

EFFECT OF ACTIVATED DOUBLE BOND  
COMPOUNDS ON DOUGH MIXING PROPERTIES

by

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B.S. Universidade Federal Rio Grande do Sul - Brasil 1969

A MASTERS' THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER IN SCIENCE

Department of Grain Science and Industry

Kansas State University

Manhattan, Kansas

1976

Approved by

  
Major Professor

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# TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION . . . . .	1
LITERATURE REVIEW . . . . .	2
Effect of Different Compounds on Dough Mixing Properties . .	2
Theories on the Actions of Different Compounds in Dough	
Mixing . . . . .	7
Thiol and Disulfide Determinations . . . . .	9
Flour Fractionation . . . . .	12
MATERIALS AND METHODS . . . . .	14
Effect of Additives . . . . .	14
Thiol and Disulfide Determinations . . . . .	15
Flour Fractionation . . . . .	16
RESULTS AND DISCUSSION . . . . .	20
Effects of Oxidants, Reducing and Thiol-Blocking Sub-	
stances on Dough Mixing Properties . . . . .	20
Effects of Fumaric Acid and Related Compounds on	
Dough Mixing Properties. . . . .	20
Combination of Compounds with Active Double Bond with	
Cysteine . . . . .	37
Thiol and Disulfide Determination. Effects of Mixing	
and Addition of Fumaric Acid, Iodate and N-Ethylmaleimide. .	41
Flour Fractions Involved in Dough Mixing Properties. . . . .	43
Systems that Reverse the Effect of Compounds with	
Activated Double Bonds . . . . .	56
Importance of the Results Found. . . . .	62

	<u>Page</u>
ACKNOWLEDGMENTS . . . . .	70
LITERATURE CITED. . . . .	71

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## INTRODUCTION

The quality of bread depends on the mixing, fermentation and baking characteristics of the dough. Mixing is the process of converting flour and water into dough by both blending and distributing the dough ingredients and developing the gluten protein into a continuous phase. Each flour has its own optimum mixing time which will provide a dough with good handling properties and a better loaf of bread.

The mixing properties of the dough depends on the environmental conditions where the wheat was grown, the milling process, and on the genetic characteristic of the wheat. In general, flours that show good mixing stability usually have a long mixing time and flours with short to medium mixing time have lower mixing tolerance. It is common practice in the bakery industry to use chemical additives to improve the baking properties of the flour and/or to reduce the mixing requirements of the dough development process. Many of these additives can cause a decrease in the mixing tolerance of the dough and thus can be a problem for the industry.

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

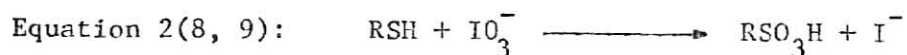
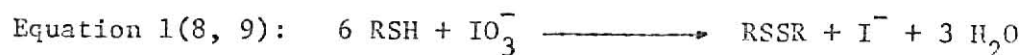
## LITERATURE REVIEW

Effect of Different Compounds on Dough Mixing Properties.

It is generally accepted that oxidants increase dough's resistance to extension, improve machining properties, and increase loaf volume. The effect of oxidants on the dough mixing properties has been studied (1-4). Weak et al. (3) found that fast acting oxidants ( $\text{KIO}_3$ , azodicarbonamide (ADA), and  $\text{KBrO}_3$  at pH 4.7) gave a rapid dough breakdown and an extremely narrow mixogram tail, while slower acting oxidants (ascorbic acid and  $\text{KBrO}_3$  at pH 6.0) did not materially affect the mixograms.

A possible explanation for the action of fast acting oxidants on the stability of the dough is that the oxidizing agents cause a considerable reduction in thiol (-SH) content and, consequently, hamper thiol-disulfide (-SH/-SS) interchange during mixing. That combined with the high speed of mixing, leads to high stresses, which causes breakage of cross-links (5). The decrease of -SH groups caused by oxidants has been well documented (6-9).

