

The Relation Between the Thiol and Disulfide Contents of Dough and its Rheological Properties

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ABSTRACT

In doughs mixed in nitrogen and with various additions of the oxidizing reagents potassium bromate or iodate, of the thiol-blocking compound N-ethyl maleimide, or of the thiol compound glutathione, or mixed in air or oxygen, thiol (-SH) and disulfide groups (-SS-) were titrated with silver nitrate. Total contents of these groups were determined in the presence of 4 and 9M urea, respectively; titrations in the absence of urea produced the contents of the reactive SH and SS groups. From creep and recovery tests at a range of shear stresses the compliance was derived, which could be divided into a viscous (permanent) and an elastic (temporary) part. In addition, extensigrams were recorded. The results show that there is no unequivocal relation between the total SH content and various rheological properties. If all oxidizing or thiol-blocking reagents are added in amounts so as to produce the same decrease in thiol content, the rheological effect of bromate is much larger than that of iodate or N-ethyl maleimide. The behavior of oxygen is intermediate between those of bromate and iodate. A small increase in thiol content owing to glutathione has about the same effect, though in opposite direction, as an equal decrease in thiol content caused by iodate or N-ethyl maleimide. In the range of additions in which rheological measurements could be performed, the ratio of reactive to total SH groups varied little; as a consequence, the above conclusions are also valid for the reactive SH content. No correlations between the total, reactive, or nonreactive SS content and the elastic properties of dough were found. For the explanation of earlier results, it was supposed that the rheologically effective SH and SS groups are only fractions of the total contents of these groups. The present results suggest that the rheologically effective fractions are even much smaller than the chemically reactive ones. If this explanation is true, the slowly acting bromate reacts more specifically with the rheologically effective fractions than do the rapidly acting iodate and N-ethyl maleimide.

Oxidation strongly affects the rheological properties of wheat-flour doughs. It is applied in practice by the addition of minute amounts of reagents like potassium bromate or potassium iodate to flours or doughs, which usually results in a considerable increase in loaf quality. A widely accepted explanation is that oxidation removes thiol groups, the presence of which is essential for the viscous deformation of dough (for a review see ref. 1, sections VI and VIII E). The decrease of the thiol content by added reagents or by atmospheric oxygen has been experimentally established (2-10). Beside this, little work on the quantitative relation between thiol content and rheological properties has been published.

An attempt to establish this relation was undertaken in previous work (10). In this, we varied the thiol content of dough by adding various amounts of potassium iodate, and characterized its rheological properties by creep and recovery tests and by extensigrams. The experimental results were compared with predictions, based on a simple model, which is explained in the discussion of the present paper. This comparison led to the suspicion that only part of the thiol groups and an even smaller fraction of the disulfide groups are rheologically effective.

The experiments reported in the present paper are an amplification of our earlier work. Because of the suspicion that not all thiol and disulfide groups are

rheologically effective, we titrated not only the total contents of these groups, but also the contents of only the chemically reactive ones. To vary the thiol and disulfide contents of the doughs, we used—besides potassium iodate—the oxidizing reagents potassium bromate and oxygen, the thiol-blocking compound N-ethyl maleimide, and, finally, glutathione, which itself carries a thiol group.

Because of the greater variety of reagents, we could bypass most of the assumptions in the earlier paper. As a consequence, the problem can be reduced to the question whether the rheological properties of dough are unequivocally determined by its thiol and disulfide contents as estimated by presently available titration techniques.

EXPERIMENTAL

In this paper, analytical results for the flour and for doughs, as well as dough compositions, are expressed on the basis of flour with 14% moisture.

We used a commercially milled, unbleached and untreated bread flour with 0.51% ash (2 h., 900°C.), 1.93% Kjeldahl N (11.0% protein), and a farinograph water absorption of 57.0%; dough development time was 5 min. Thiol and disulfide contents are reported in Table I.

Doughs were composed of 100 g. of flour, 55.5 ml. of water, and 2.0 g. of sodium chloride; for the extensigraph, twice these amounts were used. The sodium chloride and the reagents added were solved in part of the dough water. Doughs were mixed in a GRL-mixer (11) for 300 sec. at a speed of 7.1 rad. per sec. (68 r.p.m.). Mixing atmosphere was nitrogen, unless stated otherwise. The final dough temperature was 30°C. This temperature was maintained through all operations, except titration of thiol and disulfide groups and the dispersion preceding these titrations. For the storage of doughs we used a cabinet with a high humidity.

For the titration of thiol and disulfide groups, we dispersed the flour or dough (up to 5 g. of the latter) in up to 10 ml. of a 4.5M ammonium nitrate solution by mechanical stirring for about 600 sec.; occasionally a longer time, up to 1,800 sec., was necessary. After dispersion, we added the other solutions so as to make the final compositions (in moles (M) per liter) as follows:

| | <i>SH</i> | | <i>SS + SH</i> | |
|-------------------|--------------|-----------------|----------------|-----------------|
| | <i>Total</i> | <i>Reactive</i> | <i>Total</i> | <i>Reactive</i> |
| Ammonium nitrate | | 1.0 | | |
| Ammonia | | 0.05 | | |
| EDTA ^a | | 0.001 | | |
| Urea | 4 | 0 | 9 | 0 |
| Sodium sulfite | | 0 | | 0.02 |

^aEthylene diamino tetra-acetic acid or versene.

