MIXOGRAPH STUDIES. II. EFFECT OF ACTIVATED DOUBLE-BOND COMPOUNDS ON DOUGH-MIXING PROPERTIES[1]

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ABSTRACT   Cereal Chem. 55(3): 348-360

The effects of α, β-unsaturated carbonyl compounds, such as fumaric acid (FA), maleic acid, and ferulic acid (FER), on dough-mixing properties are similar to that of the SH-blocking reagent N-ethylmaleimide (NEMI). NEMI and cysteine, however, interact during dough mixing; cysteine and FA do not. Thiol (-SH) and disulfide determinations showed that mixing in air caused a decrease in -SH groups. KIO_3 and NEMI increased the loss of -SH, while FA caused no change in the loss of -SH groups. The disulfide content of dough did not change significantly as a result of addition of FA and NEMI. These results indicate that the effects of FA and related compounds cannot be explained by the thiol-disulfide interchange theory. Fractionation of the flour with water (10:1 water/flour ratio) showed that the mixing properties of the dough were affected by an interaction of the insoluble fraction, gluten plus starch (G+S), and water-soluble (WS) fractions. Further fractionation of the WS fraction indicated that the nondialyzable, heat-stable (WS_{DB}) fraction was most effective in reducing the mixing time of G+S; that fraction has been reported to contain FER. The dialyzable fraction of water solubles (WS_{DZ}) did not affect dough stability, but did reduce mixing time. An interaction of the WS_{DB} and WS_{DZ} fractions apparently caused dough breakdown. Fast-acting oxidants increased the rate of dough breakdown of G+S only when the WS fraction was present or when compounds containing activated double bonds were added. The effects of α, β-unsaturated compounds were reversed by lipoxidase present in enzyme-active soybean flour and antioxidants, which inhibit free radicals reactions. Thus, the α, β-unsaturated compounds appear to have their effect by their reaction with free radicals created in the gluten proteins during dough mixing.

In previous work (1), a mixograph was used to study the effects of oxidizing, reducing, and thiol-containing compounds on dough-mixing properties. The theory of the thiol-disulfide interchange reaction (2,3) explains most of the changes in dough-mixing properties caused by flour additives.

The effects of oxidants, sulfhydryl-containing compounds, and thiol blocking agents have been explained by their ability to oxidize the thiol groups (-SH), to cause cleavage of disulfide bonds (-SS), and to block -SH groups, respectively (2−6). That theory offers some logical conclusions based on the available data, but there are certain weaknesses in the theory in that the effects could be explained by other forms of chemical bonding and molecular interaction (7−9).

Conn and Kichline (10,11) have patented the use of fumaric acid (FA) and unsaturated alcohols to reduce the mixing time in conventional bread production. The effect of FA on the mixogram curve is similar to that caused by N-ethylmaleimide (NEMI) (1). The action of FA and related compounds is unknown. The compounds, however, reportedly react with -SH groups under certain conditions (12).

Ferulic acid (FER) has a structure similar to that of FA, but its effect on the rheological properties of the dough has not been reported. This compound has been found in the water-soluble wheat flour pentosans (13–15) and may take part in the oxidative gelation of aqueous flour extracts.

