A FARINOGRAPH TECHNIQUE FOR STUDYING GLUTEN[1]

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ABSTRACT

The farinograph was adapted to produce a reasonably normal curve for dried vital gluten and water mixed to about 81% absorption; in essence, the 50-g. bowl is used with the linkage set for the 300-g. bowl. Addition of 10 μeq. per g. gluten of 2-mercaptoethanol yields a farinogram for reduced gluten exhibiting a rapid fall in consistency such as is obtained with reduced dough. Additions of oxygen at atmospheric pressure, or of 15 or 30 μeq. per g. of N-ethylmaleimide (NEMI) after 10 min. of mixing, had little effect on the curve. But a sharp peak in consistency followed by a further gradual decrease was caused by adding 15 or 30 μeq. per g. of iodate. A second addition of 30 μeq. per g. of iodate at 20 min. after the addition of 15 μeq. per g. of iodate or NEMI had no additional effect, whereas a similar addition of NEMI produced a slight increase in the rate of breakdown. Results are discussed in terms of possible reactions with –SH and S–S groups. It is suggested that iodate creates S–S cross-links, whereas NEMI does not, and that the similarity in the improving action of the two reagents under certain conditions must result from removal of –SH groups and subsequent effects on the rate of the S–S interchange reactions.

The farinograph has been successfully used in studies of the effects on dough properties of chemicals that react with sulfhydryl (–SH) groups of flour proteins (14,15,19). The literature contains no specific reference to farinograph studies of gluten-water mixtures; however, there have been a number of farinograph and baking studies on blends of gluten with flour (1,2) or starch (10). The present paper describes a farinograph technique for mixtures of dry "vital" gluten and water and its application to studies of the effects of certain chemicals on gluten properties.

Materials and Methods

The dry "vital" gluten was a commercial sample supplied by the Ogilvie Flour Mills Company. It was prepared by the batter process (3) from a 50-50 blend of straight-grade (13.5% protein; 0.52% ash) and first clear (15.6% protein; 0.76% ash) flours milled from hard red spring wheat. It contained 5.3% moisture, and 83.2% protein, 0.90% ash, 72 μmole per g. disulfide, and 1.8 μmole per g. sulfhydryl, on a dry basis. Disulfide and sulfhydryl contents were determined by the method of Stricks and Chakravarti (18) and Sokol, Mecham, and Pence (16), respectively. All chemicals used were reagent grade.

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A reasonably normal farinograph trace was obtained by using the adjustments described by Irvine, Bradley, and Martin (13) for macaroni doughs; in essence, use of the 50-g. bowl with the farinograph linkage set as for the 300-g. bowl. Mixing was done under nitrogen and with a water absorption of about 81%.

Figures 1 and 2 show the farinograms discussed in this paper. In Fig. 1 the control (No. 1) was obtained by mixing 45.3 g. vital gluten (43 g. dry material) containing 5.3% (d.b.) moisture with 32.6 ml. of distilled water. Farinograms 2 to 7 are for the same amounts of gluten and water, and 10 μeq. 2-mercaptoethanol per g. of dry gluten added at the beginning of mixing, but for farinograms 3 to 7 a second reagent was introduced at the rate indicated after 10 min. Reduced gluten was used to accentuate the effect of reduction of S–S and oxidation (or blockage) of –SH to the extent where they would be readily shown by the farinogram. Mercaptoethanol was used instead of the more commonly used sulfite because it gives two –SH groups from each disulfide bond whereas sulfite gives a –SH group and a S–sulfonate group. Oxygen was introduced at atmospheric pressure into the farinograph bowl through the nitrogen inlets by manipulating a three-way stopcock; potassium iodate and NEMI were added in solid form. In Fig. 2, curves 3 to 6, a second addition of either iodate or NEMI at the rate of 30 μeq. per g. was made at 20 min. Curves 1 and 2 of Fig. 2 are curves 4 and 5 of Fig. 1, respectively.

Results and Discussion

Curve 1 (Fig. 1) shows that it is possible to use the farinograph to obtain for a gluten-water mixture a reasonably normal farinogram that rises to an optimum consistency and then shows a gradual breakdown on further mixing.

Curve 2 is for gluten treated with 2-mercaptoethanol. This reducing agent affects the gluten farinogram in much the same way that various reducing agents affect dough farinograms (8,12). The peak consistency is somewhat decreased, and the rate of breakdown is markedly increased. Sodium bisulfite and glutathione (reduced) produced similar effects on gluten farinograms (not shown); however, no attempt was made to determine if the effects of the different reducing agents were quantitatively equivalent.

