

# Mixograph Studies. VI. Combined Effects of Charge (pH), Activated Double-Bond Compounds, and Oxidants on Dough Mixing Properties<sup>1</sup>

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## ABSTRACT

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The effects of activated double-bond compounds, fast-acting oxidants, and pH (singly and in combination) on dough overmixing (breakdown) were investigated by mixogram curve analysis. Breakdown of flour-water and full-formula doughs was quantitated by measuring mixogram curve width at 3 min after the point of minimum mobility (optimum mixing). The effect of activated double-bond compounds was more pronounced with flour-water doughs than it was with full-formula doughs, although their effect was still clearly demonstrable in mixograms. Fast-acting oxidants, such as KIO<sub>3</sub>, were shown to oxidize a compound in the gluten-

prime starch fraction and thereby cause an accelerated breakdown of the mixogram tail (overmixing). Stability of flour-water doughs to overmixing, as measured by the mixogram tail width, gave a sigmoidal curve as a function of dough pH. Tail widths were narrow at low pH and wide at high pH. There was a plateau in the curve near the pH of flour-water slurries (~6.0). Compounds that affected curve stability were most effective in the plateau region. Strong flours were more resistant to changes in pH than were weak flours.

When wheat flour doughs are mixed beyond their optimum mixing point, a number of changes in the physical and functional properties of the dough result. For example, extensibility increases and elasticity decreases. At the same time, the dough surface, which was dry and smooth at optimum mixing, becomes wet and sticky. This phenomenon, commonly referred to as dough breakdown, is well recognized (Hoseney 1985). Several compounds accelerate the breakdown of dough. Likewise, certain conditions and compounds inhibit that breakdown. However, the mode of action of these compounds remains unclear because the mechanism of dough breakdown is, at best, poorly understood.

The objective of these studies was to more completely understand the effects of various formula ingredients and rheologically active compounds on the mixing stability of wheat flour doughs.

## MATERIALS AND METHODS

Among the compounds found to affect dough breakdown are activated double bond (ADB) compounds. These compounds, which possess a double bond  $\alpha$  to a carbonyl group are present at low levels in wheat flour, primarily as phenolic acids (Sosluski et al 1982). Schroeder and Hoseney (1978) found that the addition of exogenous ADB compounds to wheat flour caused accelerated dough breakdown during mixing. The accelerated breakdown was reversed by lipoxigenase or the free radical-scavenging antioxidant, Tenox (Hoseney et al 1980). A model proposed by Schroeder and Hoseney (1978) suggested that ADB compounds acted through a free-radical mechanism. Specifically, the ADB compounds interacted with free radicals created on gluten proteins during mixing.

A commercially milled hard wheat bread flour (11.5% protein,  $N \times 5.7$ ) was obtained from Ross Industries, Wichita, KS. A high-gluten flour, obtained from the Pillsbury, Minneapolis, MN, was determined to be a strong flour. Prime wheat starch and vital wheat gluten were supplied by Midwest Grain Processors, Atchison, KS. A hard red winter wheat flour (11.34% protein), cultivar Siouxland, was determined to be a weak flour. Tenox (20% BHA, 20% BHT in corn oil) was obtained from Eastman Chemical Products, Kingsport, TN. Enzyme-active soy flour (a source of lipoxigenase) was obtained from Farmland Soy Processors, St. Joseph, MO. All other chemicals were reagent grade.

### Full-Formula Doughs

Full-formula bread doughs were prepared by AACC method 10-10B (AACC 1983). The formula consisted of the following ingredients (percentages based on flour): flour (100%), water (optimum), sucrose (6.0%), NaCl (1.5%), hydrogenated vegetable shortening (3.0%), nonfat dry milk (4.0%), active dry yeast (0.76%).

### Dough pH

Dough (10 g) was blended with 100 ml of distilled water for 1 min. in an Osterizer blender at high speed. Three drops of octanol were then added to disperse any foam formed during blending. Solution pH was determined using a pH meter (Corning model 125).