The objective of this study was to determine the mechanisms of changes that occur in the dough-mixing process, with special emphasis on the unexplained changes caused by FA and related compounds, which reduce the mixing requirement and increase the rate of dough breakdown.
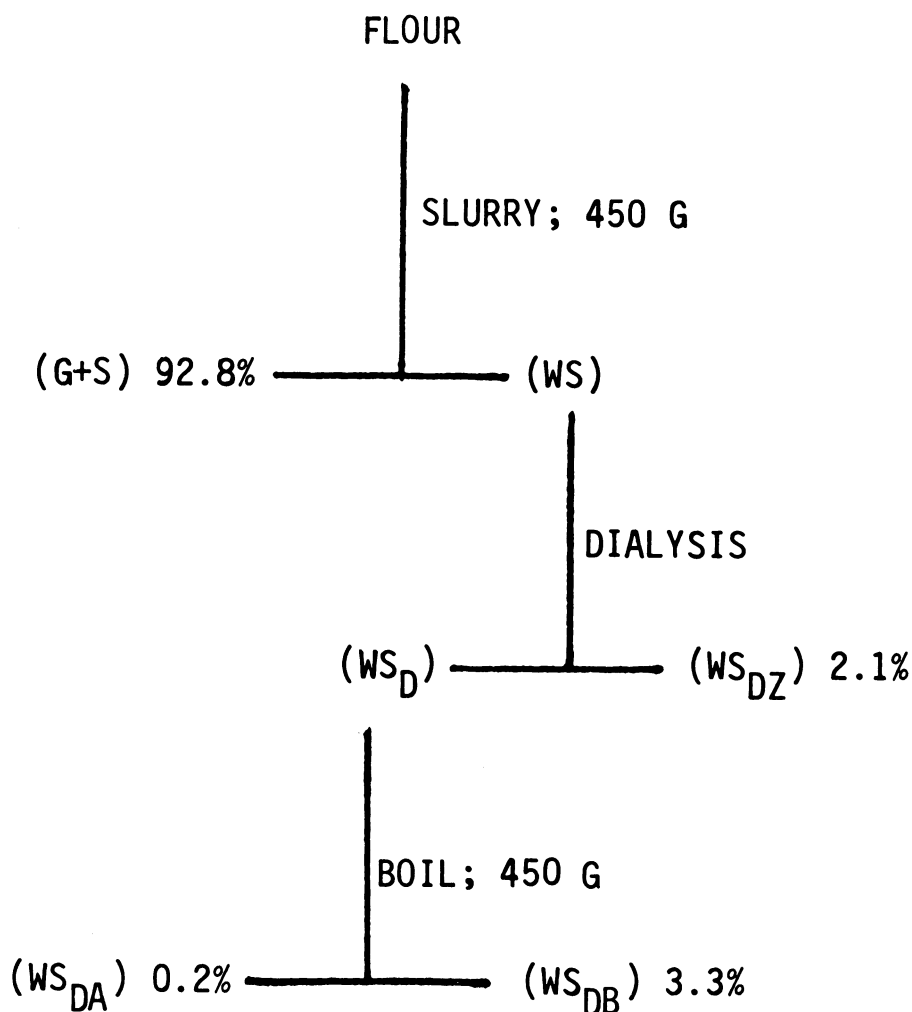

Fig. 1. Fractionation scheme employed to obtain flour fractions. Percentages of amounts recovered are based on flour weight. G+S, gluten plus starch; WS, water-soluble supernatant; WS$_D$, dialyzed fraction; WS$_{DA}$, denatured albumin fraction; WS$_{DB}$, water-soluble dialyzed and heat-stable fraction.

MATERIALS AND METHODS

Effect of Additives

A composite of experimentally milled hard red winter wheat flour with protein of 12.6% ($N \times 5.7$) and ash of 0.43% was used. All values are expressed on 14% mb. The soybean flour used was a commercial sample of enzymatically active defatted soybean flour (soy flour 200E, Far-Mar-Co., Inc., Hutchinson, KS).

All chemicals used were analytic grade. Solutions were prepared fresh daily and neutralized to pH 7 with sodium hydroxide. For interaction studies, the solutions were added to the water just before starting the mixograph. Amounts of chemicals added were expressed in parts per million based on the flour weight at 14% mb except for antioxidant studies. Tenox-4 (Eastman Chemical Products Inc.), a food-grade antioxidant containing 20% butylated hydroxytoluene (BHT) and 20% butylated hydroxyanisole (BHA) dispersed in 60% corn oil, was added on a percent basis. Pure corn oil was used as a control.

A 10-g mixograph was used according to the procedure that Finney and Shogren (16) reported. All samples were run at their optimum absorption level.

Thiol and Disulfide Determination

Sulfhydryl and disulfide determinations were made on flour and lyophilized doughs. The doughs were mixed for 1.0 min and to the peak in a 10-g mixograph at room temperature (16). Each dough was frozen immediately after mixing, lyophilized, and ground (Wiley mill) through an 80-mesh screen. The grinding has been shown not to cause loss of sulfhydryl groups (5).

The determination was done as described by Bloksma (17) except that the same concentration of urea ($9M$) was used for both thiol and disulfide determination.

Flour Fractionation

One part of flour was suspended in 10 parts of distilled water and stirred continuously for 30 min. The suspension was centrifuged for 30 min at $450 \times g$. The insoluble residue, gluten plus starch (G+S), was frozen and lyophilized. The water-soluble (WS) supernatant was further fractionated by dialysis against distilled water for 48 hr. The fraction that passed through a membrane, dialisate (WS_{DZ}), was concentrated under reduced pressure at 40°C. The fraction that remained in the membrane, dialyzed (WS_D), was boiled to coagulate the albumins and centrifuged; the supernatant was called the water-soluble dialyzed and boiled fraction (WS_{DB}), and the centrifugate, denatured albumins (WS_{DA}). All water-soluble fractions were frozen and lyophilized. G+S was ground in a micro Wiley mill to pass through an 80-mesh sieve and rehydrated to about 14% mb. The water-soluble fractions were reduced to a powder in a mortar. Figure 1 illustrates the fractionation scheme used and the amount recovered based on the flour weight.