We titrated the dispersion with 0.001M silver nitrate from a piston burett. The end point was detected amperometrically with a platinum electrode at -0.3 v. in respect of the saturated calomel electrode; after each titration, we cleaned the electrode by anodic oxidation. We performed the dispersion and titration under nitrogen at

TABLE I. THIOL AND DISULFIDE CONTENTS OF THE FLOUR, OF CONTROL DOUGHS (MIXED IN NITROGEN), AND OF DOUGHS AFTER MIXING IN AIR OR OXYGEN. EACH ENTRY SHOWS THE MEAN VALUE AND ITS STANDARD ERROR AND, IN PARENTHESES, THE NUMBER OF TITRATIONS

| Object and Mixing Atmosphere | Thiol (-SH) mM./kg. | Disulfide Plus Thiol mM./kg. | Disulfide (-SS-) ^a mM./kg. |
|------------------------------|------------------------------|------------------------------|---------------------------------------|
| | | Total | |
| Flour | 0.71±0.015 (12) | 11.7±0.18 (14) | 11.0±0.18 |
| Dough nitrogen | 0.63±0.010 (11) ^b | 10.3±0.33 (4) ^b | 9.7±0.33 |
| Dough air | 0.47±0.036 (16) ^c | 11.4±0.44 (8) | 10.9±0.44 |
| Dough oxygen | 0.42±0.046 (10) ^c | 11.0±0.10 (5) | 10.6±0.11 ^d |
| | | Reactive | |
| Flour | 0.63±0.014 (7) | 1.96±0.07 (8) | 1.33±0.07 |
| Dough nitrogen | 0.45±0.015 (4) ^b | 1.83±0.10 (5) | 1.38±0.10 |
| Dough air | 0.54±0.038 (9) | 1.91±0.05 (6) | 1.37±0.06 |
| Dough oxygen | 0.36±0.029 (8) | 1.77±0.08 (5) | 1.41±0.09 |

^aBy subtraction.

^bSignificantly ($P < 0.01$) different from flour.

^cSignificantly ($P < 0.01$) different from control dough.

^dSignificantly ($P < 0.05$) different from control dough.

20°C. We titrated test-pieces after various reaction times; the reaction time was measured from the end of mixing until the beginning of the dispersion.

For the measurements of creep and recovery we sheared a test-piece between a cone and a plate in the instrument described earlier (10). Test-pieces were allowed a reaction time of 1,800 sec., measured from the end of mixing until completion of the compression between the plate and cone. After a rest period of 600 sec., the test-piece was loaded with a constant shear stress. We recorded the shear as a function of time during 900 sec. (creep); after removal of the load, we followed the recovery for another 900 sec.

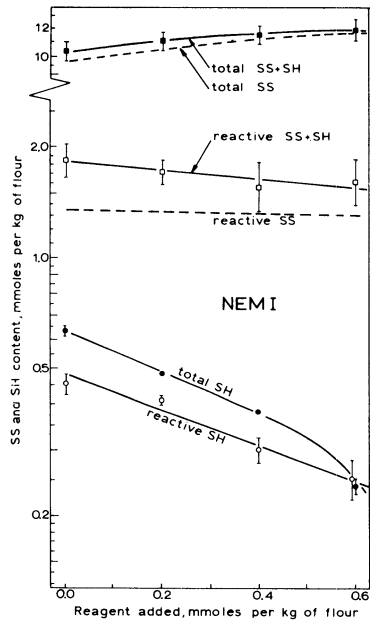
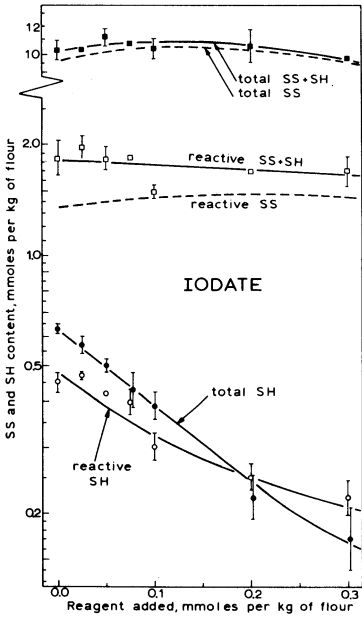
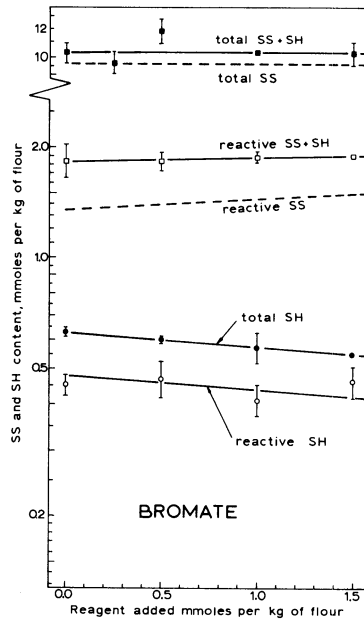
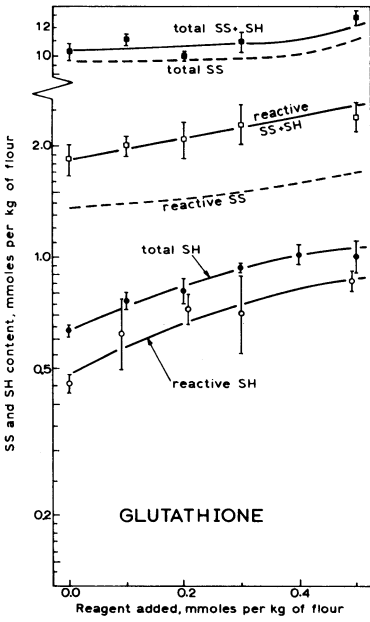
Extensigrams were recorded after 1,800-sec. reaction time (measured until completion of the shaping procedure) and 1,800-sec. rest period.

