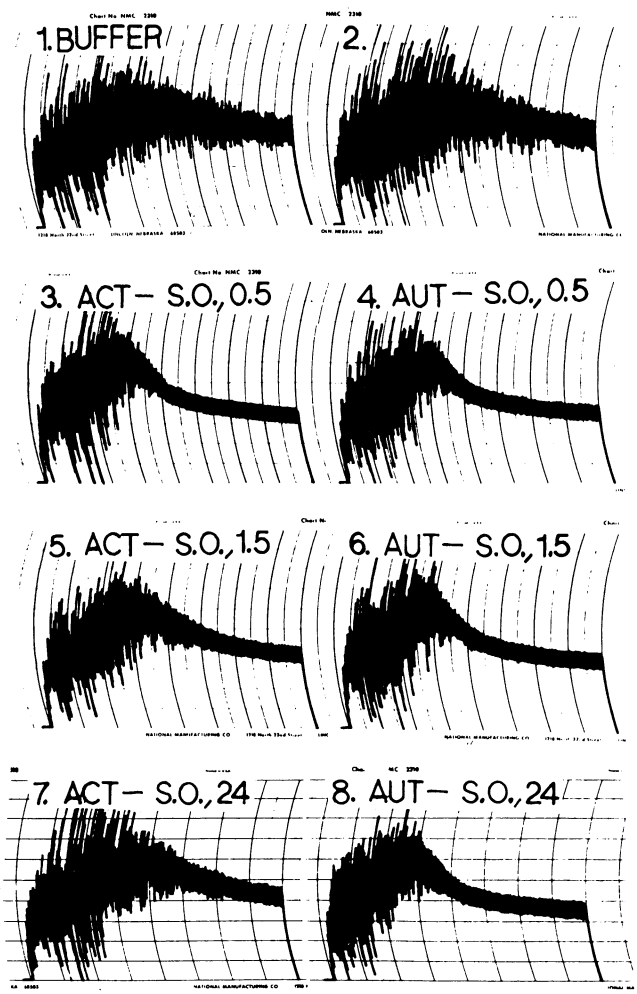

Fig. 3. The effect of sulfhydryl oxidase reaction time (time that sulfhydryl oxidase, glutathione disulfide, and horseradish peroxidase were held in combination prior to mixograph trial) on the mixogram patterns of a weak flour (TAM 105). **ACT** is active; **AUT** is autoclaved, curve 2 is a replicate of curve 1.

TABLE II
Details of Mixograph Trials Depicted in Figure 3

Mixogram No. in Figure 3[a]	Time to Attain Peak Value (min)[b]	Peak Height Obtained (resistance units)[c]	Tail Height Obtained (resistance units)[c]
1. Buffer	3.8	5.8	4.5
2. Replicate of 1	4.0	5.8	4.5
3. Active enzyme, 0.5 hr	3.0	5.9	3.5
4. Autoclaved enzyme, 0.5 hr	3.0	5.5	3.5
5. Active enzyme, 1.5 hr	3.4	5.5	4.8
6. Autoclaved enzyme, 1.5 hr	2.9	6.0	3.5
7. Active enzyme, 24 hr	4.0	5.5	4.2
8. Autoclaved enzyme, 24 hr	3.0	5.9	3.5

[a] 1. Weak flour plus 6.26 ml of H_2O. 3. Weak flour plus 0.3 units of active sulfhydryl oxidase, 2.0 units of horseradish peroxidase, and 0.8 mM GSSG in 6.2 ml of 0.047 M phosphate buffer, pH 7.0. Components, except flour, were combined and held for 0.5 hr prior to mixograph trials. 4. Same as 3 above except that enzymes were autoclaved. 5. Same as 3 above except components (except flour) were combined and held for 24 hr prior to mixograph trials. 6. Same as 5 above except that enzymes were autoclaved. 7. Same as 3 above except components (except flour) were combined and held for 24 hr prior to mixograph trials. 8. Same as 7 above except that enzymes were autoclaved.
[b] Each abscissa division on the mixogram is equal to 1 min.
[c] Each ordinate division on the mixogram has an arbitrary value of 1 resistance unit.

Bake Tests with Sulfhydryl Oxidase

With both flour types used (weak and strong), the active SO preparations had no noticeable effect on loaf volume as compared to the volumes of loaves prepared from the buffer controls. Autoclaved enzyme preparations and GSSG, together or separately, had no effect, or a slight negative effect, on loaf volume. This was presumably due to SH/S-S interchange involving either GSSG or possibly some denatured proteins with the SH/S-S groups of flour proteins. This could have caused disaggregation of some of the flour proteins of larger molecular weight.

CONCLUSIONS

One hypothesis for the action of oxidative dough strengtheners is that large aggregates of gluten protein are created through the formation of disulfide bonds between protein subunits, thereby strengthening the dough and yielding optimum loaf volumes and crumb characteristics. The purpose of this study was to test SO (an enzyme that catalyzes disulfide bond formation de novo) as a dough strengthener.