In the reconstitution study, each fraction was added to the G+S fraction proportional to the amount recovered and the combinations were mixed in the bowl before water was added.

Defatted flour was obtained by extraction with petroleum ether (bp 35–60°C) for 16 hr in a Soxhlet extractor.

RESULTS AND DISCUSSION

The effects of FA and related compounds on dough-mixing properties are similar to those caused by NEMI (1), except that NEMI is more effective in causing dough breakdown (Fig. 2). Those compounds contain a carbon-carbon double bond (-c=c-) activated by an adjacent carbonyl group, and are classified as α, β-unsaturated carbonyl compounds that have properties characteristic of both double bonds and carbonyl functional groups. The functional group that is important in dough mixing is the double bond, because the saturated homolog of FA, succinic acid, does not affect dough-mixing properties. The action of NEMI has been explained by the reaction between its -c=c- double bond and -SH groups of the wheat protein (3,4,6). Thus, NEMI reverses the action of cysteine during dough mixing. If FA and related compounds have similar properties, they
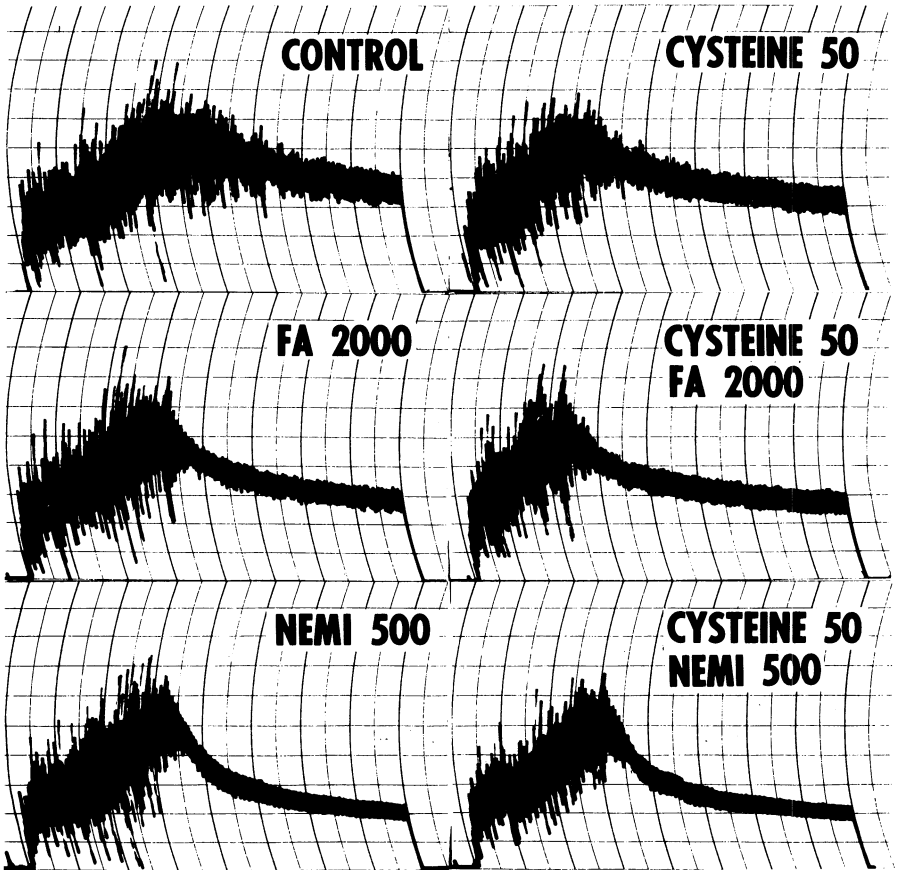

Fig. 2. Mixograms showing effects of fumaric acid (FA, 2,000 ppm), N-ethylmaleimide (NEMI, 500 ppm), and cysteine (50 ppm) and interaction of cysteine–FA and cysteine–NEMI.

should also interact with cysteine.