RESULTS

Thiol and Disulfide Contents

In the present study we report the thiol and disulfide contents after a reaction time of 3,300 sec.; this moment corresponds to the end of creep and the beginning of recovery in the rheological experiments. We titrated a number of samples, usually between 3 and 8, occasionally up to 16, from at least two doughs after various reaction times. Then we calculated by standard statistical methods (12) the linear regression between reaction time and content, and from that the estimate of the group content after 3,300-sec. reaction time, and its standard error. These estimates and their standard errors are reported in Figs. 1-4 and Table I. The slopes of the regression lines usually were slightly negative; for the total thiol content, a slow decrease in resting doughs has previously been reported (4,5,8).

From the data in Fig. 1 it follows that the increase in the contents of both total



Figs. 1-4. Thiol and disulfide contents of doughs with various additions. The lengths of the vertical lines at the experimental points correspond to the 95% confidence interval, that is, four times the standard error of estimate; if there is no vertical line, this length is smaller than the size of the sign. Dashed lines show the disulfide contents, obtained by subtraction of the thiol contents from the observed sums of disulfide and thiol contents. Fig. 1: glutathione; Fig. 2: potassium bromate; Fig. 3: potassium iodate; Fig. 4: N-ethyl maleimide.

and reactive thiol groups as a consequence of the addition of glutathione is, for small additions, 90 to 100% of the theoretical value. Observations for which there is no obvious explanation are the increase of total SS as a consequence of the addition of N-ethyl maleimide (Fig. 4), and the crossing over of the lines for total and reactive SH with large additions of iodate (Fig. 3) or N-ethyl maleimide (Fig. 4).

Creep and Recovery

General Results. In the interpretation of the recorded creep and recovery curves, the division of the total deformation into an elastic and a viscous part is an essential step. This division is based upon the assumption that the proceeding of the elastic part of the deformation is described by the mirror image of the recovery curve (13). It is illustrated in Fig. 5. This assumption is supported by our observation that the course of the recovery curve is not affected by the duration of the preceding creep; as a matter of course, the recovery in each of these experiments was only followed for the same time as the chosen duration of creep. Application of this procedure to a recorded creep-and-recovery curve results in two functions of time: the elastic and viscous part of the total deformation.

For a comparison of experiments with various shear stresses we introduce the compliance, which is the deformation divided by the stress; of these two, only the deformation is a function of time. In the same way as the deformation can be divided into a viscous and an elastic part, the compliance can be so divided. A plot of compliance after constant time against stress easily visualizes the effect of the latter; if the behavior is linear, that is, the deformation is proportional to the stress, the compliance is independent of the stress. Figures 6 and 7 are examples of such plots, and refer to the control doughs of this study. They clearly demonstrate that the viscous deformation of dough is not linear; its elastic deformation is nearly linear. Double-logarithmic plots of compliance against stress can usually, but not always, be represented by straight lines.

The effect of some of the additions or mixing atmosphere on the rheological properties is illustrated in Figs. 8-11. They demonstrate that glutathione increases both the viscous and elastic compliances, whereas oxidizing or thiol-blocking reagents decrease them.

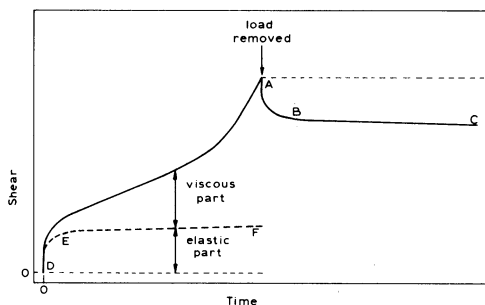


Fig. 5. Schematic representation of a creep experiment and its interpretation. Line DEF is the mirror image of ABC.

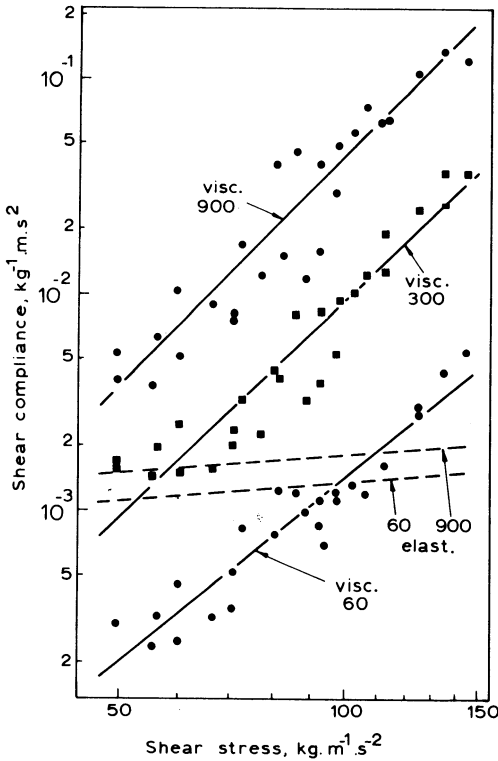


Fig. 6. Viscous compliances of control doughs after 60, 300, and 900 sec. as a function of shear stress. For comparison of the effects of stress and time on the viscous compliance, on the one hand, and on the elastic on the other, the lines for the elastic compliance after 60 and 900 sec. from Fig. 7 are shown here as dashed lines.

