COMPARISON OF THE EFFECTS OF N-ETHYLMALEIMIDE AND UREA ON RHEOLOGICAL PROPERTIES OF DOUGH[1]

M. JANKIEWICZ[2] AND Y. POMERANZ[3]

ABSTRACT

Water absorption was increased and farinograph dough development time of a wheat flour dough was reduced to one-third that of the control by addition of a 3*M* urea solution. Consistency of urea-containing doughs dropped to an extent comparable to that of doughs containing N-ethylmaleimide (NEMI). But whereas NEMI-containing doughs were sticky and highly extensible, a "putty," lacking dough structure, was formed in the presence of urea. NEMI lowered dough extension and extensigram areas; areas of urea-containing doughs were lowest. Doughs containing urea-NEMI combinations resembled urea-containing doughs. Adding urea or NEMI after the dough had developed produced results similar to those from adding the reagents at the beginning of dough development. Adding $MgSO_4$ retarded dough development, partially restored properties of urea-containing doughs, and increased dough development time about 10 times.

Rheological properties of wheat dough depend basically on the structure of gluten proteins. The role of sulfhydryl and disulfide groups in forming gluten structure has been emphasized in a number of publications (1–6), and the hypothesis of the reacting system has been developed (1,4–8). The reactivity of the sulfur-containing groups of protein explains, however, only some features of dough rheology. The important role of hydrogen bonding in the formation of specific structures of macromolecular protein systems has been well established (9,10).

Results obtained by Cook and Alsberg (11), Rose and Cook (12), and others (13–15) on gluten dispersibility in urea solutions suggested the importance of hydrogen bonding in wheat-dough structure. Holme

[1]Manuscript received June 19, 1964. Co-operative investigations between Crops Research Division, Agricultural Research Service, U.S. Dept. Agr., and Dept. Flour and Feed Milling Industries, Kansas State Univ. Contribution No. 483, Kansas Agricultural Experiment Station, Manhattan; a report of work done under contract with U.S. Dept. Agr. and authorized by the Research and Marketing Act of 1946. This contract was supervised by the Western Research and Development Div., Agr. Research Service.

[2]Postdoctoral fellow, participant in the Exchange Program organized by the Institute of International Education under the auspices of the U.S. Department of State. Present address: Department of Grain Technology, College of Agriculture, Poznan, Poland.

[3]Research Technologist (Cereal), ARS, U.S. Dept. Agr.

and Briggs (16) and Beckwith *et al.* (17) presented evidence that amide groups function as interaction sites in wheat gluten proteins. In our study, the effects of blocking the sulfhydryl groups with N-ethylmaleimide (NEMI) and of the presence of urea on the rheological properties of dough were investigated.

Materials and Methods

Untreated flour was experimentally milled from a composite grist of several hard winter wheat varieties grown in 1963 at a number of locations throughout the Great Plains. Certain chemical and baking properties of the flour composite, on 14% moisture basis, follow: ash 0.42%, protein 12.8%; 100 g. flour had a bromate requirement of 3 mg., water absorption 61.7%, mixing time 3⅛ min.; and a loaf volume, 950 cc. Chemical analyses were performed according to AACC *Cereal Laboratory Methods* (18). Baking tests were done as described by Finney and Barmore (19,20).

Farinograms were made by mixing 50 g. flour (14% moisture basis) in a small bowl with sufficient distilled water or sodium pyrophosphate buffer (0.01*M*, pH 7.0) to give a maximum dough consistency centered around the 500-B.U. line. Solutions contained either 0.001*M* NEMI or 3.0*M* urea, or combinations of both. All molar (*M*) denotations refer to concentrations in liquid added to flour of 14% moisture.

The levels of NEMI added were selected to give approximately a tenfold excess needed to block available sulfhydryl groups of wheat-flour proteins. Dill and Alsberg (15) have shown that at least 2.0*M* urea solutions were necessary to affect gliadin solubility. This agrees with our studies on effects of buffered solutions of urea on wheat-protein properties, to be reported elsewhere.

Adding a 3.0*M* urea solution affected the amount of liquid which had to be added during farinograph determination.[4] Corrections for the water absorption change were made in part of the experiments, as indicated in "Results and Discussion."

NEMI and urea were added either as solutions during dough formation and development or after the dough was developed for 5 min. This time period was required for maximum development of the control, buffer-dough system. If introduced to the developed doughs, NEMI and urea were first dissolved respectively in 1-ml. and 2.8-ml. portions of buffer; concentration of the reagents in final doughs was identical with the controls. Magnesium sulfate, used in some of the experiments in concentration of 0.63*M* was added to the dough in solid form.