The effects of cysteine (50 ppm), FA (2,000 ppm), NEMI (500 ppm), and certain of their combinations during dough mixing are shown in Fig. 2. The results show clearly that NEMI and cysteine interacted during dough mixing, because the effect of a mixture of cysteine and NEMI on the mixing curve is essentially the same as that found with NEMI alone. FA and cysteine, however, did not interact, because the combination of FA and cysteine showed an additive effect in reducing mixing time and in causing dough breakdown after the peak. FER and maleic acid gave results similar to those with FA.

Those studies indicated that FA and its related compounds do not exert their effects on dough-mixing properties by the same -SH reaction scheme as that proposed for NEMI (2,6). Those results, however, do not eliminate the possibility that the action of FA and related compounds is caused by their reaction with some specific -SH groups present in a dough system. There is also a possibility that those compounds exert their effects via reactions with -SS groups. If either is true, α, β-unsaturated carbonyl compounds should decrease the -SH or -SS groups or both of the dough.

Thiol and Disulfide Determination: Effects of Mixing and Adding FA, KOI₃, and NEMI

The results of the -SH and -SS group determinations are shown in Table I. The control flour results are mean values of six determinations. For the other samples, results of at least two determinations were averaged. The standard

TABLE I
Thiol and Disulfide Determinations of Flour and
Dough at Different Mixing Times. Effect of FA,[a] KIO₃, and NEMI[b]

Treatment	-SH[c] (μmoles/g[d])		-SS[e] (μmoles/g[d])	
	Found	Change	Found	Change
Control flour	0.58	...	11.10	...
1-Min mixing				
Control dough	0.44	−0.14	11.26	+0.16
+ 2,000 ppm FA	0.41	−0.17	11.17	+0.07
+ 30 ppm KIO₃	0.22	−0.36	11.26	+0.16
Mixed to peak				
Control dough	0.32	−0.26	11.20	+0.10
+ 2,000 ppm FA	0.31	−0.27	11.08	−0.02
+ 4,000 ppm FA	0.38	−0.20	11.16	+0.06
+ 30 ppm KIO₃	0.14	−0.44	11.42	+0.32
+ 60 ppm KIO₃	0.05	−0.53	11.58	+0.48
+ 2,000 FA + 30 KIO₃	0.14	−0.44	11.56	+0.46
+ 500 ppm NEMI	0.00	−0.58	11.16	+0.06
Standard deviation	0.03		0.12	

[a]FA = fumaric acid.
[b]NEMI = N-ethylmaleimide.
[c]-SH = thiol.
[d]Flour dry basis.
[e]-SS = disulfide.

deviations were 0.03 and 0.12 μmoles/g for -SH and -SS determinations, respectively. Values shown as changes in Table I are differences between the value for each sample and the control flour.

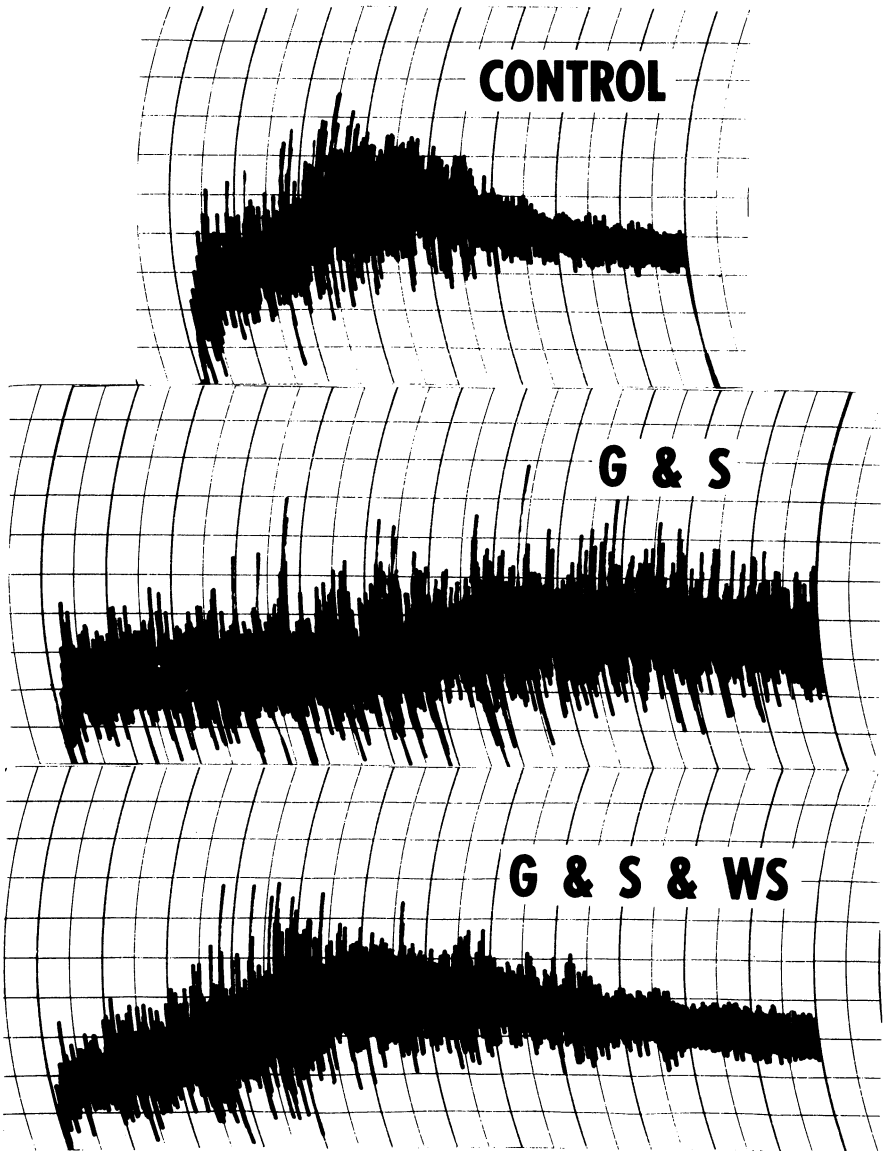Mixing in air caused a decrease in -SH for all samples; the loss of -SH for