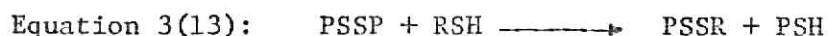
The oxidation of sulfhydryl compounds by iodate may proceed between the two extremes, as proposed by Hird and Yates (8, 9):



The ratio RSH to  $\text{IO}_3^-$  could thus vary between 6 and 1. For cysteine and glutathione the ratios were found to be 4; for thiolated gelatin and reduced proteins of flour, they were between 3.7 and 4.8 (8, 9).

Tsen and Bushuk (6) showed that in iodate treated doughs mixed in nitrogen, -SH losses paralleled iodate losses, given a -SH/iodate molar ratio of about 6 for low iodate concentrations and 4 for iodate concentrations well in excess of -SH content of the flour.

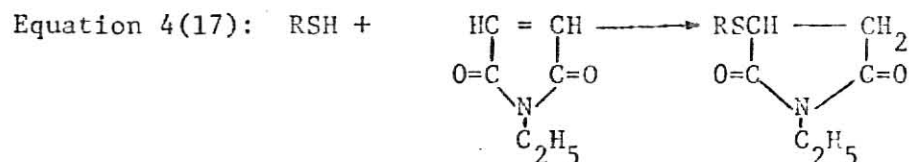
The effects of certain sulfhydryl containing compounds on dough mixing are well documented (3, 10, 11, 12). Most experiments reported in the literature have been performed by adding the amino acid cysteine or the tripeptide glutathione to the dough (5). Cysteine reduces mixing time and at high levels cause dough breakdown (3, 10). The degree that mixing time is decreased appears to depend on the amount of cysteine added rather than flour type (13). The action of the -SH compounds is explained by many researchers as cleavage of inter- and intra-molecular disulfide bonds of gluten protein. The reactions that take place by addition of -SH compounds was proposed by Meredith and Hlynka (13).



This theory is supported by the fact that protein solubility increases after treatment with thiol-reducing reagents (14).

The action of thiol blocking agents can also be related to the thiol-disulfide theory. N-ethylmaleimide (NEMI) is the compound of this class that has been studied most extensively (5). It reduces dough development time and increases peak high of the farinogram; after the peak, the farinogram shows a rapid decrease in consistency (4, 13, 15). The effect of NEMI on the mixograph curve is similar to that found in the farinogram (3, 16). Small amounts of NEMI (0.5 micro-mole/g. of flour) can cause mixing changes which could be

explained by inhibition of thiol-disulfide exchange (5, 15). The chemical reaction of NEMI and a thiol group is shown below:



The reduction of the thiol content of the dough by NEMI has indeed been demonstrated (18, 19). Meredith and Bushuk (15) showed that in addition to the reaction with -SH groups, NEMI reacts with another functional group. That functional group reacts with iodate either very slowly or not at all. This secondary reaction, which seems to occur during mixing and only after all the -SH groups have been removed or blocked, has a marked effect on the tolerance of the dough to mixing (15, 20). Indirect evidence suggest that the secondary reaction involves the SS-bonds (15, 20). The theory that the action of NEMI is due to its reaction with the -SH groups of the flour has been questioned (22) because NEMI is not specific toward sulfhydryl compounds as had previously been supposed (21). The reaction of NEMI with imidazole and its derivatives, and with the alpha-amino group of peptides has been reported (21). The reaction of NEMI and imidazole groups may cause the disruption of ionic interactions involving histidine groups (22).

The use of fumaric acid (FA) and unsaturated alcohols to reduce the mixing time in conventional bread production has been patented by Conn and Kickline (23, 24). The effect of fumaric acid on the mixogram curve is similar to that caused by NEMI (3). The action of FA and related compounds is unknown, although, the compounds are reported to react with thiol groups under certain conditions (25).

The effect of ferulic acid on the rheological properties of the dough has not been reported. This compound has been found esterified to the water soluble wheat flour pentosans (26-28) and may take part in the oxidative gelation of aqueous flour extracts and therefore maybe an other type of compound in wheat flour readily attacked by oxidizing agents (26). Ferulic acid can reportedly form diferulic acid thus cross-link polysaccharide chains (27). In the oxidized crude pentosans fraction, diferulic acid was detected (27). Ferulic acid has also been reported to be present in the starch tailing fraction and is probably bound by ester linkage to the insoluble pentosans (29).

