# Reactive and Total Sulfhydryl and Disulfide Contents of Flours of Different Mixing Properties[1]

**C. C. TSEN** and **W. BUSHUK,** Grain Research Laboratory,
Winnipeg 2, Manitoba

**ABSTRACT**

Total and reactive SH and S–S contents were determined for seven flours having dough development times between 25 (very strong) and 2 (weak) min. Total and reactive SH contents and the ratio reactive/total SH increased with decreasing strength. Total S–S contents decreased slightly whereas the reactive S–S and the ratio reactive/total S–S increased with decreasing strength. For the flours examined, mixing strength appears to be inversely related to reactive SH and S–S contents.

It is now well established that the improver action of oxidizing agents on bread flour results from their reaction with sulfhydryl (SH) groups. Furthermore, for optimum improver effect only a fraction of the total SH groups are oxidized. Closely related to the SH groups are the disulfide (S–S) groups. These are important, for two reasons. First, they form strong cross-links between polypeptide chains and hence play an important role in the structure of doughs. Secondly, by reacting with SH groups, they can interchange and hence provide for mobility to the relatively semirigid structure of the dough. The participation of S–S interchanges in the rheological properties of dough was first suggested by Goldstein (1) in 1957 but as yet has not been confirmed by direct experimental evidence.

It was demonstrated previously (2) from this Laboratory that in dough, not all the SH groups are accessible to iodate, a commonly used oxidizing improver. The phenomenon of variable SH reactivity has been demonstrated in other proteins. It seems that for a specific set of conditions only a fraction of the total SH will react, and these have been called accessible, reactive, available, or free SH groups.

A somewhat similar situation apparently exists with S–S groups. In this case, however, differences in reactivity could also arise from the fact that two types of S–S groups can exist—the intra- and interchain groups. From the reaction of the S–S groups of a number of different proteins, Cecil and Wake (3) postulated that the interchain S–S groups react with sulfite in aqueous solution, whereas most of the intrachain groups are not reduced under these conditions. In relation to dough, it seems logical that the interchain S–S groups would be the more important ones in the rheological properties.

To examine further the role of SH and S–S groups in dough structure, it seemed desirable to determine the reactive SH and S–S contents for a series of flours covering a range of flour strength, in the hope that these would be better correlated with rheological properties than the total contents (4). The results obtained are discussed in this paper.

---

[1]Paper No. 268 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Manitoba. Presented at the 52nd annual meeting, Los Angeles, April 1967.

Present address of Tsen: American Institute of Baking, Chicago, Ill. 60611. Present address of Bushuk: Dept. of Plant Science, University of Manitoba, Winnipeg 19, Manitoba.

## MATERIALS AND METHODS

The characteristics of the flours used in this study are given in Table I. All were unbleached and contained no improvers. The wheats were selected to provide a wide range of farinograph mixing properties, and were milled on a laboratory Buhler mill. All are of the 1964-65 crop. The first four are hard red spring wheats and the last three are soft white winters.

TABLE I
DESCRIPTION OF THE FLOURS

| VARIETY | ORIGIN | ASH | PROTEIN | D.D.T.[a] | STRENGTH CLASSIFICATION |
|---|---|---|---|---|---|
| | | %[b] | %[b] | min. | |
| Minnesota II-54-29 | Minnesota | 0.46 | 16.6 | 25 | Very strong |
| Pembina | Manitoba | 0.48 | 13.1 | 10 | Very strong |
| Thatcher | Manitoba | 0.46 | 14.6 | 7.0 | Strong |
| Prairie Pride | Manitoba | 0.43 | 11.3 | 5.0 | Weak |
| Richmond | Ontario | 0.49 | 12.6 | 2.5 | Weak |
| Talbot | Ontario | 0.48 | 11.4 | 2.0 | Weak |
| Genesee | Ontario | 0.48 | 11.0 | 2.0 | Weak |

[a] Farinograph dough development time = time in min. to reach farinogram peak height.
[b] 14% m.b.

Doughs were mixed in the 50-g. farinograph mixer at 63 r.p.m. and 30°C., with 50 g. flour (14% m.b.). Absorptions were adjusted to give a consistency of 500 B.U. The actual absorptions used were 67.8, 62.0, 64.6, 65.4, 55.5, 56.9, and 56.9% for Minnesota II-54-29, Pembina, Prairie Pride, Thatcher, Genesee, Richmond, and Talbot flours, respectively. Figure 1 shows the farinograms of flour-water doughs prepared from these flours.