Fig. 3. Mixograms of gluten plus starch (G+S) fraction and G+S plus water soluble (WS) fraction.

samples mixed to the peak was greater than that for samples mixed for 1 min. Mixing the sample with KIO_3 increased the loss of -SH. At a KIO_3 level of 60 ppm and mixed to the peak, almost all -SH disappeared.

Mixing a dough containing NEMI caused a total loss of -SH groups. Doughs mixed with FA, however, gave -SH values essentially equal to those for samples mixed without FA. Those results showed that FA did not react with the -SH groups of the flour.

The values of -SS groups represent those originally present in the flour, plus any generated during mixing. The only values significantly different from those of the control were doughs that were mixed to the peak with KIO_3. In those samples, there were significant increases in the total -SS groups. Those increases were of the same magnitude as the loss of -SH groups, suggesting that the loss in -SH was by an oxidation of -SH to -SS groups.

The complete loss of -SH groups for the sample mixed with NEMI, together with the nonsignificant increase in -SS content, suggested that the disappearance of -SH groups was due to their reaction with NEMI. No significant changes were found in the total -SS group for standard doughs or doughs containing FA.

Flour Fractions Involved in Dough-Mixing Properties

Figure 3 shows that the water-insoluble fraction of flour, G+S, had a mixing time of 8 min and a greater dough stability than did the original flour. When the WS fraction was added back to the G+S, the mixing properties of the reconstituted flour were similar to those of the original flour. These results agree with previous reports that the water fractionation technique does not change the flour properties (18−20) and that the dough-mixing properties are caused by an interaction of the water-insoluble and water-soluble fractions (19,21−23). The longer mixing time of the reconstituted flour compared with the control flour can be attributed to the fractionating techniques and to the mixing required to
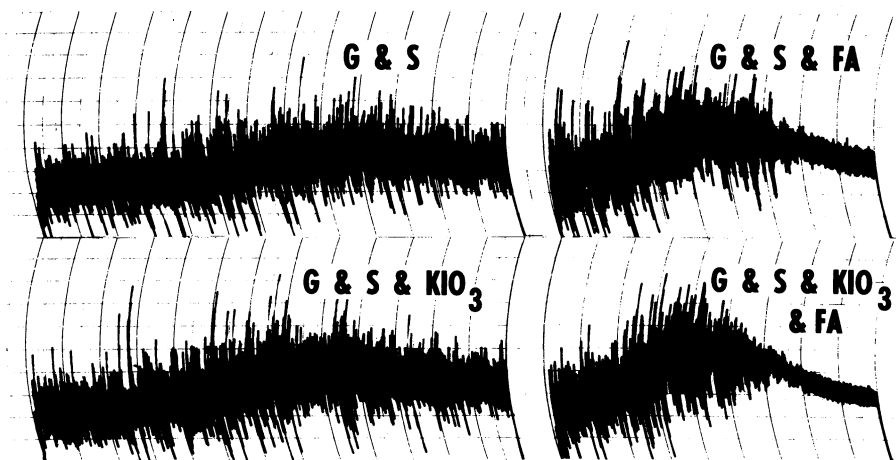

Fig. 4. Mixograms showing effects of potassium iodate (KIO_3, 30 ppm), fumaric acid (FA, 2,000 ppm), and their combination on gluten plus starch (G+S).

reestablish the protein association and interaction that existed in the original flour (22).