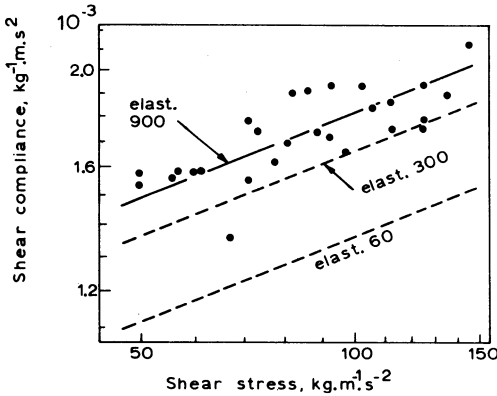
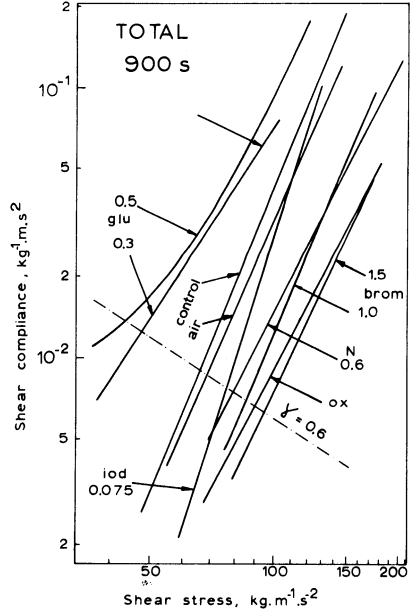
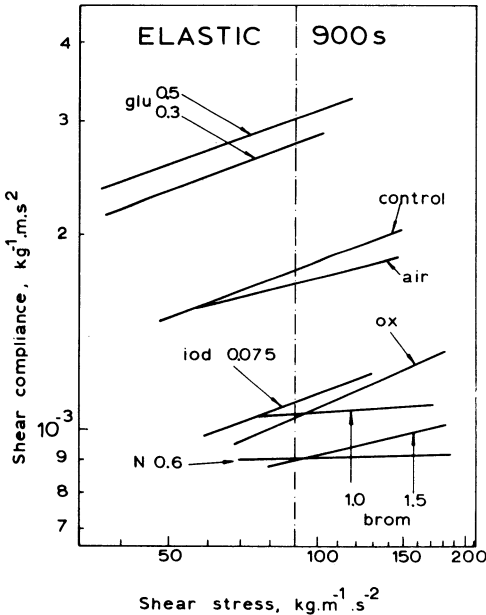
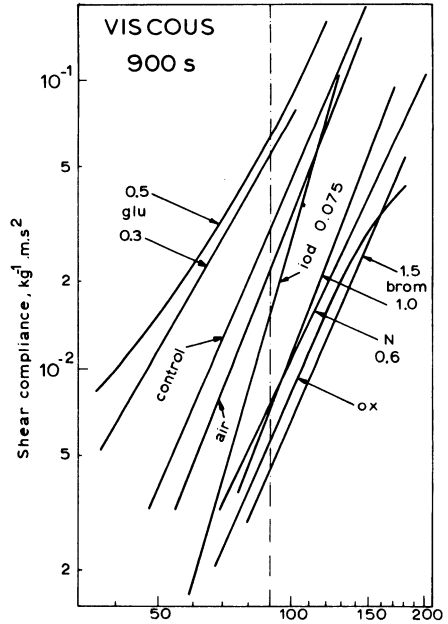
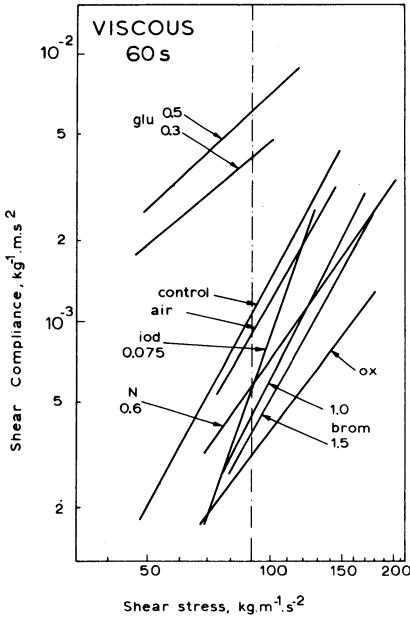


Fig. 7. Full line and experimental points: elastic compliance of control doughs after 900 sec. as a function of shear stress. Dashed lines are elastic compliance after 60 and 300 sec. (experimental points not shown).



Figs. 8-11. Compliances as a function of shear stress. Reagents are indicated by the following abbreviations: glu = glutathione, brom = bromate, iod = iodate, N = N-ethyl maleimide, ox = mixing in oxygen. The figures indicate amounts added in mM. per kg. of flour. Fig. 8: viscous compliance after 60 sec.; Fig. 9: the same after 900 sec.; Fig. 10: elastic compliance after 900 sec.; Fig. 11: total compliance after 900 sec.

For a quantitative comparison with thiol and disulfide contents we plotted in Figs. 12-15 various compliances against the amounts of the reagents added. In these graphs a larger number of different amounts of each of the reagents is shown than could be done in Figs. 8-11. The effect of mixing in air or oxygen is shown in Table II.

Figures 12-15 are obtained by making a cross-section through graphs like Figs. 8-11, either at constant shear stress or at constant deformation.