The changes in the farinogram produced by subsequent additions of oxygen, iodate, and NEMI to the reduced gluten are quite interesting (see curves 3 to 7). Oxygen, at atmospheric pressure, had only slight effect; the amount of oxygen that is actually mixed into the

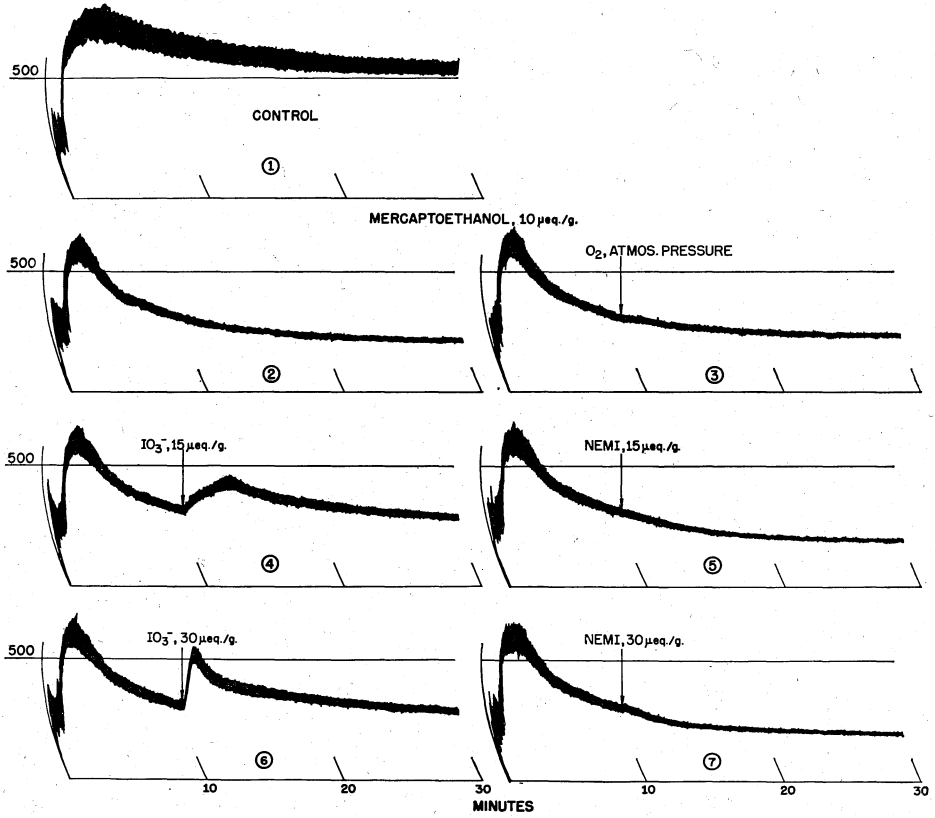

Fig. 1. Gluten farinograms. 1, Control, 45.3 g. gluten (5.3% moisture) and 32.6 ml. water; 2, control plus 10 μeq. per g. mercaptoethanol; 3 to 7, control plus 10 μeq. per g. mercaptoethanol plus the following at 10 min.: 3, oxygen at atmospheric pressure; 4, 15 μeq. per g. iodate; 5, 15 μeq. per g. NEMI; 6, 30 μeq. per g. iodate; 7, 30 μeq. per g. NEMI.

relatively fluid gluten batter is probably too small to produce any marked changes. Additions of iodate to the reduced gluten (curves 4 and 6) produced a sudden increase in consistency (the rate depending on the amount of iodate added). The final consistency (e.g. at 30 min.) is essentially the same for the two concentrations of iodate added at 10 min. Addition of NEMI at the lower concentration level had no effect on the farinogram of the reduced gluten (curve 5). When the concentration of NEMI was doubled, a slight increase in the rate of breakdown was actually observed (curve 7).

A second addition, 30 μeq. per g. of iodate at 20 min. (curve 3, Fig. 2), had no further effect on the iodate-treated reduced gluten.