Figure 4 shows that KIO_3 had no effect on the mixogram of the G+S fraction. FA, however, was effective in shortening mixing time and increasing the rate of dough breakdown of that fraction. When a combination of KIO_3 and FA were added in combination with G+S, the mixogram showed that dough breakdown was much greater. Similar results were obtained when NEMI or FER was substituted for FA. Thus, apparently fast-acting oxidants (such as KIO_3) need the WS fraction or compounds with an activated double-bond system to be effective.

To determine what in the WS fraction was responsible for the reduced mixing time and for the increased rate of dough breakdown, the WS fractions were fractionated by the scheme shown in Fig. 1. The fractions and their combinations were added back to G+S and mixograms determined in the presence of 30 ppm of KIO_3 (Fig. 5). The WS_{DB} fraction was the most effective in reducing mixing time and increasing the rate of dough breakdown. This fraction reportedly contains FER. The WS_{DZ} fraction had little effect on the rate of dough breakdown, but did reduce the mixing time, while the WS_{DA} fraction affected neither mixing time nor the dough stability (possibly because the albumins were denatured). Dough breakdown apparently is increased by both the WS_{DB} and WS_{DZ} fractions, because the fully reconstituted dough showed a greater rate of breakdown than with either of those fractions separately.

The results of adding KIO_3 and FA to G+S (Fig. 4) raised some doubts that the
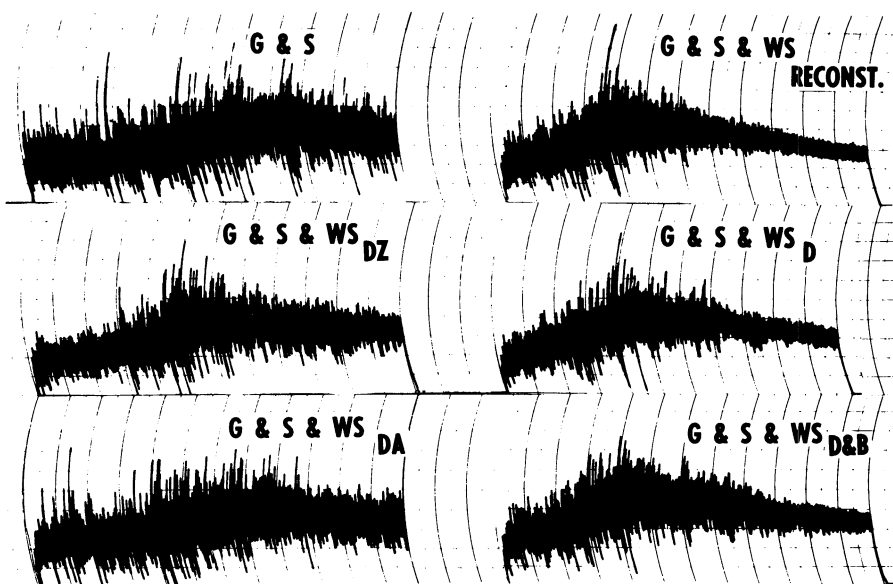

Fig. 5. Mixograms showing effects of water-soluble (WS) fractions on gluten plus starch (G+S). Water-soluble dialysate (WS_{DZ}) (2.1%), water-soluble dialyzed and boiled (WS_{DB}) (3.3%); WS reconstituted total water solubles (5.6%).

effects of oxidants and NEMI are the result of their reaction with -SH groups of the flour protein. If the G+S fraction has good mixing stability because the -SH groups are effective in thiol-disulfide interchange, why did KIO_3 not cause a rapid rate of dough breakdown? If G+S contains no -SH groups effective in thiol-disulfide interchange, why were compounds with active double bonds still effective?

The results obtained suggest that the effect of compounds with activated double bonds on the mixing time reduction and dough breakdown depends on their interaction with some group or radical created in the G+S fraction of flour during dough mixing. The fact that fast-acting oxidants increased dough breakdown only when those activated double-bond compounds were present indicated that the action of the oxidant was to create a functional group or radical that will interact with the added or indigenous activated double-bond compounds. There is evidence that free radicals form during dough mixing (24) and activated double-bond compounds can undergo one electron reduction by radicals (25). Therefore, during dough mixing certain reactions could be initiated by a free radical mechanism.