We did so at constant shear stress for the viscous and elastic parts of the compliance. The range of shear stresses applied varied slightly with the type and amount of reagent; the range from 80 to 100 N. per m² (kg. \cdot m⁻¹ \cdot s⁻²) was common to all treatments. We chose 90 N. per m² for the preparation of Figs. 12-15. Since in each of the Figs. 8-11 the lines are more or less parallel, another choice of stress would have resulted mainly in a different level of the lines in Figs. 12-15, but hardly in a different shape. The relative positions of the various reagents would not change very much either. The only obvious exception to the parallelism are the lines for the viscous compliance after 60 sec. for doughs with glutathione (see Fig. 8).

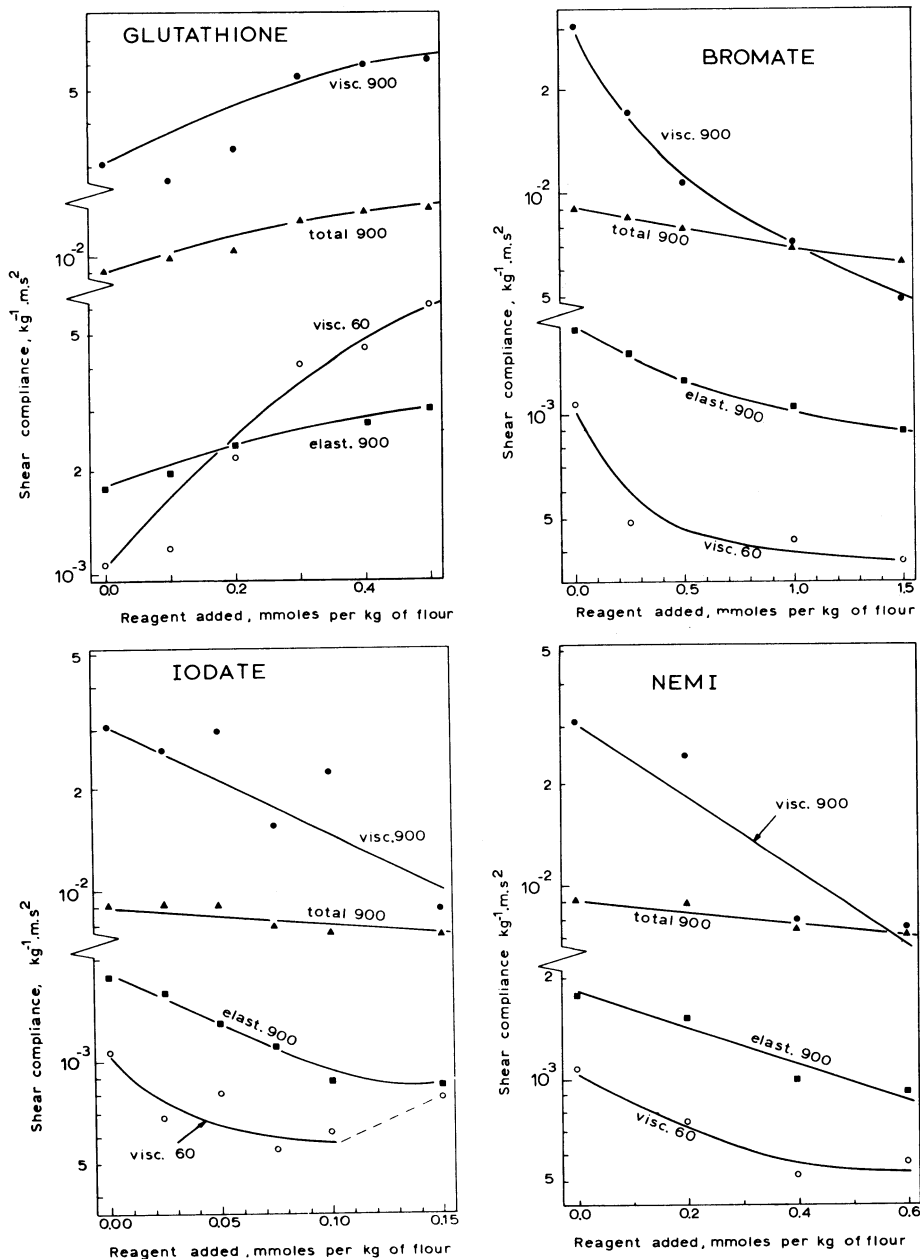
One can also argue that test-pieces should be compared at the same deformation instead of at the same stress. In fact, extensigram heights are often compared at constant extension (14,15). If one does so, the division of the total deformation into an elastic and a viscous part loses most of its meaning. Therefore we plotted in Figs. 12-15 the total compliance after 900 sec. and at a constant shear of 0.60 against the amount of reagent added. We chose this value for the shear since it corresponds to the extension during 900 sec. of tin proof in a bakery (16). The curves at constant shear are obtained by making a cross-section, as indicated in Fig. 11. Measured at constant shear, the effect of the reagents on the viscous or total compliance is much smaller than if measured at constant stress. This is due to the steep slopes of the curves of compliance against stress (Figs. 8,9, and 11).