### Mixing Under Nitrogen

Flour (10 g, 14% mb) was placed in a mixograph bowl, and the bowl was sealed with a two-hole 15 rubber stopper. After air was removed from the bowl and flour by evacuation, they were flushed with dry N<sub>2</sub> (three times). Water for the mixograms was degassed by boiling and stored under a blanket of N<sub>2</sub> until use. Evacuated and N<sub>2</sub>-flushed bowls containing flour were placed in a 10-g mixograph enclosed in a gas-tight glove bag. The glove bag was sealed, and the entire assembly was flushed with dry N<sub>2</sub> (two times). Mixograms were produced according to Hoseney et al (1980).

### Determination of Mixing Stability

Using the 10-g mixograph procedure of Finney and Shogren (1972), doughs were mixed for 9 min, unless the effects of extended mixing time were being studied. Dough stability was assessed by measuring the width of the mixogram tail 3 min after the mixing peak (point of minimum mobility). Thus, larger values

Chemical oxidation can also promote dough breakdown during mixing. A dough containing a fast-acting oxidant, such as KIO<sub>3</sub>, breaks down more rapidly than a dough lacking KIO<sub>3</sub>, simply mixed in air (Hoseney et al 1980). Atmospheric oxygen also has an oxidative effect, because a dough mixed in a nitrogen atmosphere (without O<sub>2</sub>) exhibits increased tolerance to overmixing (Baker and Mize 1937).

Finally, charge, specifically the charge on gluten protein molecules, affects the rate of dough breakdown. The accelerated breakdown caused by KIO<sub>3</sub> or ferulic acid can be reversed by the addition of sodium chloride or sodium dodecyl sulfate (SDS) molecules capable of interacting with or shielding charged sites on proteins (Danno and Hoseney 1982). Hoseney and Brown (1983) investigated the effects of pH (and, therefore, charge) alone on dough mixing and breakdown. They found that decreasing dough pH from a native flour value of 6.12 decreased mixing stability, whereas increasing pH increased both mixing time and stability.

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reflected less dough breakdown. The means of three independent determinations are reported. Standard deviations were 1 mm for tail widths < 30 mm and 2 mm for tail widths > 30 mm. This was determined to be the curve parameter most responsive to mixing stability.

#### Ethyl Acetate Extraction

Flour and ethyl acetate (1:3, w/v) were stirred with a magnetic stirrer under  $N_2$  for 30 min, after which the solvent was recovered by centrifugation. The extraction was repeated three times. The extracts were pooled and stored under  $N_2$  until use.

#### Thin-Layer Chromatography of Ethyl Acetate Solubles

Silica gel plates (20 × 20 cm), obtained from EM Reagents, Cincinnati, OH, were used to separate the compounds removed from flour by ethyl acetate extraction. Two solvent systems were employed: benzene-methanol-acetic acid at 90:16:8 and at 94:4:2. Easily oxidizable compounds were detected with phosphomolybdic acid spray reagent (Alltech Associates, Deerfield, IL).

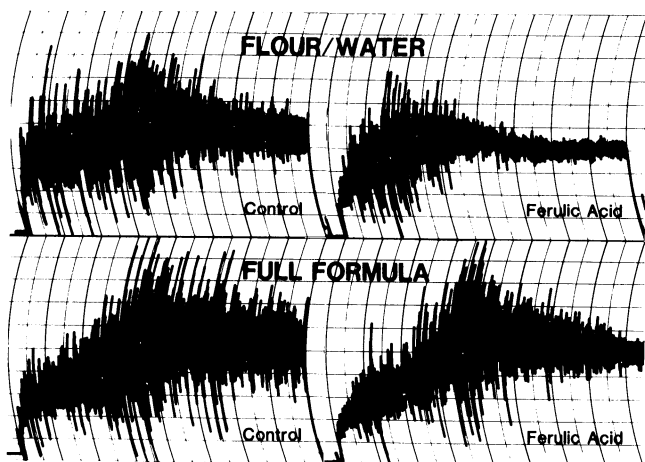


Fig. 1. Effects of activated double-bond compounds on the mixing properties of flour-water and full-formula doughs.

