## COMMUNICATION TO THE EDITOR

### Changes in Sulfhydryl Groups of Flour during Storage

DEAR SIR:

The important role of the sulfhydryl groups in determining the properties of flour doughs (2,4,6) prompted us to investigate the changes in the sulfhydryl contents of flours subjected to prolonged storage. Incidental material was available from a long-term study of the effect of packaging material on the storage life of flour (3). Since no information appears available on this topic, results of this short investigation are presented and discussed below.

Four series of flours were selected for the present tests. Two of these series of flours had fully retained their baking strength during storage; the other two series had progressively and markedly deteriorated in baking strength throughout the storage period. All four series of flours had been stored in identical packages. The samples for which data are presented below represent two of the four series flours, one which had fully retained baking strength and one which had undergone marked deterioration. One series of flours had been dried down to 8% moisture before being placed in storage; the other series was stored at the original moisture content, 14.5%. Both series of flours were stored at a constant temperature of 24°C. They were packed in 100-lb. lots, each in a cotton bag overlaid with a laminated heat-sealed moisture barrier material and, in turn, in an outer bag of jute. (These were the 100-lb. barrier bags of the main storage experiment (3).)

At the end of each year during a period of 6 years of storage, one bag of flour of each series was removed from storage. The flour was repacked in paint-type cans of 1-gal. capacity and, after testing for other purposes, had, up to the present experiments, been maintained in cold storage at a temperature of 1° to 2°C.

Sulfhydryl groups in flour were determined according to the method of Sokol, Mecham, and Pence (5) with the following modifications: First, a Goldfisch extraction beaker with a closely fitted cover was used as the titrating vessel. Second, during the dispersion of the sample and the titration, an ice-water bath was not used; instead, nitrogen was flushed continuously into the beaker to minimize oxidation by atmospheric oxygen. The titrations were done in 6*M* urea and in water, yielding presumably total sulfhydryl and accessible sulfhydryl values. Each individual sulfhydryl value was obtained from at least duplicate titrations, and is reported as $\mu$eq./g. of flour (dry basis).

Figure 1 shows that significantly higher sulfhydryl values are ob-

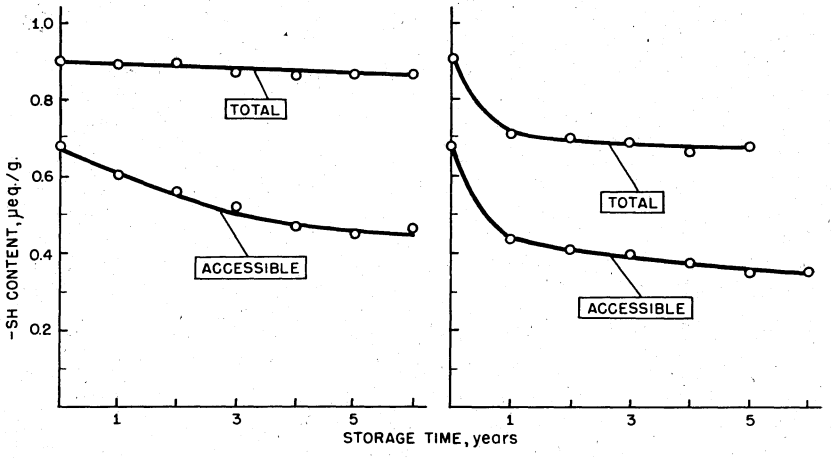


Fig. 1. Changes in sulfhydryl groups of flour during storage. Left: Low-moisture flour (8.0% moisture initially). Right: Normal-moisture flour (14.5% moisture initially).

tained in the presence of urea (the "total" sulfhydryl curve) than in the presence of buffer only (the "accessible" sulfhydryl curve). This is in contrast to the results of Sokol *et al.* (5), who noted approximately equal sulfhydryl values in the presence or absence of urea. We have confirmed our observation not only for all of the stored flours of this study but also for a number of freshly milled flours as well. Such higher values in the presence of urea may result from a more complete dispersion of flour particles than is the case in buffer alone.

Alternatively, urea may cause an unfolding of the structure of the flour protein, thus exposing to the titrant some previously unreactive or masked sulfhydryl groups. This hypothesis has been proposed by Benesch *et al.* (1) for bovine and human serum albumin.

Figure 1 shows that, for the low-moisture flour (left graph), the total sulfhydryl content decreased only slightly during storage. In contrast, the accessible sulfhydryl content of the low-moisture flour decreased more markedly during storage. For the normal-moisture flour (right graph), a marked rapid initial decrease in both total and accessible sulfhydryl groups occurred within the first year of storage. Subsequent changes, however, tended to parallel those occurring for the low-moisture flour.

Changes in the total and accessible sulfhydryl groups essentially similar to those illustrated in Fig. 1 were observed for the other two series of flours in this study; i.e., flour of 8% moisture and flour of 14.5% moisture stored out-of-doors at mean annual temperature of between 7° and 10°C. As noted earlier, all four series of flours were stored in identical packages.

The following postulates may account, at least in part, for the results illustrated in Fig. 1. The gradual decrease in both accessible and total sulfhydryl groups of flour during storage may be due to the oxidation, to a disulfide bond, of two sulfhydryl groups that are in sufficiently close proximity to react with one another. Alternatively, a single isolated sulfhydryl group may be oxidized to a sulfoxide. The greater extent of change in accessible and total sulfhydryl content for the normal moisture flow may indicate that moisture facilitates the sulfhydryl oxidation.

The changes in the sulfhydryl content of the flours as shown in Fig. 1 are most interesting when considered in relation to baking behavior. The low-moisture flours fully retained baking strength during the storage experiment. Moreover, baking tests made coincidentally with the sulfhydryl determinations of this experiment showed that the low-moisture flours have still fully retained their baking strength. On the other hand, normal-moisture flours underwent a progressive deterioration in loaf volume during storage and, in fact, the current baking tests show that these flours have continued to deteriorate.

From the foregoing results it does not seem possible to infer a quantitative relation between changes in the sulfhydryl groups of flour and changes in baking behavior. In this study the difference in sulfhydryl levels between the low- and the normal-moisture flours is relatively small, yet the baking behavior of these flours is drastically differ-

ent. If the sulfhydryl groups of stored flour constitute a major factor in the deterioration in baking properties, then perhaps there may be a limiting or threshold sulfhydryl value below which flour sulfhydryl must fall before deterioration in loaf properties sets in. On the other hand, it is recognized that constituents of these flours, other than the sulfhydryl groups, may possibly be the limiting factor in determining baking behavior.

Further work in this area should prove most interesting.

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