Shape of Recovery Curve. In Fig. 7 the lines for the elastic compliance after 60 and 300 sec. are parallel to that for 900 sec. This means that the shape of the recovery curve is independent of the shear stress. We found that it is also hardly

TABLE II. SHEAR COMPLIANCES AND EXTENSIGRAPH RESISTANCES OF CONTROL DOUGHS (MIXED IN NITROGEN) AND OF DOUGHS AFTER MIXING IN AIR OR OXYGEN

| Mixing Atmosphere | Shear Compliance, kg. ⁻¹ \cdot m. \cdot sec. ² | | | | Extensigraph Resistance ^a B.U. |
|-------------------|---|--------------------------------|-------------------------------|-------------------------------|--|
| | Shear stress = 90 kg. \cdot m. ⁻¹ \cdot sec. ⁻² | | Elastic 900 sec. | Total 900 sec. | |
| | Viscous 60 sec. | Viscous 900 sec. | | | |
| Nitrogen | 11 \times 10 ⁻⁴ | 3.0 \times 10 ⁻² | 1.8 \times 10 ⁻³ | 9.1 \times 10 ⁻³ | 165 \pm 4 (9) |
| Air | 8.9 \times 10 ⁻⁴ | 2.2 \times 10 ⁻² | 1.7 \times 10 ⁻³ | 8.7 \times 10 ⁻³ | 194 \pm 6 (4) |
| Oxygen | 3.1 \times 10 ⁻⁴ | 0.56 \times 10 ⁻² | 1.0 \times 10 ⁻³ | 6.7 \times 10 ⁻³ | 717 \pm 52 (6) |

^aResistance measured after 30 mm. kymograph paper transport (4.6 sec.) with correction for sample-holder displacement. 1 B.U. = 12.3 mN. Each entry shows the mean value; its standard error; and, in parentheses, the number of test-pieces stretched.



Figs. 12-15. Compliances of doughs with various additions. Viscous and elastic compliances are measured at a shear stress of 90 N/m^2 , the total compliance at a shear of 0.60 . Fig. 12: glutathione; Fig. 13: potassium bromate; Fig. 14: potassium iodate; Fig. 15: N-ethyl maleimide.

affected by added reagents. If the recovery after 900 sec. is taken as 100%, the recoveries after shorter time intervals are: for 60 sec., 72 to 76%; and for 300 sec., 88 to 93%. The only exceptions are experiments with large amounts of iodate (>0.1 mM. per kg.) or N-ethyl maleimide (0.6 mM. per kg.), with which the recoveries after 60 and 300 sec. were only 68 and 86%, respectively.

Because of the constant shape of the recovery curve, a single figure, for instance the elastic compliance after 900 sec., is sufficient for a comparison of the elastic properties of the various doughs at a chosen shear stress or deformation.

Extensigrams

The effects of added reagents and of the mixing atmosphere on the resistance in the extensigraph are shown in Fig. 16 and Table II. All reagents used, including glutathione, decreased the extensibility. Doughs with more than 0.2 mM. per kg. of glutathione could not be shaped.

DISCUSSION

The stiffening of dough as a consequence of oxidation or thiol blocking, and the reverse effect of compounds with thiol groups can qualitatively be explained if it is assumed that the rigidity of dough is, at least partly, owed to disulfide cross-links in the network of protein molecules that fills its continuous phase or gluten phase. By

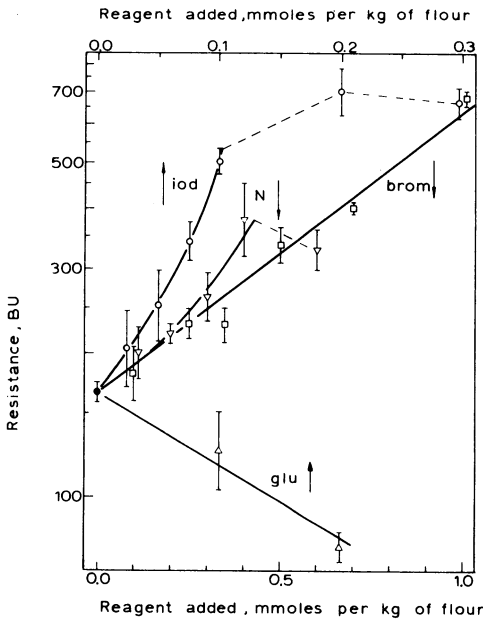


Fig. 16. Resistances against extension, as measured with the Brabender extensigraph, with various additions. Abbreviations as in Figs. 8-11. Arrows indicate which of the horizontal scales applies to the respective curves. The lengths of the vertical lines at the experimental points correspond to the 95% confidence interval; that is, four times the standard error of the mean value. Resistances were measured after 30 mm. kymograph paper transport (4.6 sec.) with correction for sample-holder displacement. 1 B.U. = 12.3 mN.

analogy to rubber, one expects that, at a given stress, the elastic (or temporary) deformation decreases with an increasing number of cross-links. The elastic deformation of rubber is, under certain conditions, inversely proportional to the number of cross-links per unit volume (17); a similar behavior might be expected for gluten or dough too.

