

COMMUNICATION TO THE EDITOR

Presence and Probable Role of Thioctic Acid in Wheat Flour

DEAR SIR:

Chemists have long sought an explanation of the phenomena involved in the oxidation of flour, but no acceptable theory has been proposed. It is generally believed (3,5,9) that some of the sulfhydryl groups of the flour proteins are oxidized to form disulfide bridges between the peptide chains of the flour proteins or that an exchange between sulfhydryl and disulfide bonds of the proteins is involved. Evidence (2,7) would also indicate that some compound in the lipid fraction (which is closely associated with the protein) is somehow concerned in the oxidation. But the site of the reactive sulfhydryl groups, the sequence of reactions, and the over-all mechanism have remained obscure.

We have identified thioctic acid in the acid-hydrolyzed extract of flour. We believe this compound is concerned in the maturing of wheat flour.

Evidence of the presence of thioctic acid is as follows: Glacial acetic acid was added to a water extract of a straight-grade flour (37 g.) to give a 5% solution (pH 3). The solution was heated 45 minutes in a boiling-water bath and extracted with 30 ml. n-butanol (centrifugation was necessary to destroy colloidal dispersion). The clear butanol extract gave an absorbance at $334\text{ m}\mu$ of 0.40. Addition of 0.01 ml. of tertiary butyl hydroperoxide reduced this value to 0.30 in 17 minutes. Thioctic acid has an absorption maximum at $334\text{ m}\mu$ which is lost on oxidation to the monoxide or further stages (1).

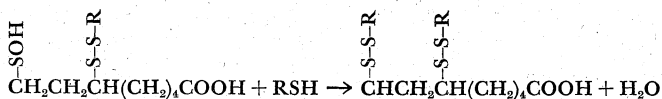
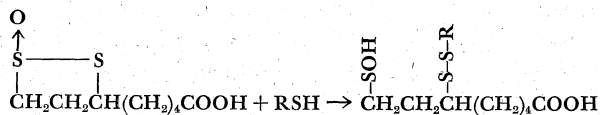
An extract of flour (250 g. flour and 500 ml. water) was made 1.0N with hydrochloric acid and heated 3 hours in a boiling-water bath. The hydrolysate was extracted with 150 ml. benzene and the benzene solution was then extracted with two 100-ml. portions of 5% sodium bicarbonate. The bicarbonate solution was acidified with 40 ml. concentrated hydrochloric acid and then extracted with 50 ml. benzene. One milliliter of the benzene extract was spotted on Whatman No. 1 paper and chromatographed (ascending), employing 1% acetic acid as solvent. After immersion in 0.1N potassium permanganate, a spot showed an R_f value of 0.7. A drop of 30% hydrogen peroxide added to 2 ml. of the benzene extract and similarly chromatographed gave a spot at $R_f = 0.84$. Synthetic dl-thioctic acid gives

identical values under these conditions. The same procedure, employing water-saturated butanol, gave R_f values of 0.95 for the original and 0.63 for the oxidized solution. Literature reports (6) values of 0.9 for unoxidized thiocetic acid and 0.6 for oxidized thiocetic acid (thiocetic monoxide).

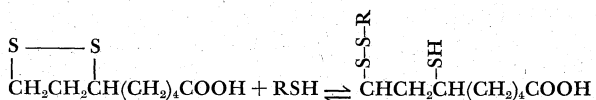
We have also found that, although parachloromercuribenzoate (PCMB) reacts with free $-SH$ groups, these $-SH$ groups can be measured in the presence of PCMB using the amperometric procedure of Kolthoff, Stricks, and Morren (4). Equal titration values for $-SH$ are given in the presence or absence of PCMB with mercuric chloride as titrant. Presumably, mercuric chloride displaces the PCMB from the sulfhydryl groups, the mercuric chloride having a stronger affinity for $-SH$ than PCMB. This provides a means of protecting $-SH$ groups from the time of their solution to the time of measurement. Use is made of this technique in the measurement of the sulfhydryl groups of flour.

Ten grams of a straight-grade, spring wheat flour were dispersed in 200 ml. of 8M urea (purged of oxygen) and then made 0.05M to borax and 0.5M to potassium chloride. The dispersion was amperometrically titrated with 0.001M mercuric chloride using the rotating platinum electrode. A value of 0.0018% $-SH$ was obtained. The same procedure, using 200 ml. 8M urea containing 5 micromoles PCMB, gave a value of 0.0020% $-SH$. The water-soluble $-SH$ measured 0.0009% (quenched with PCMB) and 0.0003% (unquenched). When PCMB is used as a quenching agent, the sulfhydryl content of flour is found to be about equally divided between the water-soluble proteins (albumins and globulins) and the more insoluble gluten proteins. The higher value of $-SH$ in the presence of PCMB is consistently observed in the measurement of total $-SH$ of flour. Since the possibility of air oxidation was excluded by careful purging of all solutions with nitrogen, there is some other factor which lowers the $-SH$ content of flour in the absence of a blocking agent. The divergence of values reported in the literature for water-soluble $-SH$ groups in flour may be due to oxidation of the $-SH$ groups by thiocetic monoxide. Other investigators (8) have demonstrated a higher value of flour $-SH$ in the cold rather than at room temperature. This has been confirmed in this laboratory. The monoxide of thiocetic acid could, conceivably, be the compound which reacts with flour $-SH$ at room temperature, lowering its measurement but decreasing it less at lower temperatures because of less reactivity and not lowering the measurement at all in the presence of a blocking agent such as PCMB.

One could expect the following two-stage reactions:



The following reaction reported by Calvin (1) to be rapid and reversible at pH 8 may also occur at the pH of dough.



Presumably all the sulfhydryl groups compete for the thioctic monoxide sites.

The extent of crosslinking and its consequent effect on the physical properties of a given dough would depend on:

- a) The number of thioctic monoxide sites available,
- b) The total number of sulfhydryl groups and their distribution, and
- c) The working of the dough and the exchange of sulfhydryl with disulfide groups.

The addition of an oxidant, either air or one of the commonly employed maturing agents, would increase the number of thioctic monoxide sites and these, in turn, would decrease the number of reactive SH groups. The correct amount of oxidant and work would produce the proper balance to give the crosslinking in the dough most desirable for optimum baking characteristics.

The sodium salt of thioctic acid and its monoxide used at 20 mg. percent markedly increase the extensibility of dough and decrease the resistance to extension, as measured on the extensigraph.

We are attempting to isolate thioctic acid from wheat flour. The fact of its presence and its ease of oxidation allows a more reasonable explanation of flour maturing than has been possible heretofore, but the over-all mechanism needs further proof.

LELAND DAHLE
BETTY SULLIVAN

*Research Laboratories,
Russell-Miller Milling Company,
Minneapolis, Minnesota*

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