The use of enzyme-active soy flour as bleaching agent has been patented by Hass and Bohn (30). Enzymatically active soy flour is used extensively in the commercial preparation of white bread (31, 32). The enzymes chiefly concerned are the lipoxidases, although proteinases and beta-amylases play a minor role (31). The reaction involved in the bleaching action is a coupled oxidation of carotene and unsaturated fatty acid by atmospheric oxygen (33). Besides its bleaching effect, lipoxidase increase the mixing tolerance of the dough (31, 33). Koch (34) found that if flour is defatted before mixing into a dough, lipoxidase addition has no effect, and if the lipids are extracted from the flour, treated with lipoxidase, and sprayed back onto the flour, the mixing tolerance is reduced. A possible explanation for the observed results will be that some intermediates formed during the enzymatic oxidation, possibly free radicals, react with some constituents

of flour protein (34). An increase in the loss of flour -SH group due to the presence of lipoxidase and fat peroxides has been used as evidence that the improvement caused by enzyme-active soy flour is due to the oxidation of sulfhydryl groups (31, 34, 35, 36).

The effect of ascorbic acid (AA) on dough mixing properties is more controversial. AA itself is a reducing substance but exerts its improving action as an oxidizing agent (37). L-ascorbic acid is purported to be oxidized by an enzyme to form dehydroascorbic acid, which acts as an oxidant (32). In the continuous bread production system, ascorbic acid reduces mixing speed requirements and produces softer doughs (37, 38). Johnston and Mauseth have patented the use of ascorbic acid in continuous bread production (39). Weak et al. (3) studied the effect of ascorbic acid on the mixograph curve in air and CO<sub>2</sub> atmospheres and showed that the only discerable effect of ascorbic acid is a reduction of peak height. This effect could be related to the softer and drier doughs described by Johnston and Mauseth (38) using ascorbic acid in continuous bread production (3). Tsen (40) proposed that the improving action of ascorbic acid is due to the oxidation of -SH groups in dough by dehydro-ascorbic acid formed during dough mixing. However, Zenter (41) has shown that neither reduction of disulfide bonds nor blocking of thiol groups can be attributed to ascorbic acid. Kuninori and Matsumoto (42) did not detect any changes in -SH groups due to the addition of dehydroascorbic acid. Zenter (41) suggested that ascorbic acid acts as a hydrogen bonding breaking reagent similar to urea.

The effect of pH on the mixing properties of dough has been reported previously. Earlier results are summarized by Tanaka et al. (43), who observed that not only pH but also salt concentration altered dough mixing properties. Decreasing the dough pH (5.8 to 4.2) in the absence of salt increased both the maximum consistency of the dough and the rate of breakdown; however, the resistance to extension decreases as pH decreases in the presence of salt (43). The mixing time rapidly increases for doughs mixed in air as the pH increases to 9 and rapidly decreases from pH 9 to 10 (43, 44). In a nitrogen atmosphere, mixing time stays essentially constant through the pH range 4 to 10 (43, 44). The farinograph stability of doughs mixed in air increases with increases in pH from 4 to 9 and drops off sharply from pH 9 to 10 (43, 44). In a nitrogen atmosphere, farinograph stability remains the same (or tends to drop off) for all pH levels other than the normally found during mixing (43). These results have been interpreted in terms of an increase in the sulfhydryl-disulfide (SS) interchange with increasing pH (44, 45).

#### Theories on the Actions of Different Compounds in Dough Mixing

As we have seen in the first part of this review, most of the changes in dough mixing properties caused by flour additives have been explained by the theory of the thiol-disulfide interchange reactions. This theory was first suggested by Goldstein (46) and was based on the facts that NEMI, a thiol blocking reagent which does not form disulfide

crosslinks, has effects on rheological properties similar to those caused by oxidizing reagents, and that, in general, the deformation of the dough is predominantly permanent or viscous (19). These observations can only be explained if the -SS crosslinks are not permanent and if there is a constant interchange reaction with -SH groups of the protein network (19).