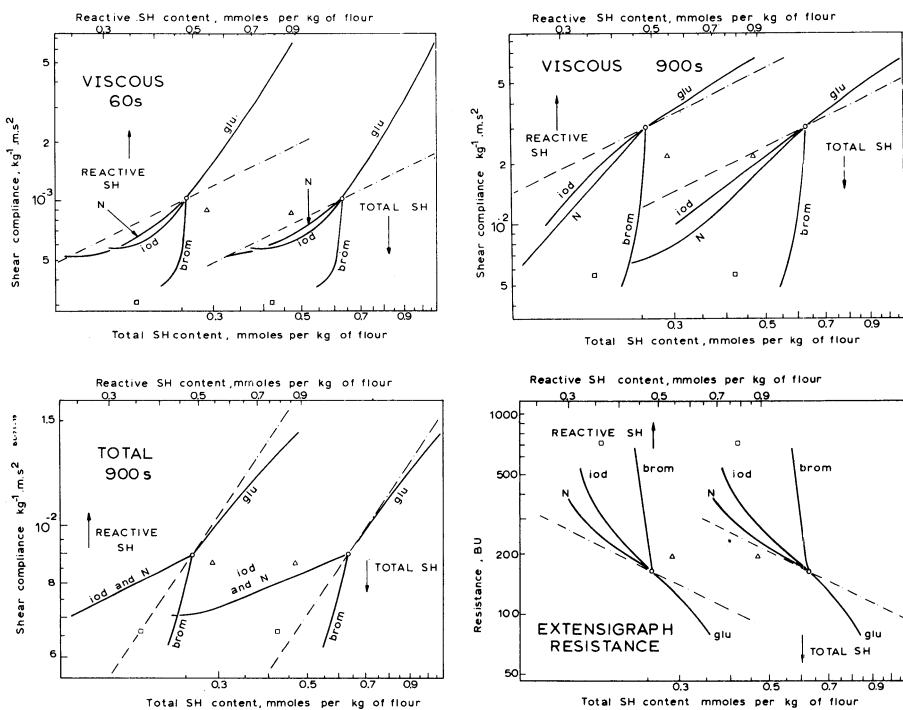
Dough shows, in addition to its elastic deformation, a considerable viscous (or permanent) one, as is illustrated in Fig. 5. This can only occur if the cross-links are broken and re-formed. To explain this, it is assumed that an interchange reaction between thiol and disulfide groups proceeds (18; for a review see ref. 1, section VI). The actual occurrence of such a reaction has been demonstrated by chemical experiments (19-26). This explains why, at a given stress, the viscous deformation of dough decreases with decreasing thiol content, irrespective whether the latter is from oxidation or thiol blocking. If the rate of the interchange reaction is proportional to the thiol content, it may be expected that the viscous deformation too is proportional to the thiol content.

In previous work (10) we found neither an inverse proportionality between the disulfide content of dough and its elastic deformation, nor a direct one between the thiol content and the viscous deformation. As a possible explanation, we suggested that only fractions of the analytically determined thiol and disulfide groups are rheologically effective. Rheologically effective disulfide bonds are those that restrict the elastic deformation; similarly, rheologically effective thiol groups are those that allow for the viscous deformation. Chemically reactive thiol groups probably participate faster in the interchange reaction with disulfide bonds and will, therefore, also be rheologically more effective. For this reason, in the present study we titrated thiol groups both in the presence and absence of urea; in its absence a more reactive fraction of the thiol groups is measured. A similar relation between chemical reactivity and rheological effectivity cannot be expected for the disulfide bonds. Nevertheless, out of curiosity, we titrated both the total and reactive disulfide bonds.

The model that we used in our previous work (10), and which is outlined in the preceding paragraphs, is linear in every respect. Apart from the proportionalities that were already explicitly mentioned, it contains proportionality between the properties of the continuous or gluten phase and the corresponding property of the dough. If all these proportionalities are valid, why would the deformation not be proportional to the stress? The observation that stress and deformation are not proportional shows that our original approach was too ambitious.

This can also be illustrated by another argument. According to the model, it does not make any difference whether one takes the effect of a reagent on the compliance at constant stress or at constant deformation. Figure 11, however, shows that it makes, in fact, a very large difference. If the compliance at constant stress is proportional to the content of a particular group, the compliance at constant deformation cannot be so, and the reverse.

Because of these considerations we did not focus our attention to possible proportionalities between compliances and analytical results. Instead, we reduced the problem to the question whether, other factors being constant, the rheological properties of dough are unequivocally determined by its thiol and disulfide content. To answer this, we constructed graphs of various rheological properties against total



Figs. 17-20. Compliances or extensigraph resistance as a function of the total or reactive thiol content. Circle: control dough (mixed in nitrogen). Triangle: dough mixed in air. Square: dough mixed in oxygen. Abbreviations: glu = glutathione, brom = bromate, iod = iodate, N = N-ethyl maleimide. Dash-dot lines correspond to direct proportionality between thiol content and compliance, or inverse proportionality between thiol content and resistance. Fig. 17: viscous compliance at a shear stress of 90 N./m² and after 60 sec.; Fig. 18: the same after 900 sec.; Fig. 19: total compliance after 900 sec. and at a shear of 0.60; Fig. 20: extensigraph resistance after 30 mm. kymograph paper transport.

or reactive thiol or disulfide content. For this purpose we took one curve from Figs. 1-4, and a corresponding one from Figs. 12-16. From the three properties involved, we eliminated the amount of reagent added, and thus obtained the relation between rheological property and group content. Results are shown in Figs. 17-22. By combining data from Tables I and II, we obtained points for doughs mixed in air and oxygen, which are also plotted in these figures. As a reminiscence of previous, more ambitious plans, the dash-dot lines in the figures correspond to direct or inverse proportionality.