[4] Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Extensigrams were obtained by the procedure described by Villegas *et al.* (21). Doughs were prepared with either 2% sodium chloride, 0.01*M* sodium pyrophosphate buffer of pH 7.0, or the buffer containing either 0.001*M* NEMI or 3.0*M* urea, or combinations of both. Doughs were prepared in the large farinograph bowl from 300 g. of flour and solutions in amounts calculated to obtain a maximum dough consistency centered around the 500-B.U. line. When urea was used, the volume of buffer was the same as in controls. The doughs were mixed 5 min.; then two 150-g. portions were scaled, rounded 20 times, moulded into dough cylinders, and placed in the extensigraph cabinets maintained at 30°C. Curves were drawn for duplicate doughs at 45, 90, and 135 min.

Sodium pyrophosphate buffer was prepared as described by Coates and Simmonds (22). All the reagents used were of analytical reagent grade.

Results and Discussion

The absorption and farinogram characteristics of the flour were unchanged if a buffer solution was substituted for water; adding a 3*M* urea solution affected the amount of liquid which had to be added to obtain a farinograph curve centered around the 500-B.U. line.

Significant modifications of dough properties were caused by adding NEMI (Fig. 1, curve C). The rate of development of the NEMI-con-

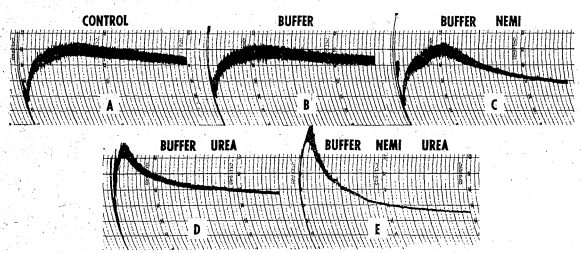

Fig. 1. Effects of 3.0*M* urea and 0.001*M* NEMI on farinograph characteristics of doughs prepared with 0.01*M* sodium pyrophosphate buffer, pH 7.0.

taining dough was similar to that of water or buffer doughs. After maximum dough consistency was reached, consistency of the NEMI-containing dough decreased faster than did consistency of the control dough; this agrees with previously reported results (3,4). Adding urea, as shown in curve D, shortened dough development time to about one-third of the time required to reach maximum dough consistency in control, and enhanced rate of consistency drop beyond the maximum. The dough prepared with both NEMI and urea (curve E) had essen-

tially the characteristics of the urea-containing dough, but the consistency drop beyond maximum was steeper. The alteration of dough structure caused by adding urea was so extensive that adding NEMI had no additional effect. This was true despite the fact that the presence of urea exposed additional –SH groups to the blocking action of NEMI.

The extensigrams of doughs containing the buffer, NEMI, urea, or NEMI and urea are shown in Fig. 2. The use of the buffer instead of