Jones and Carnegie (47) proposed a hypothesis that recognizes the apparent importance of thiol-disulfide exchange reactions, but does not involve the polymerization of proteins by disulfide linkages. The change from dry flour to wet dough will necessitate conformational re-arrangements in the proteins. The rate at which intramolecular disulfide bonds can be formed will influence the rate of conformational rearrangement, and thiol groups will affect the kinetics of disulfide forming.

There are some research findings which do not support the thiol-disulfide theory. First of all, the similarity of effects between -SH-blocking reagents (NEMI) and fast-acting oxidants is true only for normal mixing times and relatively low reagent concentrations (20).

Sullivan and Dahle (48) in a study of the improving action of formamidine disulfide concluded that although disulfide interchange provided a plausible explanation for the action of improvers, however, their results cast doubt that appreciable interchange occurs at the normal pH range of dough. Kuninori and Sullivan (49) used radioactive glutathione ( $G^{35}SH$ ) and found no evidence of increased reaction rates



with pH increases from 5.0 to 9.6 and/or oxygen removal in a flour slurry system. In a dough system they found incorporation of 29 to 35.4%  $G^{35}SH$  into gluten protein with mixing times from 5 to 30 min. By assuming that -SS-cleavage occurred with this incorporation of  $G^{35}SH$ , one can estimate that approximately 2% or less of gluten SS bonds are broken during normal dough mixing. The authors pointed out that it seems most likely that the major part of the energy employed in the mixing breaks the hydrogen bonds of gluten and starch. Subsequently, certain SS bonds are stretched and strained which makes them more vulnerable to attack by SH groups and changes the configuration and arrangement of the protein structure (49).

In addition to pH, other questions on the soundness of the theory arise from the low number of -SH groups involved in protein conformation and protein size (50). Stanley et al. (51) found evidence that no disulfide bonds are broken or formed during dough preparation.

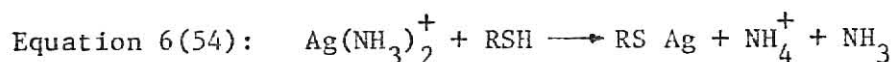
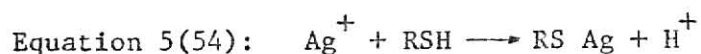
In summary, it is difficult to assess the true importance of thiol-disulfide exchange on dough properties. There are certain weaknesses in the theory which could possibly be explained by other forms of chemical bonding and molecular interaction. On the other hand, the theory does offer some logical conclusions to the data available.

#### Thiol and Disulfide Determinations

For many years progress in the study of sulfhydryl radical chemistry in flour and dough has been hampered by limitations of the analytical

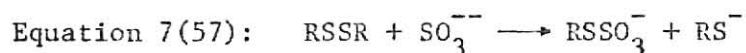
methods available. Flour and dough contain small amounts of readily oxidizable substances which interfere in those analytical methods that depend upon oxidizing agents (18). Many investigators showed that starch may also be an interfering substance (18).

The method introduced by Benesch and Benesch (52) and by Kolthoff, Stricks, and Moren (53), which depend upon the combination of silver or mercury salts with the sulfhydryl group, obviates the difficulties inherent in the use of oxidizing agents. The precision of the titration was increased also by the use of the amperometric technique (52, 53). In the absence of chloride, the titration of -SH groups with silver nitrate can be carried out in acid or neutral medium (54). The interference of chloride and bromide ions can be eliminated if the titration is carried out in an ammoniacal medium (54). The reactions involved in the titration in neutral or acid medium or in the presence of ammonia are:



The stoichiometric reaction of -SH groups and silver nitrate form an involuble silver mercaptide which, in an amperometric determination, limits current flow until there is an excess of silver ions in solution. At this point the diffusion current of silver ions to the rotating platinum electrode rises sharply in proportion to the concentration of these ions in the solution. The end point of the titration is obtained graphically by plotting current readings against volume of silver nitrate

solution added. The end point is determined by the intersection of two straight lines (52). The same principles have been used for the determination of disulfide groups (55, 56). The disulfide groups are reduced to thiol groups by the action of sodium sulfide and then measured by reaction with silver nitrate (55, 56). The reaction of disulfide groups with sulfite is:



These methods have been used with success and studied in detail with regard to pH, temperature, urea concentration and titration atmosphere (55, 56, 58, 59). The specificity of the method has been demonstrated (59).

Available information on the -SH and -SS contents of wheat flours, doughs and protein were reviewed by Mechan (60). The SH content of patent and straight-grade flours has been found to be near 1  $\mu\text{eq}$  per g. of flour or 4 to 19  $\mu\text{eq}$  per g. protein (60). The SS contents range from 7.4 to 16.9  $\mu\text{eq}$  per g. flour, or 83 to 130  $\mu\text{eq}$  per g. protein (60). There is no doubt about the decrease of SH groups during dough mixing (7, 60, 61, 62) and that oxidants and NEHL increase the rate of disappearance (4, 5, 7, 18). More controversy exist about the changes of SS during dough mixing. Axford et al (63) and Tanaka and Bushuk (7) found no change in the number of disulfide groups with mixing different flours in air and nitrogen. Tsen and Bushuk (6) concluded that doughs which show a marked decrease in resistance to extension, as measured with the extensigraph (high iodate treatment and prolonged mixing in air or oxygen), show a definitive loss of S-S as

well as the expected loss of -SH. Bloksma (62) found no correlations between the total, reactive, or nonreactive SS content and the elastic properties of dough.

Sokol et al (64) found that stability values taken from farinograms correlated well with the sulfhydryl-loss rate constant for three hard red spring, three white, and two hard red winter wheat flours, and for two durum flours supplemented with albumin. Nonsupplemented durums and two hard red winter wheat flours giving atypical farinograms did not show this relationship (64). Bloksma (62) found no unequivocal relation between the total SH content and various rheological properties. Smith and Mullen (69) concluded, after a study of -SH and SS content of flour fractions, that mixing properties were not controlled by the total amount of these functional groups.

#### Flour Fractionation

The fractionation of flour with water has been used largely as a tool to characterize the roles of each fraction in the baking and rheological properties of the dough. That the fractionation and reconstitution techniques do not damage the wheat protein has been well demonstrated (65-69).

The amount of water solubles extracted varies widely with the technique and flour:water ratio used. Mattern and Sandstedt (66), using a 1:10 flour:water ratio, recovered 9.9% solubles from a spring wheat blend and from winter wheat samples a range of 8.61 to 10.01% solubles. Finney (65) recovered 3.61% solubles from the gluten wash water from Kharkof, a winter wheat. Prince et al. (70) recovered 5.91%

and 6.31% solubles from the gluten wash water for two spring wheats. Shuey and Gilles (71) reported that the protein content and yield of water-solubles increased with increased dilution and that the material extracted at the ratio of 10:1 (water:flour) influenced the mixing characteristic of the flour. Hoseney et al (67) determined the starch-gel electrophoretic patterns of extracts made with flour-to-water ratios of 1:3, 1:5, 1:10, 1:20, and 1:50 and found that as the flour-water ratio decreased and, thereby decreasing the salt concentration, more and more gliadin was solubilized. They also found several changes in the rapidly moving bands (67).

Mattern and Sandstedt (66) found that the principal factor responsible for determining the mixing requirement of wheat flours was water-soluble, was precipitated by low concentrations of ammonium sulfate, and has a high amide nitrogen content which indicates it consists largely of gliadin. Smith and Mullen (69) used the farinograph to study the mixing properties of doughs prepared from salt-soluble, water soluble and protein-starch residue fractions of short- and long-mixing flours. They found that salt-soluble fractions (albumins and globulins) had little effect upon mixing characteristics, and that protein-starch residues (glutenins) had long-mixing requirements. The addition of water-solubles (containing gliadins) markedly shortened the mixing requirements. They concluded that the mixing differences of short- and long-mixing flours appear to be determined by undefined characteristics of the protein-starch residues and the quantity and molecular-weight distribution of the water-solubles (69).