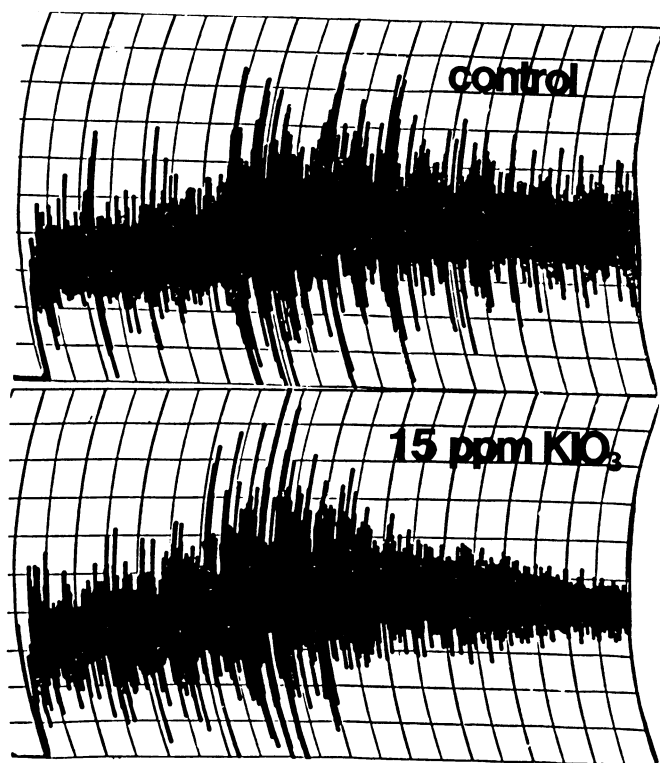


Fig. 2. Effect of  $KIO_3$  on the mixing characteristics of gluten-starch doughs.

## RESULTS AND DISCUSSION

Those compounds shown previously to affect the breakdown of flour-water doughs (Weak et al 1977, Schroeder and Hosney 1978, Hosney et al 1980, Hosney 1985) were used here at the concentrations shown to produce optimum effects.

#### ADB Compounds

Previous studies on the action of ADB compounds in doughs have used flour-water doughs as the experimental system. The additional materials present in a full-formula dough might cancel or, at least, modify the effects observed with flour-water doughs. To test this, mixograph studies were undertaken to determine the effects of ferulic acid (an ADB compound) on the breakdown of full-formula doughs (Fig. 1). Comparing the mixograms shows that the including breadmaking formula ingredients produced doughs more resistant to overmixing than their flour-water counterparts. Although the accelerated breakdown that accompanies ADB presence was more pronounced in flour-water doughs, it was also clearly evident in the full-formula doughs (Fig. 1). The action of ADB compounds in full-formula doughs suggests that the phenomenon could be of significance in the breadmaking process.

#### Oxidation

Fast-acting oxidants, such as  $KIO_3$ , cause accelerated breakdown of flour-water doughs (Weak et al 1977). Schroeder and Hosney (1978) have shown that removal of the water-soluble fraction resulted in a flour that, when mixed with no added oxidant, gave enhanced mixing stability. Surprisingly, in this study, when the water-soluble fraction of flour was removed, oxidation still accelerated the breakdown of gluten-total starch flours (Fig. 2). To ensure that the action of oxidants did not release a bound form of some endogenous ADB from water-insoluble pentosans, this same study was repeated with commercial gluten plus prime starch. The results are shown in Figure 3. Even without the water-insoluble pentosan fraction (i.e., the difference between the total starch and prime starch fractions), oxidation by  $KIO_3$  was active, either affecting the gluten-starch fraction directly or affecting a compound bound to that fraction.

Hydroquinones can become ADB compounds upon oxidation. When added to flour at 100 ppm and mixed in air (Fig. 4), hydroquinone was extremely effective at accelerating dough breakdown. However, when its oxidation was prevented by mixing under  $N_2$ , its activity was abolished. A number of hydroquinone-related compounds have been reported as natural constituents of wheat (Vuataz, 1950, Bungenberg de Jong et al 1953, Graveland et al 1984).