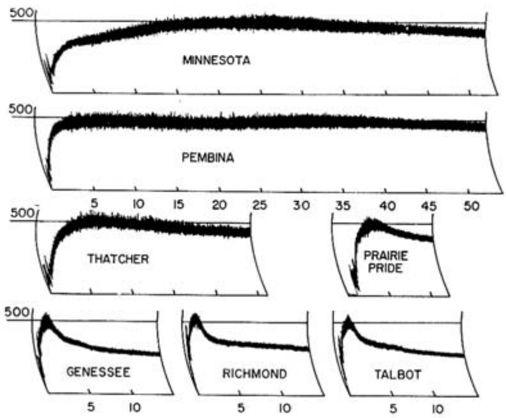


Fig. 1. Farinograms for the seven flours used in the determination of SH and S–S contents.

Separate analyses were used to determine the SH content and the combined SH and S–S (after reduction) content in flour. The S–S content was then determined by difference.

Reactive and total SH contents were determined by amperometric titrations with $AgNO_3$ under nitrogen at −0.10 volt *vs.* the saturated calomel electrode. The SH groups measured by titration in a medium containing 6*M* urea, is considered as the total SH content; the groups, titrated without urea, is the reactive SH content. The titration method was described previously (5).

Total S–S contents were determined by reducing S–S groups with sulfite in the presence of 6*M* urea and then titrating for total SH with $AgNO_3$ by the method of Kolthoff, Shore, Tan, and Matsuoka (6). Reactive disulfide contents were determined by reducing disulfide groups with sulfite in the absence of urea and titrating at 0°C. to prevent the further reduction following addition of $AgNO_3$, as recommended by Cecil and Wake (3). The silver nitrate titration with the platinum electrode gives somewhat lower results for S–S contents from those obtained previously by the ethyl mercuric chloride titration with the use of the dropping mercury electrode (4). The reason for this discrepancy is not known.

## RESULTS AND DISCUSSION

### SH Contents

Total and reactive SH contents, expressed in μeq. per g. of flour and per g. of protein for the seven flours are given in Table II. The last column gives the percentage of the SH groups that are reactive. The seven wheat varieties are listed in order of decreasing farinograph dough development time, referred to as mixing strength in this paper. The last three varieties have essentially the same mixing strength, so that the order shown is quite arbitrary. However, as can be seen from the farinograms in Fig. 1, the seven varieties give a relatively wide range of mixing strengths. For the purpose of this discussion only the contents (of SH and S–S) on the basis of protein content will be considered; the data on flour basis are included for the purpose of comparison with published results.

TABLE II
SULFHYDRYL CONTENTS

| FLOUR | TOTAL SH CONTENT | | REACTIVE SH CONTENT | | REACTIVE SH × 100 / Total SH |
|---|---|---|---|---|---|
| | Flour Basis | Protein Basis | Flour Basis | Protein Basis | |
| | *μeq./g.* | *μeq./g.* | *μeq./g.* | *μeq./g.* | %[a] |
| Minnesota II-54-29 | 1.21 | 6.26 | 0.73 | 3.78 | 60.4 |
| Pembina | 1.21 | 7.95 | 0.80 | 5.26 | 66.2 |
| Thatcher | 1.32 | 7.80 | 0.98 | 5.79 | 74.2 |
| Prairie Pride | 1.07 | 8.17 | 0.82 | 6.26 | 76.6 |
| Richmond | 1.62 | 11.06 | 1.52 | 10.38 | 93.9 |
| Talbot | 1.55 | 11.69 | 1.30 | 9.80 | 83.8 |
| Genesee | 1.70 | 13.29 | 1.43 | 11.18 | 84.1 |

[a] Protein basis.

Table II shows that the total and reactive SH contents and the percentage of reactive SH tend to increase with decreasing mixing strength. Total SH contents increase gradually from 6.26 to 13.29 μeq. per g.; reactive SH contents increase from 3.78 to 11.18 μeq. per g. The percentage of reactive SH increases from 60.4 for the strongest flour to 93.9% for one of the three weak flours. Although the data of Table II show several minor exceptions, the trends indicated here are quite definite and significant.

### S-S Contents

Table III gives the analogous S–S contents of the seven flours. The trends here are not as definite as for SH contents in Table II. There is suggestion that the total S–S decreases slightly with decreasing strength whereas the reactive S–S increases. However, the trends are not definite and there are many exceptions to this generalization. With two minor exceptions, the trend for the percentage of reactive S–S is to decrease with decreasing strength.