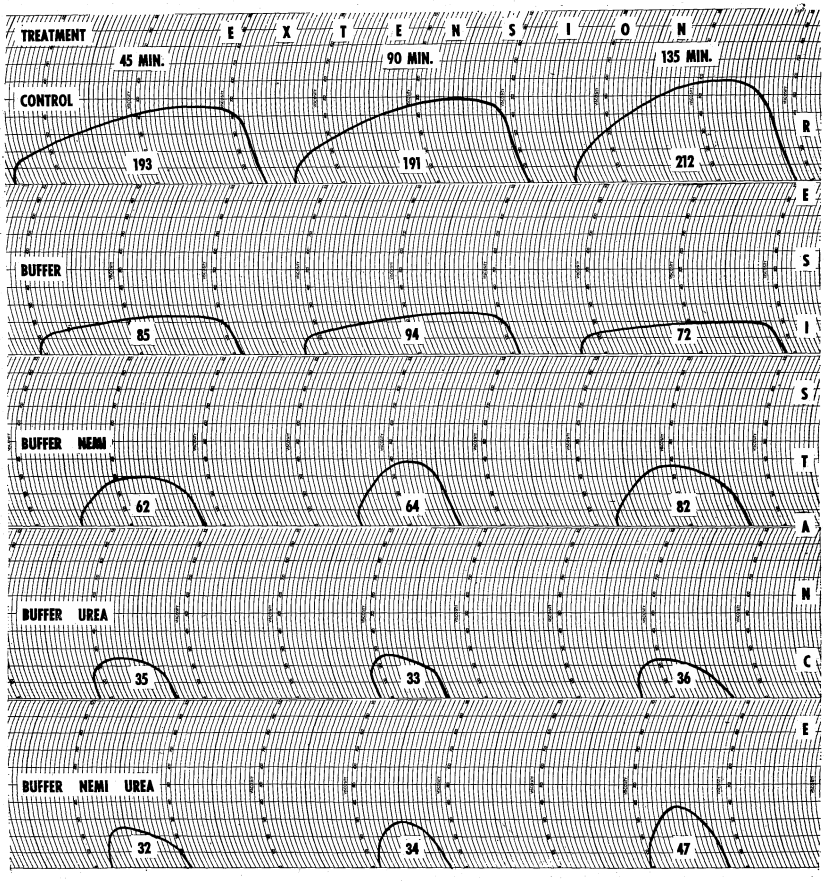

Fig. 2. Effects of 3.0*M* urea and 0.001*M* NEMI on extensigraph characteristics of doughs prepared with 0.01*M* sodium pyrophosphate buffer, pH 7.0. (Figures inscribed into the extensigrams denote areas in cm.^{2}.)

a sodium chloride solution changed the extension only slightly but lowered dough resistance considerably. Areas of the buffered-dough

extensigrams were only approximately half those of the control. Presence of NEMI in the buffer increased dough resistance slightly compared with the buffered dough, but substantially decreased extension and extensigram areas. Adding urea to the buffer lowered dramatically both extension of the dough and areas of extensigrams. Whereas adding NEMI resulted in the formation of a sticky, highly extensible dough, adding urea caused formation of a creamy mass lacking normal dough structure.

The effects of adding NEMI and urea on rheological properties of developed dough systems are presented in Fig. 3. Farinograms A, C,

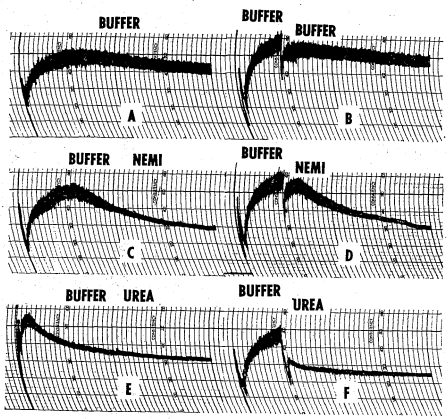

Fig 3. Effects of adding 0.001*M* NEMI and 3.0*M* urea on farinograph characteristics of doughs developed for 5 min. with 0.01*M* pyrophosphate buffer, pH 7.0. Curves A, C, and E were obtained by the regular procedure; in B, D, and F the buffer, NEMI, and urea, respectively, were added after 5-min. mixing time.

and E were obtained from doughs developed with buffer solutions for 5 min., after which 1.0 ml. buffer, 1.0 ml. NEMI, or 2.8 ml. urea, respectively, was added; the total amount of liquid in the final three doughs was the same as in the controls. NEMI caused weakening of the dough, similar to that resulting from adding the reagent directly to flour (compare curves C and D). The urea action was almost immediate and caused breaking of the dough structure in a few seconds (curve F). Such action of urea might be expected from the results obtained by Cook and Alsberg (11), who demonstrated highly intensive dispersion of gluten proteins in the presence of urea.

As there has been no agreement in the literature on whether changes induced by urea in gluten structure are connected with irreversible protein denaturation (11,15), the nature of the urea effect was studied. Farinograms in Fig. 4 demonstrate the reversible character of the urea-

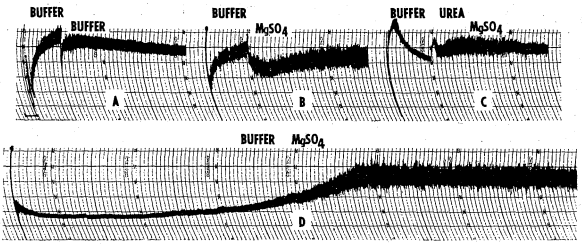

Fig. 4. Effects of adding 0.63*M* magnesium sulfate on the farinograph characteristics of developed dough, 3.0*M* urea-containing dough, and undeveloped dough. Top row, curves of doughs mixed for 5 min. with buffer or buffer and urea solutions, respectively. Additional buffer solutions or solid $MgSO_4$ added after dough development for 5 min. Curve D was obtained from a dough mixed with a buffer solution and solid $MgSO_4$.

affected modification of dough properties. Adding magnesium sulfate to the dough developed in the presence of urea caused rapid restoration of almost normal rheological properties of the system (curve C). Magnesium sulfate added to the dough developed without urea caused minimal changes in farinograph consistency but increased the width of the farinogram band appreciably.

Such an effect of magnesium sulfate seems related to its ability to "salt-out" the macromolecular proteins, as demonstrated earlier (11,12). Cook and Alsberg (11) and Rose and Cook (12) recommended the use of magnesium sulfate as an effective precipitant for the gluten proteins dispersed in different solvent systems. According to Holme and Briggs (16), 2*M* to 3*M* concentrations of urea solubilize gliadin by bonding with hydrogen bonding sites on the gliadin molecules. In the presence of urea the reacting sites are masked to intermolecular cross-linking. In the absence of urea, intermolecular cross-linking takes place and gliadin solubility is decreased. As a result of adding salts to such solutions, intermolecular bonding becomes again predominant, and gliadin precipitates from the solution.