In an effort to determine whether the compound affecting the mixing stability was, in fact, hydroquinone, thin-layer chromatograms of ethyl acetate extracts were tested with phosphomolybdic acid reagent. Although several easily oxidized compounds were detected in the chromatograms, none of the detected spots corresponded with the authentic hydroquinone standard. Therefore,

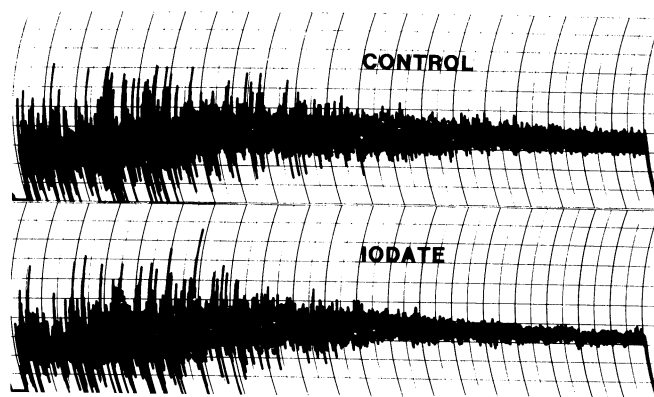


Fig. 3. Effects of  $KIO_3$  on the mixing characteristics of commercial gluten plus prime starch doughs.

hydroquinone could not be the endogenous agent responsible for dough breakdown.

### Multiple Factor Studies

A series of experiments was undertaken to show how dough oxidation, protein charge (a function of pH), and the presence of ADB compounds interacted to affect dough breakdown. Measurements of the stability of a control flour as a function of pH (Fig. 5) gave a roughly sigmoidal curve that showed both the known strengthening effect of high pH and the weakening effect of low pH (Hoseney and Brown 1983). Also noteworthy is the fact that the curve had a region (pH 6-8) over which dough stability was relatively unaffected by changes in pH. The plateau of the curve corresponded, roughly, to the pH range (~pH 6) normally encountered in simple, flour-water dough systems.

The interaction between free radical scavengers and pH was studied by the addition of 2% Tenox to flours at various pHs. When compared with the control, the Tenox-treated dough (Fig.

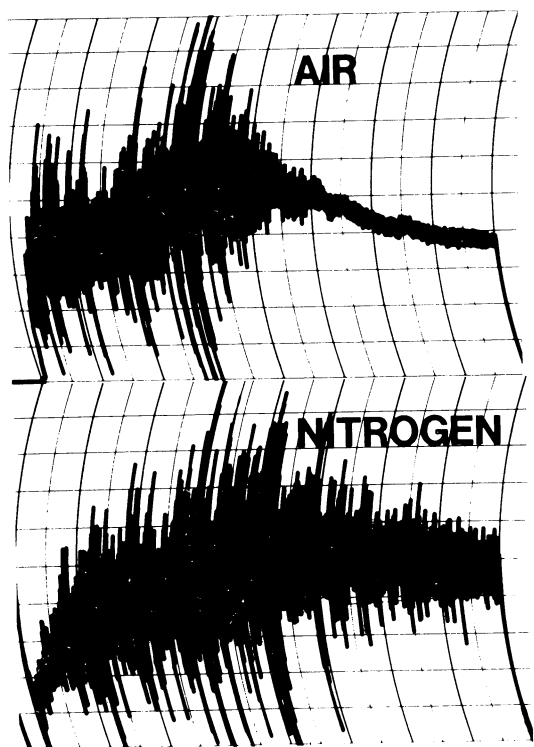


Fig. 4. Effects of 100 ppm of hydroquinone on properties of flour-water dough mixed under air and  $N_2$ .