Figures 17 and 18 show that the viscous compliance is not unequivocally determined by either the reactive or total thiol contents. If bromate is used as reagent, a small decrease in thiol content causes a considerable reduction of the compliance. If one wants to produce the same rheological effect by means of iodate or N-ethyl maleimide, the thiol content must be decreased to a much lower level. The behavior of oxygen is intermediate between that of bromate and iodate. Changes in thiol content by the addition of glutathione have about the same effect, though in the opposite direction, as when iodate or N-ethyl maleimide are used. Use of the reactive thiol content instead of the total content causes a parallel shift of

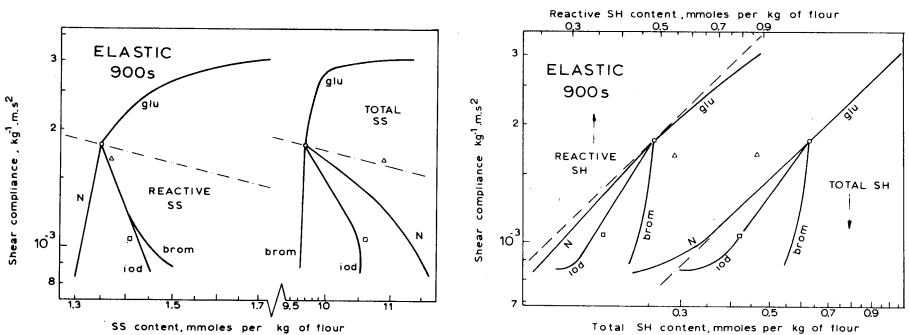
the figure, while its shape remains essentially the same. This is owing to the fact that, in the range where rheological measurements could be made, the ratio of reactive to total thiol content is nearly constant, as is shown in Figs. 1-4.

The total compliance in Fig. 19 shows essentially the same behavior as its viscous component. The rheological effects are smaller, as can be concluded from the position of the drawn lines relative to the dash-dot line; this is, however, owing to the fact that the total compliance has been taken at constant deformation instead of at constant stress (see Results).

For a comparison of Fig. 20 for the extensigraph resistance with the previous three, top and bottom have to be interchanged. Despite the fact that the extensigraph resistance is also taken at constant deformation, Fig. 20 is more similar to the graphs for the viscous compliance (Figs. 17 and 18) than to that for the total compliance (Fig. 19).

Figure 21 shows that, contrary to the theory, there is no correlation between the total or reactive disulfide content and the elastic compliance. If the elastic compliance is plotted against the nonreactive disulfide content (total minus reactive) the figure (not shown) is essentially similar to that for the total disulfide content. Figure 22 shows that the correlation of the elastic compliance with the thiol content is, unexpectedly, better than with the disulfide content; the graph is similar to those for the viscous compliance (Figs. 17 and 18).

Because of these results one can choose between two possibilities. The first is to drop completely the hypothesis that disulfide bonds restrict the elastic deformation of dough, and that thiol-disulfide interchange reactions are a condition for its viscous deformation. Because of the striking rheological effects of the specific reagents, and the absence of an alternative explanation, this first choice does not appear justified. The second is to conclude that our analytical procedure did not measure the rheologically effective groups. In the earlier paper (10) we arrived at this conclusion for the total thiol or disulfide contents; the present results show that it is also valid for the titrations of the reactive groups only (in the absence of urea), and for the nonreactive disulfide bonds. The rheologically effective groups are probably only a small fraction of the groups that we titrated.



Figs. 21-22. Elastic compliance at a shear stress of 90 N./m^2 and after 900 sec. as a function of total or reactive thiol or disulfide content. Symbols and abbreviations as in Figs. 17-20. Dash-dot lines correspond to direct proportionality between thiol content and compliance or inverse proportionality between disulfide content and compliance. Fig. 21: disulfide content; Fig. 22: thiol content.

The rheologically effective thiol groups may be those that form part of small peptides. Tkachuk recently found three thiol peptides in flour, two of which could be identified as L-cysteinylglycine and glutathione; in flour, their total quantity amounts to between 0.1 and 8 μM . per kg. of flour; that is, less than 1% of the total thiol content (27,28). They react with potassium iodate and with iodoacetic acid (29). The latter compound is a very efficient flour-improving agent; its optimum addition in baking tests is only 30 μM . per kg. (30). That means that so small a decrease in thiol content is sufficient to cause a considerable rheological effect, provided that it is due to the disappearance of rheologically effective groups.

Other reagents in appropriate amounts can cause a larger decrease in total thiol content with a smaller rheological effect, if the decrease is to a large extent owing to the disappearance of rheologically ineffective groups. Figures 17 to 19, inclusive, and 22 show that there are considerable differences between bromate, on one hand, and iodate and N-ethyl maleimide, on the other. Bromate apparently reacts more specifically with the rheologically effective groups, since, for the same rheological effect, and therefore the same decrease in effective thiol content, the decrease in total thiol content is smaller with bromate than with iodate or N-ethyl maleimide. If the reagents are added in such amounts as to produce the same decrease in titratable thiol content, the rheological effect of bromate is much larger than that of the other two reagents. This conclusion is not enfeebled by the fact that more equivalents of bromate than of the other two reagents must be added to produce this effect. The difference in specificity is not related to the type of reaction (oxidation or thiol-blocking). It may be related to the rate of reaction: iodate and N-ethyl maleimide act rapidly, whereas bromate acts slowly (31).

The hypothesis that small thiol peptides like glutathione are the rheologically effective compounds does not explain why the effect of a decrease in total or reactive thiol content caused by bromate is so much larger than that of an equal increase owing to glutathione. One would expect that in Figs. 17-20 the initial slope of the bromate line would be equal to or smaller than that of the glutathione line. For an explanation of this observation, more knowledge is required of the transformation of naturally present or added glutathione during mixing and reaction time.

Further progress of our understanding of the role of thiol and disulfide groups in dough rheology asks for techniques that can measure thiol and disulfide contents of specific protein and peptide fractions.

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