TABLE III
DISULFIDE CONTENTS

| Flour | Total S–S Content | | Reactive S–S Content | | Reactive S–S × 100 / Total S–S |
|---|---|---|---|---|---|
| | Flour Basis | Protein Basis | Flour Basis | Protein Basis | |
| | μeq./g. | μeq./g. | μeq./g. | μeq./g. | %[a] |
| Minnesota II-54-29 | 16.97 | 87.84 | 3.00 | 15.53 | 17.68 |
| Pembina | 12.66 | 83.24 | 2.32 | 15.25 | 18.32 |
| Thatcher | 13.40 | 79.20 | 2.35 | 13.89 | 17.54 |
| Prairie Pride | 11.50 | 87.86 | 2.04 | 15.59 | 17.74 |
| Richmond | 12.24 | 83.55 | 2.66 | 18.15 | 21.72 |
| Talbot | 10.95 | 82.58 | 3.00 | 22.63 | 27.40 |
| Genesee | 9.93 | 77.64 | 2.52 | 19.70 | 25.37 |

[a] Protein basis.

## GENERAL DISCUSSION

The results presented here add further support to the already published information on the involvement of SH and S–S groups in the rheological properties of doughs. If this involvement is mainly through the interchange of S–S groups, then it might be speculated that the reactive SH and S–S groups should play a more important role than the unreactive groups. Results obtained in this study support this view, since both reactive SH and S–S increase with decreasing strength, whereas total S–S decreases with decreasing strength.

The observed variations in SH content seem reasonable; total, reactive, and percentage of reactive SH groups increase with decreasing strength. This supports the view that the SH group plays an important role in the rheological properties of dough (3,7). More SH groups, particularly the reactive ones, should facilitate the interchange of S–S bonds in dough. As a result, the dough would be more extensible in the extensigraph or show a weakening in the farinograph. The reduction of S–S bonds by the flour SH groups, as in the first step of the interchange, could also lead to protein depolymerization. This mechanism, which remains to be confirmed, could lead to a decrease in dough strength.

On the other hand, total S–S appears to decrease slightly with decreasing strength, whereas the reactive S–S content increases (protein basis or as percentage of total S–S) with decreasing strength. If the reactive S–S groups are the interchain cross-links as postulated by Cecil and Wake (3), then the protein in weaker flour would have more of this type of S–S bond than the protein from stronger flour. This seems contrary to the known facts on the chemical and physical properties of the proteins from strong and weak flours. A more reasonable explanation is that weaker flours contain more watersoluble protein than the strong flours, even though they are usually lower in protein content. This explanation is in general agreement with the results of Smith and Mullen (8) on the relation of protein solubility to mixing properties.

It is not possible to explain the mechanism by which urea increases the reactivity or accessibility of SH and S–S groups in flour dispersions on the basis of available information. Physiochemical studies are necessary to determine if this effect results from the unfolding of the protein molecules or from the dissociation of aggregates comprising a large number of molecules.

### Acknowledgment

Technical assistance of R. A. McLeod is gratefully acknowledged.

### Literature Cited

1. GOLDSTEIN, S. Sulfhydryl- und Disulfidgruppen der Klebereiweisse und ihre Beziehung zur Backfähigheit der Brotmehl. Mitt. Gebiete Lebensm. Hyg. (Bern) **48:** 87–93 (1957).
2. BUSHUK, W. Accessible sulfhydryl groups in dough. Cereal Chem. **38:** 438–448 (1961).
3. CECIL, R., and WAKE, R. G. The reactions of inter- and intra-chain disulphide bonds in proteins with sulphite. Biochem. J. **82:** 401–406 (1962).
4. TSEN, C. C., and ANDERSON, J. A. Determination of sulfhydryl and disulfide groups in flour and their relation to wheat quality. Cereal Chem. **40:** 314–323 (1963).
5. TSEN, C. C., and HLYNKA, I. Flour lipids and oxidation of sulfhydryl groups in dough. Cereal Chem. **40:** 145–153 (1963).
6. KOLTHOFF, I. M., SHORE, W. S., TAN, B. H., and MATSUOKA, M. A critical study of amperometric titration methods for sulfhydryl determination in native and denatured bovine serum albumin. Anal. Biochem. **12:** 497–508 (1965).
7. FRATER, R., HIRD, F. J. R., MOSS, H. J., and YATES, J. R. A role for thiol and disulphide groups in determining the rheological properties of dough made from wheaten flour. Nature **186:** 451–454 (1960).
8. SMITH, D. E., and MULLEN, J. D. Studies on short- and long-mixing flours. II. Relationship of solubility and electrophoretic composition of flour proteins to mixing properties. Cereal Chem. **42:** 275–287 (1965).

[Received May 29, 1967. Accepted October 24, 1967]