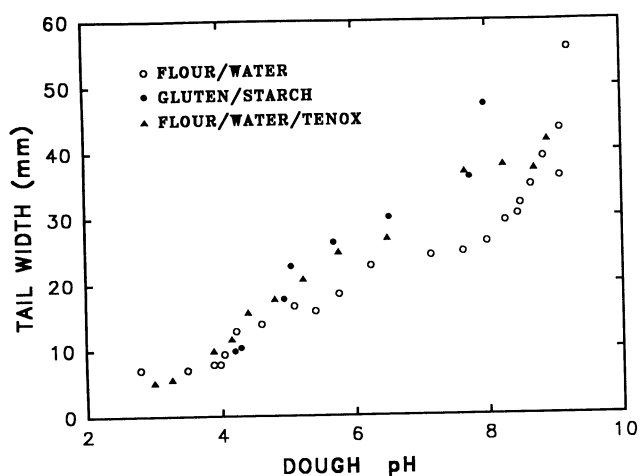


Fig. 5. Dough pH vs. mixing stability (mixogram tail width) for flour-water dough, gluten-starch dough, and flour-water dough plus 2% Tenox.

5) was stronger (greater tail widths) at most pHs tested. This was reflected as a vertical shift in the stability curve. Tenox is thought to eliminate gluten thyl radicals so that ADB compounds can no longer react. When a gluten-starch dough was tested (Fig. 5), the response was similar to that of the flour-water-Tenox system, even though the latter system possessed both ADBs and thyl free radicals.

Studying the effects of oxidants as a function of pH (Fig. 6) demonstrated that mixing under  $N_2$  (without oxidation) eliminated the level region in the pH response curve and made the curve linear over the pH range tested. This effect was more prominent at pH 6 and above. The presence of the oxidant,  $KIO_3$ , at 15 ppm resulted in a downward shift of the curve. Thus, dough was weaker (smaller tail width) at any given pH. In this case, the response was most pronounced at those pHs corresponding to the plateau region of the curve.

When an ADB compound (100 ppm of ferulic acid) was added to a dough, the pH versus stability curve (Fig. 7) retained a shape that was similar to that of the control but shifted down (weakened) and to the right. Chemical oxidation ( $KIO_3$ ) plus ADB compounds weakened the curve further but caused no apparent shift to higher or lower pHs. Although ADB compounds and oxidants appeared to have an additive effect in shifting flour response to pH, the effects are differentiable. Oxidants shift the curve principally up or down, whereas ADB compounds cause both vertical and horizontal displacement of the curve.

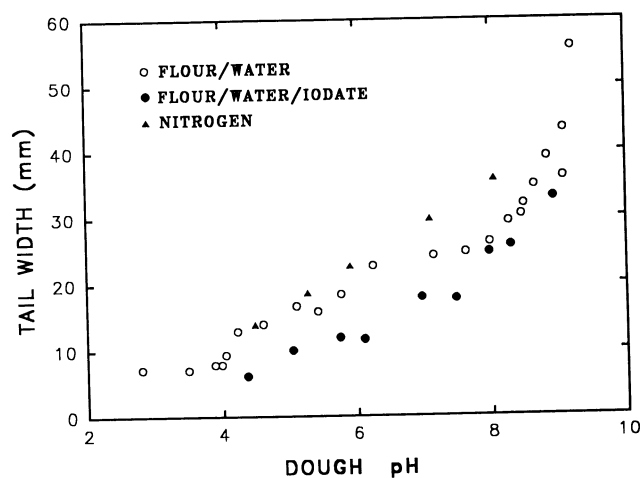


Fig. 6. Effect of pH on dough response to oxidation for flour-water dough control (○), control plus 15 ppm of  $KIO_3$  (●), control plus 15 ppm of  $KIO_3$  mixed under  $N_2$  (▲).