Magnesium sulfate, when added to flour simultaneously with the buffer (Fig. 4, curve D), caused extremely slow development of dough structure. Protein imbibition and formation of a characteristic gluten structure of the dough were detectable only after a 20-min. mixing period; consistency of the dough was normal after 40 min. The effect of magnesium sulfate on the farinograph curve was opposite to that of urea.

Results of this study point to a major role of hydrogen bonding in solubilization of wheat proteins and formation of dough structure. In the absence of these forces, no normal dough can be formed. The possibility of rapid breakdown and of reversible restoration of the

gluten structure supports previous suggestions (11–13) concerning high lability of that system. Better understanding of the hydrogen bonding in wheat gluten should extend and amplify our knowledge and lead to a more detailed picture of the structure of wheat proteins.

Acknowledgment

The authors gratefully acknowledge the help of K. F. Finney, who provided the sample of wheat flour used in this study.

Literature Cited

1. AXFORD, D. W. E., CAMPBELL, J. D., and ELTON, G. A. H. Disulphide groups in flour proteins. J. Sci. Food Agr. **13:** 73–78 (1962).
2. BUSHUK, W., and HLYNKA, I. The effect of iodate and N-ethylmaleimide on extensigraph properties of dough. Cereal Chem. **39:** 189–195 (1962).
3. MECHAM, D. K. Effects of sulfhydryl-blocking reagents on the mixing characteristics of dough. Cereal Chem. **36:** 134–145 (1959).
4. 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).
5. TKACHUK, R., and HLYNKA, I. Reactions of flour protein sulfhydryl with N-ethylmaleimide and iodate. Cereal Chem. **40:** 704–716 (1963).
6. TSEN, C. C., and BUSHUK, W. Changes in sulfhydryl and disulfide contents of doughs during mixing under various conditions. Cereal Chem. **40:** 399–408 (1963).
7. FRATER, A., 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. MAURITZEN, C. A. M., and STEWART, P. Disulphide-sulphhydryl exchange in dough. Nature **197:** 48–49 (1963).
9. REITHEL, F. J. The dissociation and association of protein structures. Advan. Protein Chem. **18:** 124–226 (1963).
10. SCHERAGA, H. Protein structure. Academic Press: New York (1961).
11. COOK, W. H., and ALSBERG, C. L. Preparation of glutenin in urea solutions. Can. J. Research **5:** 355–376 (1931).
12. ROSE, R. C., and COOK, W. H. Viscosity of gluten dispersed in alkali, acid, and neutral solvents. Can. J. Research **12:** 63–81 (1935).
13. BECKWITH, A. C., WALL, J. S., and DIMLER, R. J. Effect of disulfide bonds on molecular interactions of wheat gluten proteins. Federation Proc. **22:** No. 1122 (1963).
14. BURK, N. F. Osmotic pressure, molecular weight and stability of gliadin. J. Biol. Chem. **124:** 49–70 (1938).
15. DILL, D. B., and ALSBERG, C. L. The preparation, solubility and specific rotation of wheat gliadin. J. Biol. Chem. **65:** 279–304 (1925).
16. HOLME, J., and BRIGGS, D. R. Studies on the physical nature of gliadin. Cereal Chem. **36:** 321–340 (1959).
17. BECKWITH, A. C., WALL, J. S., and DIMLER, R. J. Amide groups as interaction sites in wheat gluten proteins: Effects of amide ester conversion. Arch. Biochem. Biophys. **103:** 319–330 (1963).
18. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (7th ed.). The Association: St. Paul, Minnesota (1962).
19. FINNEY, K. F., and BARMORE, M. A. Yeast variability in wheat variety test baking. Cereal Chem. **20:** 194–200 (1943).
20. FINNEY, K. F., and BARMORE, M. A. Optimum vs. fixed mixing time at various potassium bromate levels in experimental bread baking. Cereal Chem. **22:** 244–254 (1945).
21. VILLEGAS, EVANGELINA, POMERANZ, Y., and SHELLENBERGER, J. A. Effects of thiolated gelatins and glutathione on rheological properties of wheat doughs. Cereal Chem. **40:** 694–703 (1963).
22. COATES, J. H., and SIMMONDS, D. H. Proteins of wheat and flour. Extraction, fractionation, and chromatography of the buffer-soluble proteins of flour. Cereal Chem. **38:** 256–272 (1961).