Systems That Reverse the Effect of Compounds With Activated Double Bonds

Enzyme-active soy flour (soy) increases dough stability. It has been proposed that the stability is increased because free radicals formed during peroxidation of lipids interact with certain flour constituents (26). Results discussed in the previous section showed that compounds with activated double bonds were involved in rapid dough breakdown. If free radicals formed by the soy enzymes acting on lipids do interact with activated double-bond compounds during dough mixing, then the effect of FA should be reversed. Figure 6 shows that 2% soy completely reverses the effect of FA in a normal flour system (left column). When the same study was performed with defatted flour (right column), the lipoxidase enzymes of soy did not have substrate to create free radicals, and therefore, FA was still effective.

If compounds with activated double bonds interact with flour constituents by a free radical mechanism, the use of antioxidants that are free radical scavengers should reverse the effect of those compounds. Figure 7 shows that the effect of FA was reversed by the presence of BHT and BHA in the system. The inert ingredient (corn oil) of the Tenox did not affect the mixing properties of flour. Antioxidants not only reversed the reaction that caused rapid dough breakdown but also increased the mixing time. The data suggest that a free radical mechanism may be involved in the dough development process.

One possible mechanism to explain the effect of compounds with activated double bonds is that during dough mixing, certain -SS bonds will be strained and break, thus forming free radicals that may react with added or indigenous activated double-bond compounds. The results found in the determination of -SS groups (Table I), however, do not support that hypothesis.

The presence of FA did not cause significant decreases in -SS groups. It should be noted that when FA was present in the system, the values found for -SS groups were lower than those found for samples without FA. The differences, however, were within experimental error and thus were not significant. On the other hand, if only 2% or less of gluten SS bonds are broken during dough mixing, as

proposed by Kuninori and Sullivan (27), finding significant differences would be difficult because the error involved in the determination is of the same magnitude.

SUMMARY

Because of its ability to block the -SH groups of the gluten protein, NEMI has been used as a representative of a class of compounds that change rheological properties of dough (2,6). Up to a certain limit, there is a correlation between the disappearance of -SH groups and changes in dough properties (2,6). A good correlation by itself, however, does not prove that the change in -SH group
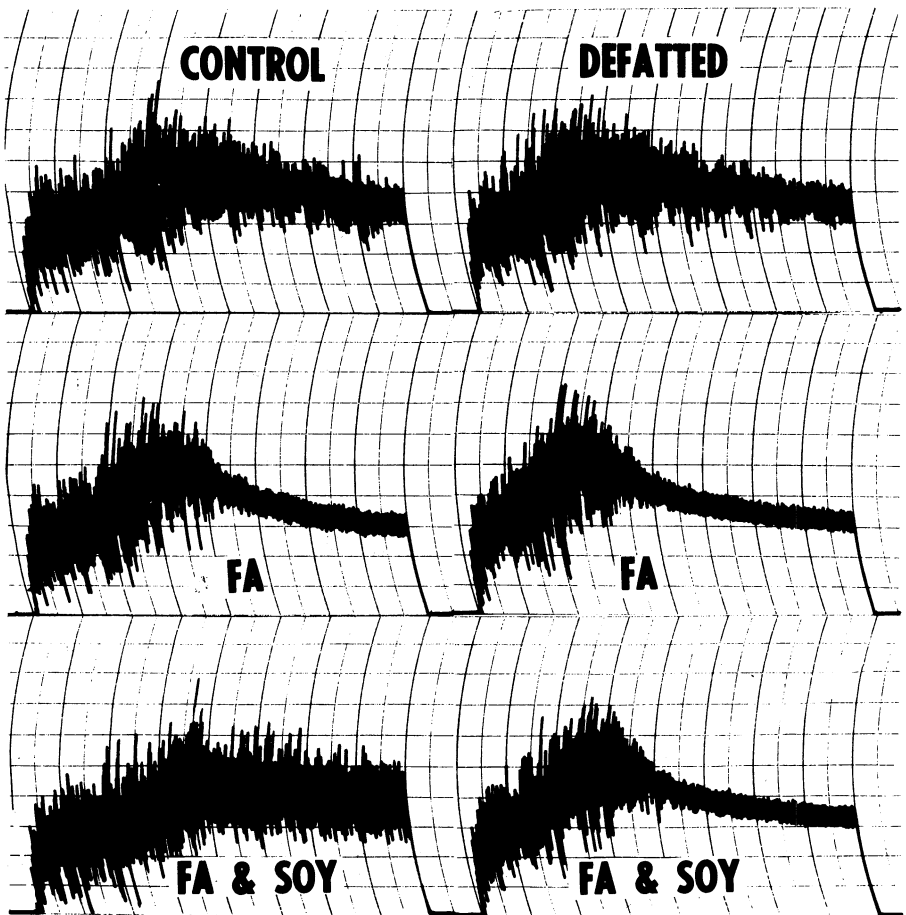