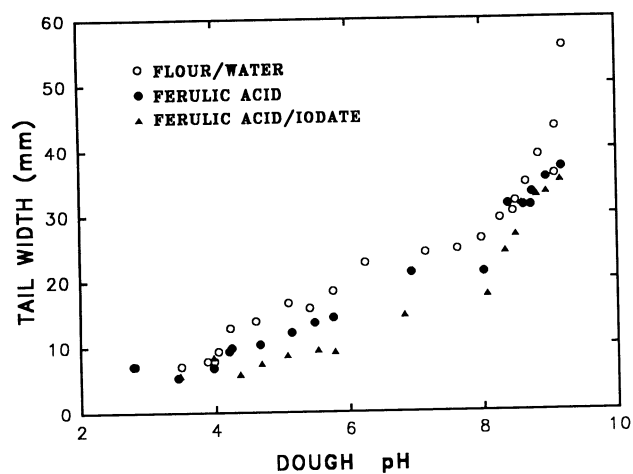


Fig. 7. Effect of pH on dough response to ferulic acid and  $KIO_3$  for flour-water dough control (○), control plus 100 ppm of ferulic acid (●), control plus 100 ppm of ferulic acid plus 15 ppm of  $KIO_3$  (▲).

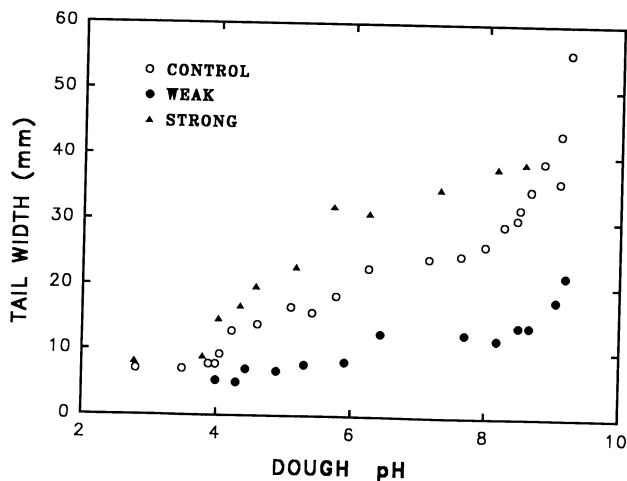


Fig. 8. Effect of dough pH on the stability of flours of differing strengths. ○ = control (Ross), ● = weak (Siouxland), △ = strong (high gluten).

As dough pH was reduced below 6, differences in tail width were reduced (Fig. 7). Below pH 4, no difference was apparent between treatments. This suggests that, at high hydrogen-ion concentration (i.e., low pH), the positive charge generated on the protein is the primary factor affecting dough stability.

#### Responses of Flours of Differing Strengths

The response curves of flours of different strengths (Fig. 8) showed differing effects of pH. A strong flour was stronger at any given pH than was the control HRW flour; the curve was shifted up relative to that of the control. On the other hand, a weak flour gave a curve that was shifted down relative to that of the control (i.e., it had narrower tail widths at any pH).

The different responses of strong and weak flours to changes in pH suggest potentially different protein compositions. Strong flour had greater resistance to dough breakdown in the presence of a positive charge (low pH) than did weak flours. This suggests that the proteins of strong flours have less potential positive charge or fewer positively charged amino acids (a fact not borne out by amino acid analyses) than do the proteins of weak flours. Alternatively, the proteins of strong flours may have stronger interactions with each other and, thereby, resist the effects of charge.

#### CONCLUSIONS

The results of these apparently diverse studies, when taken together with previously published work, add to our understanding of the phenomenon of dough overmixing (breakdown) in several fundamental ways. First, the action of ADB compounds, well-characterized in flour-water systems, was found to persist,

only slightly abated, in full-formula bread dough systems. Second, the ability of the fast-acting oxidant  $KIO_3$  to accelerate dough breakdown persisted in the absence of flour's water-soluble fraction. Thus gluten plus starch, which does not break down significantly when mixed, undergoes rapid breakdown in the presence of  $KIO_3$ . Third, these investigations provided strong evidence that the endogenous agent responsible for the breakdown of intact flour is not hydroquinone, a molecule shown to be very active in the phenomenon. Our understanding of the role played by pH in affecting dough breakdown was expanded significantly by finding a pH range over which dough was relatively unresponsive to changes in pH. The weakening effects of ADBs and fast-acting oxidants on dough were most pronounced in that range. Finally, flours of greater dough strength gave more stable mixograms in the same pH range.

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