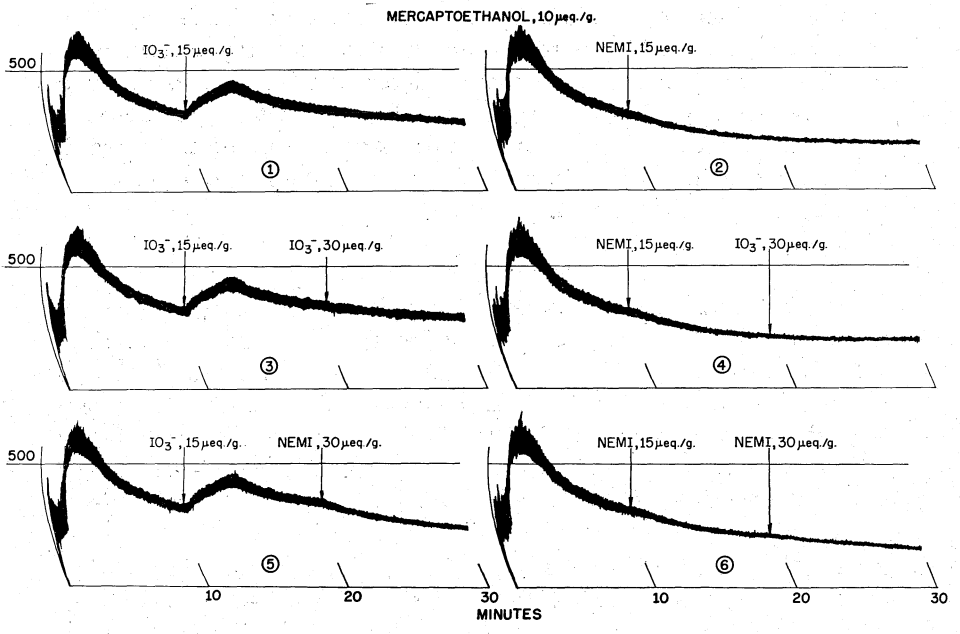

Fig. 2. Gluten farinograms. All curves, 45.3 g. gluten (5.3% moisture), 32.6 ml. water, and 10 μeq. per g. mercaptoethanol; 1, plus 15 μeq. per g. iodate at 10 min.; 2, 15 μeq. per g. NEMI at 10 min.; 3, plus 15 μeq. per g. iodate at 10 min. plus 30 μeq. per g. iodate at 20 min.; 4, plus 15 μeq. per g. NEMI at 10 min. plus 30 μeq. per g. iodate at 20 min.; 5, plus 15 μeq. per g. iodate at 10 min. plus 30 μeq. per g. NEMI at 20 min.; 6, plus 15 μeq. per g. NEMI at 10 min. plus 30 μeq. per g. NEMI at 20 min.

Accordingly, it appears that the 15 μeq. per g. of iodate added at 10 min. was sufficient to complete the reaction with all the accessible –SH groups. The absence of an effect due to a second addition of iodate at 20 min. is not due to the degree of breakdown but seems to be due to the absence of certain reactive groups (presumably –SH). If the first addition of iodate at 10 min. is omitted the addition at 20 min. produces a rise in consistency (results not shown). Furthermore, if the degree of breakdown is increased by the addition of a larger amount of 2-mercaptoethanol (30 μeq. per g.) to the extent where the consistency at 15 min. is 150 B.U., an increase in consistency is still obtained when iodate is added.

Addition of 30 μeq. per g. of NEMI (curve 5) to the iodate-treated reduced gluten produced a slight increase in the rate of breakdown. As in the case of iodate-treated reduced gluten, the second addition of iodate to NEMI-treated reduced gluten (curve 4) had no effect. This agrees with the previous report that the reaction of iodate in doughs

can be completely inhibited by prior addition of NEMI (5). The second addition of NEMI to a reduced gluten already treated with NEMI (curve 6) produced a further, detectable increase in the rate of breakdown. Although it might be difficult to detect the effects of the second addition of NEMI in the farinograms reproduced in Fig. 2, direct superposition of the original farinograms does indeed show that the effects indicated are real.

General Discussion

Previous experience with flour-water doughs (6) suggests that all the mercaptoethanol would react during the first 3 min. of mixing. But the amount added was equivalent to about one-seventh of the disulfide content, so that some S–S bonds should remain in the gluten. The decrease in consistency after the peak is reached is probably caused by rupture of S–S bonds through reaction with –SH groups (7,8). Accessibility of S–S bonds, similar to that observed for –SH groups (5), may also condition this time-dependent reaction.

The sudden rise in consistency of the reduced gluten on addition of iodate may well be caused by formation of new S–S cross-linkages, as has already been demonstrated by analytical methods (11). Since the curve does not rise to the control level, the new bonds appear to make a smaller contribution than those originally present. This agrees with the observation of Freilich and Frey (9) for bread doughs, that effects of reducing agents on loaf volume cannot be completely overcome by subsequent addition of oxidizing agents. Further mixing of the iodate-treated gluten causes a decrease in consistency, presumably through the interchange reaction; for there is evidence[3] that iodate does not react with all –SH groups in dough, even at relatively high iodate/–SH ratios. The possibility of physical rupture of S–S cross-linkages (4) in relatively fluid gluten batters is discounted.