Fig. 6. Interaction of fumaric acid (FA, 2,000 ppm) and enzyme-active soy flour (soy, 2% flour basis) in normal flour (left column) and in defatted flour (right column).

concentration is the cause of changes in dough properties. Other workers (6,21) have suggested that the effect of NEMI is not due to its ability to block -SH groups. The results reported here show that FA and related compounds have effects similar to those of NEMI on dough-mixing properties but that they do not react with the -SH groups of the flour. Thus, NEMI's effect on dough properties is likely by a mechanism other than blocking -SH groups. The use of FA and related compounds will be useful to determine what groups are involved in dough rheology changes, because the results will not be masked by changes in -SH groups.

From a practical viewpoint, FA and related compounds have been shown to be effective in reducing the dough-mixing requirement. Their effect is not reversed by the oxidants commonly used in the baking industry. One of the problems that must be solved is the rapid rate of dough breakdown that is associated with the
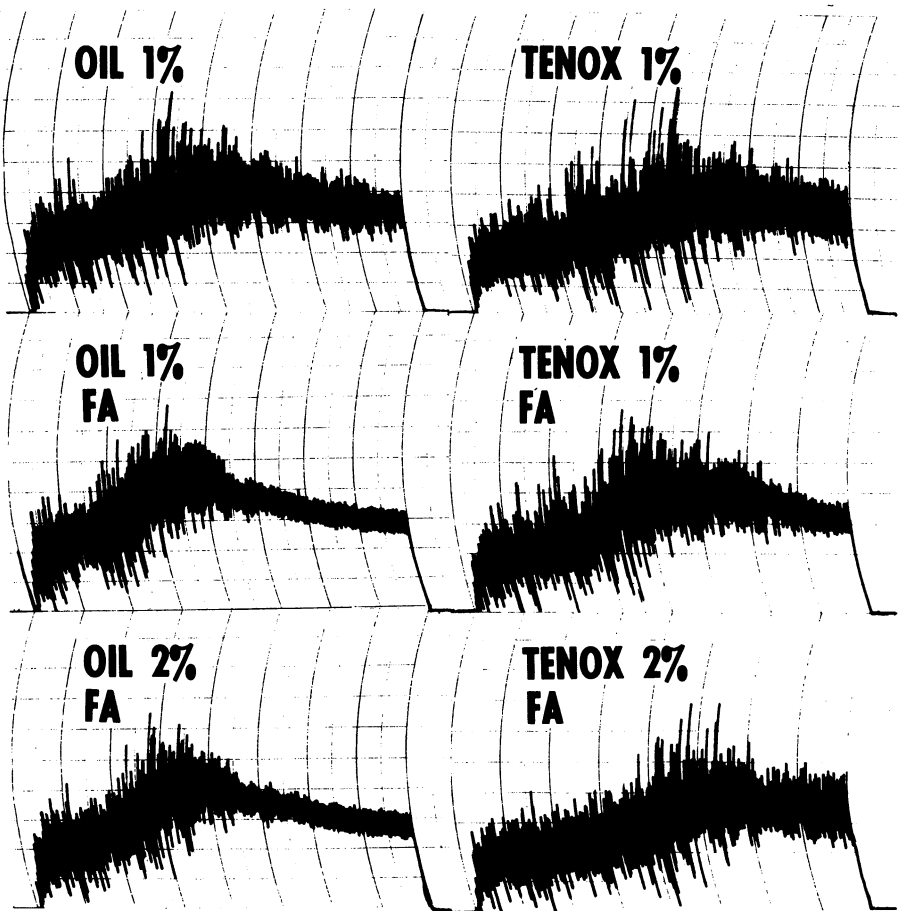

Fig. 7. Mixograms showing effects of Tenox and corn oil (1% and 2% flour basis) and their interaction with fumaric acid (FA, 2,000 ppm).

reduction of mixing time. The lipoxidase system present in enzyme-active soy flour appears to overcome the problem of rapid dough breakdown, but it also lengthens the mixing time.

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