COMMUNICATION TO THE EDITOR

Studies with Radioactive Tracers. VI. The Oxidation of Sulfhydryl Groups of Gluten by Bromate and Iodate

DEAR SIR:

The possible involvement of the sulfhydryl group in the action of oxidizing improvers has held the interest of cereal chemists for many years (3,6). A principal obstacle to an unequivocal demonstration of the participation of sulfhydryls in improver action has been the low level at which sulfhydryls occur in flour and the consequent analytical difficulties in tracing changes in sulfyhdryl content (5). This Communication gives a preliminary report on our attempts to show, by the radioactive tracer technique, the destruction of part of the sulfhydryls in gluten by bromate and iodate.

The method for the estimation of sulfhydryl content is based on our finding that the adduct formed between N-ethylmaleimide (NEMI) and L-cysteine, S-(N-ethylsuccinimido)-L-cysteine (I), is recoverable after hydrolysis in 6*N* hydrochloric acid at 100°–103°C. for 18

hours (4). If an S^{35}-labeled protein were allowed to react with NEMI and then hydrolyzed, any sulfhydryl groups originally present in cysteine residues should give rise to S^{35}-labeled I, which may be isolated with the aid of ordinary I as carrier. It is of interest to point out that prolonged acid hydrolysis of I at higher temperature will result in its destruction to give S-succinyl-L-cysteine (2)[1]. However, we have found by trials with radioactive adduct I, prepared from N-ethylmaleimide-1-C^{14} and L-cysteine, that recovery of I is essentially constant at 81 ± 1% after hydrolysis of I alone, or with added gluten, in 6*N* hydrochloric acid at 100°–103°C. for 18 hours.

In the present experiments, carrier-free sulfur-35 as sulfate was injected into maturing Thatcher wheat about 20 days before harvest. The harvested kernels were milled to provide radioactive flour. Fractionation studies showed that various protein fractions do contain S^{35}, indicating incorporation of the injected activity into flour proteins. Samples of dough were prepared, each from 15 g. of active flour and 7 ml. of water containing no improver, 50 p.p.m. potassium bromate or 50 p.p.m. potassium iodate; they were allowed to stand for 3 hours, then thoroughly mixed with a solution of 400 p.p.m. NEMI in 2 ml. of distilled water, and then allowed to stand one more hour. The gluten was then prepared in the usual manner (1) and dried at 100°C. A 1-g. sample of dried gluten was pulverized and hydrolyzed at 100°–103°C. for 18 hours in 50 ml. of 6*N* hydrochloric acid. The hydrolysate was concentrated under reduced pressure, filtered, and made up to a volume of 25 ml. Nonradioactive NEMI-cysteine adduct (I) equivalent to 1 mg. per ml. of solution was added as carrier to aid in its subsequent development and separation by paper chromatography.

While one-dimensional ascending paper chromatography was effective in separating I from cystine (4), it was found that in order to separate I from methionine as well, it was necessary to use two-dimensional ascending paper chromatography, run at right angles. The solvent systems used were 1-butanol, pyridine, acetic acid, water (BPAW) at the ratio of 30:20:6:24, and water-saturated phenol (454 g. phenol and 113.5 ml. water). The chromatogram for 5λ of hydrolysate was run on Whatman No. 1 filter paper first in the BPAW system for about 18 hours, dried in air, and then run in the second solvent for about 20 hours. After air-drying again, the chromatogram was developed with 0.1% ninhydrin in acetone. The spots correspond-

[1] Tkachuk, R., and Hlynka, I. Tracer studies of the reaction of flour protein sulfhydryl with iodate, bromate, and N-ethylmaleimide. Presented at the 47th annual meeting, St. Louis, Mo., May 1962.

ing to cystine and adduct (I) were cut out and the S^{35} activities measured in a windowless gas-flow counter. The observed counts for the cystine spots were of the order of 5,000–7,000 c.p.m. while the activities of the spots for adduct (I) were in the range of 100–300 c.p.m., which were several times as high as background and could be quite accurately determined. The activity of I relative to that of cystine was taken as a measure of the sulfhydryl-to-disulfide ratio in the gluten. The results obtained with flours derived from two crops of wheat grown about 1 year apart are as follows.

Treatment	*Sulfhydryl:Disulfide*	
	Crop 1	*Crop 2*
No improver	4.02:100	4.44:100
50 p.p.m. BrO_3	3.67:100	3.70:100
50 p.p.m. IO_3	0.86:100	2.72:100

It can be noted that treatment with either bromate or iodate caused some decrease in the sulfhydryl-to-disulfide ratio in the gluten. The magnitude of this decrease is fairly small for bromate, being of the order of 10–15% for the two flours studied. For the treatment with iodate, the loss of sulfhydryl appears to be quite definite, the sulfhydryl content being decreased by about 80 and 40%, respectively, for crops 1 and 2. These results, therefore, corroborate the idea that at least part of the sulfhydryl groups of gluten is destroyed by oxidative improvers.

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