There is little doubt that, under the experimental conditions used, NEMI reacts with –SH groups. Direct evidence of this reaction under similar conditions has been reported recently (20). In addition, it is suspected that when NEMI is present in excess of the accessible –SH groups, it can increase the rate of cleavage of disulfide bonds (17). In the case of gluten-water mixture this would lead to a decrease in consistency (see curve 7, Fig. 1, and curves 5 and 6, Fig. 2). The results presented suggest that, whereas the reaction of iodate with –SH groups produces cross-links (S–S), the reaction of NEMI does not. Accordingly the similarity in the improving action of these two reagents

[3] C. C. Tsen, Grain Research Laboratory, Winnipeg; unpublished results.

under certain conditions must result from the removal of –SH groups and subsequent effects on the rate of the S–S interchange reactions.

Literature Cited

1. AITKEN, T. R., and ANDERSON, J. A. Testing the baking strength of gluten concentrates. Cereal Chem. **20:** 79–81 (1943).
2. AITKEN, T. R., and GEDDES, W. F. The relation between protein content and strength of gluten-enriched flours. Cereal Chem. **16:** 223–231 (1939).
3. ANDERSON, R. A., PFEIFER, V. F., and LANCASTER, E. B. Continuous batter process for separating gluten from wheat flour. Cereal Chem. **35:** 449–457 (1958).
4. AXFORD, D. W. E., CAMPBELL, J. D., and ELTON, G. A. H. Changes in apparent disulphide content of doughs on mechanical development. Chem. & Ind. (London) **1962:** 407–408.
5. BUSHUK, W. Accessible sulfhydryl groups in dough. Cereal Chem. **38:** 438–448 (1961).
6. BUSHUK, W., and HLYNKA, I. The bromate reaction in dough. IV. Effect of reducing agents. Cereal Chem. **38:** 309–316 (1961).
7. DE DEKEN, R. H., and DE DEKEN-GRENSON, M. Étude du gluten de froment. II. Action des agents réducteurs. Biochim. Biophys. Acta **16:** 566–569 (1955).
8. 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).
9. FREILICH, J., and FREY, C. N. Dough oxidation and mixing studies. VI. Effects of oxidizing agents in the presence of reducing matter. Cereal Chem. **21:** 241–251 (1944).
10. HARRIS, R. H., and SIBBITT, L. D. The comparative baking qualities of hard red spring wheat starches and glutens as prepared by the gluten-starch blend baking method. Cereal Chem. **19:** 763–772 (1942).
11. HIRD, F. J. R., and YATES, J. R. The oxidation of protein thiol groups by iodate, bromate and persulfate. J. Sci. Food Agr. (in press).
12. HLYNKA, I. Effect of bisulfite, acetaldehyde, and similar reagents on the physical properties of dough and gluten. Cereal Chem. **26:** 307–316 (1949).
13. IRVINE, G. N., BRADLEY, J. W., and MARTIN, G. C. A farinograph technique for macaroni doughs. Cereal Chem. **38:** 153–164 (1961).
14. MECHAM, D. K. Effects of sulfhydryl-blocking reagents on the mixing characteristics of doughs. Cereal Chem. **36:** 134–145 (1959).
15. MEREDITH, P., and BUSHUK, W. The effects of iodate, N-ethylmaleimide, and oxygen on the mixing tolerance of doughs. Cereal Chem. **39:** 411–426 (1962).
16. SOKOL, H. A., MECHAM, D. K., and PENCE, J. W. Sulfhydryl losses during mixing of doughs: Comparison of flours having various mixing characteristics. Cereal Chem. **37:** 739–748 (1960).
17. SPACKMAN, D. H., STEIN, W. H., and MOORE, S. The disulfide bonds of ribonuclease. J. Biol. Chem. **235:** 648–659 (1960).
18. STRICKS, W., and CHAKRAVARTI, S. K. Amperometric titration of sulfhydryl and disulfide groups with organic mercury compounds at the rotated dropping mercury indicator electrode. Anal. Chem. **33:** 194–199 (1961).
19. SULLIVAN, BETTY, DAHLE, L., and NELSON, O. R. The oxidation of wheat flour. II. Effect of sulfhydryl-blocking agents. Cereal Chem. **38:** 281–291 (1961).
20. TKACHUK, R., and HLYNKA, I. Tracer study of the reaction of flour protein sulfhydryl with iodate, bromate, and N-ethylmaleimide. Abstr., AACC 47th annual meeting, St. Louis, Mo. (1962).