In flour-buffer suspensions, and in mixograph and bake trials, no evidence was obtained to indicate that active SO catalyzes formation of disulfide bonds in dough, increases mixing tolerance, or increases loaf volume. Because the optimum pH for activity of SO is 6.8–7.0 (Janolino and Swaisgood 1975), one would expect that the flour-buffer trials would have provided ample opportunity for SO to exert its catalytic effects, provided it is capable of acting on flour proteins (pH was approximately 6.8 and lowest pH for SO activity is 5.5; Janolino and Swaisgood 1975). Even if SO had exerted a catalytic effect in the flour-buffer suspensions, a less dramatic effect would be expected in the mixograph and bake trials because the pH of these doughs was approximately 6.0. Assuming that the pH levels prevailing in the test systems did not preclude SO from being active, then two other factors may have contributed to its inability to strengthen dough. First, SO may be unable to catalyze formation of disulfide bonds in the systems tested. If this is so, and if a net increase in the number of disulfide bonds has an important bearing on dough strengthening, then the cause of SO's failure is obvious. The inability of SO to catalyze a net increase in the number of disulfide bonds might have occurred because the thiol groups were insufficiently accessible to the large enzyme molecule, because enzyme specificity precluded formation of the enzyme-substrate complex, or because those thiol groups influenced by SO were not sufficiently abundant to provide a high probability of proper positioning for disulfide formation.

Secondly, SO does not catalyze thiol-disulfide interchange reactions (Janolino and Swaisgood 1975). If these reactions are of major importance in dough strengthening, then SO would not be expected to promote dough strengthening.

ACKNOWLEDGMENTS

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison. The valuable guidance and assistance of Merle D. Shogren of the Grain Marketing Research Center, Manhattan, KS, during the conduct of the mixograph and baking tests is gratefully acknowledged.

LITERATURE CITED

BEVERIDGE, T., TOMA, S. J., and NAKAI, S. 1974. Determination of SH- and SS-groups in some food proteins using Ellman's reagent. J. Food Sci. 9:49.

BLOKSMA, A. H. 1978. Rheology and chemistry of dough. Pages 523-584 in: Wheat Chemistry and Technology. Y. Pomeranz, ed. American Association of Cereal Chemists: St. Paul, MN.

CLARE, D. A., BLAKISTONE, B. A., SWAISGOOD, H. E., and HORTON, H. R. 1981. Sulfhydryl-oxidase-catalyzed conversion of xanthine dehydrogenase to xanthine oxidase. Arch. Biochem. Biophys. 211:44.

FAUBION, J. M., and HOSENEY, R. C. 1981. Lipoxygenase: Its biochemistry and role in breadmaking. Cereal Chem. 58:175.

FINNEY, K. F. 1984. An optimized, straight-dough, bread-making method after 44 years. Cereal Chem. 61:20.

FINNEY, K. F., and SHOGREN, M. D. 1972. A ten-gram mixograph for determining and predicting functional properties of wheat flours. Bakers Dig. 46(2):32.

GALAL, A.M., VARRIANO-MARSTON, E., and JOHNSON, J. A. 1978. Rheological dough properties as affected by organic acids and salts. Cereal Chem. 55:683.

HARDHAM, J. F. 1981. The determination of total and reactive sulfhydryl of whey protein concentrates. Austr. J. Dairy Tech. 36:153.

HOSENEY, R.C., RAO, H., FAUBION, J., and SIDU, J. 1980. Mixograph studies. IV. The mechanism by which lipoxygenase increases mixing tolerances. Cereal Chem. 57:163.

JANOLINO, V. G., SLIWKOWSKI, M. X., SWAISGOOD, H. E., and HORTON, H. R. 1978. Catalytic effect of sulfhydryl oxidase on the formation of three-dimensional structure in chymotrypsinogen A. Arch. Biochem. Biophys. 191:269.

JANOLINO, V. G., and SWAISGOOD, H. E. 1975. Isolation and characterization of sulfhydryl oxidase from bovine milk. J. Biol. Chem. 250:2532.

KACZKOWSKI, J., and MIELESZKO, T. 1980. The role of disulfide bonds and their localization in wheat protein molecules. Ann. Technol. Agric. 29:377.

KOBASHI, K. 1968. Catalytic oxidation of sulfhydryl groups by *o*-phenanthroline copper complex. Biochem. Biophys. Acta 158:239.

LASZTITY, R. 1980. Correlation between chemical structure and rheological properties of gluten. Ann. Technol. Agric. 29:339.

SCHROEDER, L. F., and HOSENEY, R. C. 1978. Mixograph studies. II. Effect of activated double bond compounds on dough-mixing properties. Cereal Chem. 55:348.

STAUFFER, C. E. 1983. Dough conditioners. Cereal Foods World 28:729.

SLIWKOWSKI, M. X., STRANDHOLM, I. J., and SWAISGOOD, H. E. 1980. An improved method for quantitation of sulfhydryl concentration in turbid samples. J. Food Sci. 45:317.

SWAISGOOD, H. E., and ABRAHAM, P. 1980. Oxygen activation by sulfhydryl oxidase and the enzyme's interaction with peroxidase. J. Dairy Sci. 63:1205.

SWAISGOOD, H. E., and HORTON, H. R. 1980. Sulfhydryl oxidase: Oxidation of sulfhydryl groups and the formation of three-dimensional structure in proteins. Pages 205-222 in: Sulphur in Biology, Ciba Foundation Symp. 72. K. Elliott and J. Whelan, eds. Excerpta Medica: Amsterdam.

[Received May 30, 1986. Revision received January 26, 1987. Accepted January 28, 1987.]