Shogren et al. (72) found that as the gliadins were removed and glutenins were concentrated, mixing time of the water insoluble protein fraction greatly increased. That evidence alone is not sufficient to conclude that either the gliadins or glutenins determine mixing time, because it is likely that other factors are involved (72). Mattern and Sandstedt (66) found that addition of pentosans to water extracted spring wheat flour reduced the mixing time considerable. Next to water, pentosans are the most effective hydrogen bond-forming components in wheat flour (61). The total (soluble and insoluble) pentosan content of flour is only about 1.51%, but pentosans can absorb 15 times their own weight of water, so up to 23% of the dough water could be bound to pentosans (61).

## MATERIALS AND METHODS

### Effect of Additives

A composite of experimentally milled hard red winter wheat flour with protein of 12.6% (N x 5.7) and ash of 0.43% was used for all experiments except for the study of the effect of potassium iodate, for which a flour of 12.3% protein and 0.44% ash was used. All values are expressed on 14% M.B. The soybean flour used was a commercial sample of enzymatically active defatted soybean flour (soy flour 200E, FAR-MAR-CO, Hutchinson, Kansas).

All chemicals used were analytical 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 (ppm) based on the flour weight at 14% M.B. 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 ten-gram mixograph was used with the procedure reported by Finney and Shogren (73). All samples were run at their optimum absorption level (73).

#### Thiol and Disulfide Determinations

Sulfhydryl and disulfide determination were carried out 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 (73). Each dough was frozen, immediately after mixing and then lyophilized, and ground (Wiley mill) through an 80-mesh screen. The grinding has been shown not cause loss of sulfhydryl groups (64).

For titration of thiol and disulfide groups, flour or dough was dispersed with a magnetic stirrer into 20 ml of buffer solution and followed by the addition of 35 ml of urea or 35 ml of urea plus 1 ml of sodium sulfide for the determination of SH groups or -SH plus SS groups, respectively. The final dispersion had the following composition: a) buffer: ammonium nitrate 1.0 M, ammonia 0.05 M, ethylene-diaminetetraacetic acid (EDTA) 0.001 M; b) urea: 9 M and c) sodium sulfite: 0.02 M (for the determination of SH + SS only). The titration was with 0.001 M

silver nitrate with a micro buret. The end point was detected amperometrically with a platinum electrode at -0.3 v. with respect to the saturated calomel electrode. A Fisher Electropode model 65 was used. After each titration, the electrode was cleaned by anodic oxidation. One gram of sample was used for the SH determination and 0.2 g for the determination of SH plus SS. The dispersion and titration were conducted in an atmosphere of nitrogen to avoid oxidation of SH groups. Solutions of silver nitrate and sodium sulfite were prepared fresh daily. This method has been used successfully by Bloksma (62). However in that work, different urea concentrations for the SH (4 M) and SH plus -SS groups (9 M) determinations were used.

#### Flour Fractionation

One part of flour was suspended in 10 parts of distilled water and stirred continuously for 30 min (Fig. 1). The suspension was centrifuged for 30 min. at 450 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 hours. The fraction that passed through the membrane, dializate ( $WS_{DZ}$ ), was concentrated under reduced pressure at 40°C. The fraction that remains in the membrane, dialyzed ( $WS_D$ ), was boiled to coagulate the albumins and centrifugated; the supernatant was called water soluble dialyzed and boiled ( $WS_{DB}$ ) and the centrifugate, denaturated albumins ( $WS_{DA}$ ). All the water soluble fractions were frozen and lyophilized. G + S was ground in a micro Willey mill to pass through a 80-mesh sieve and rehydrated to about 14% moisture. The water soluble fractions were

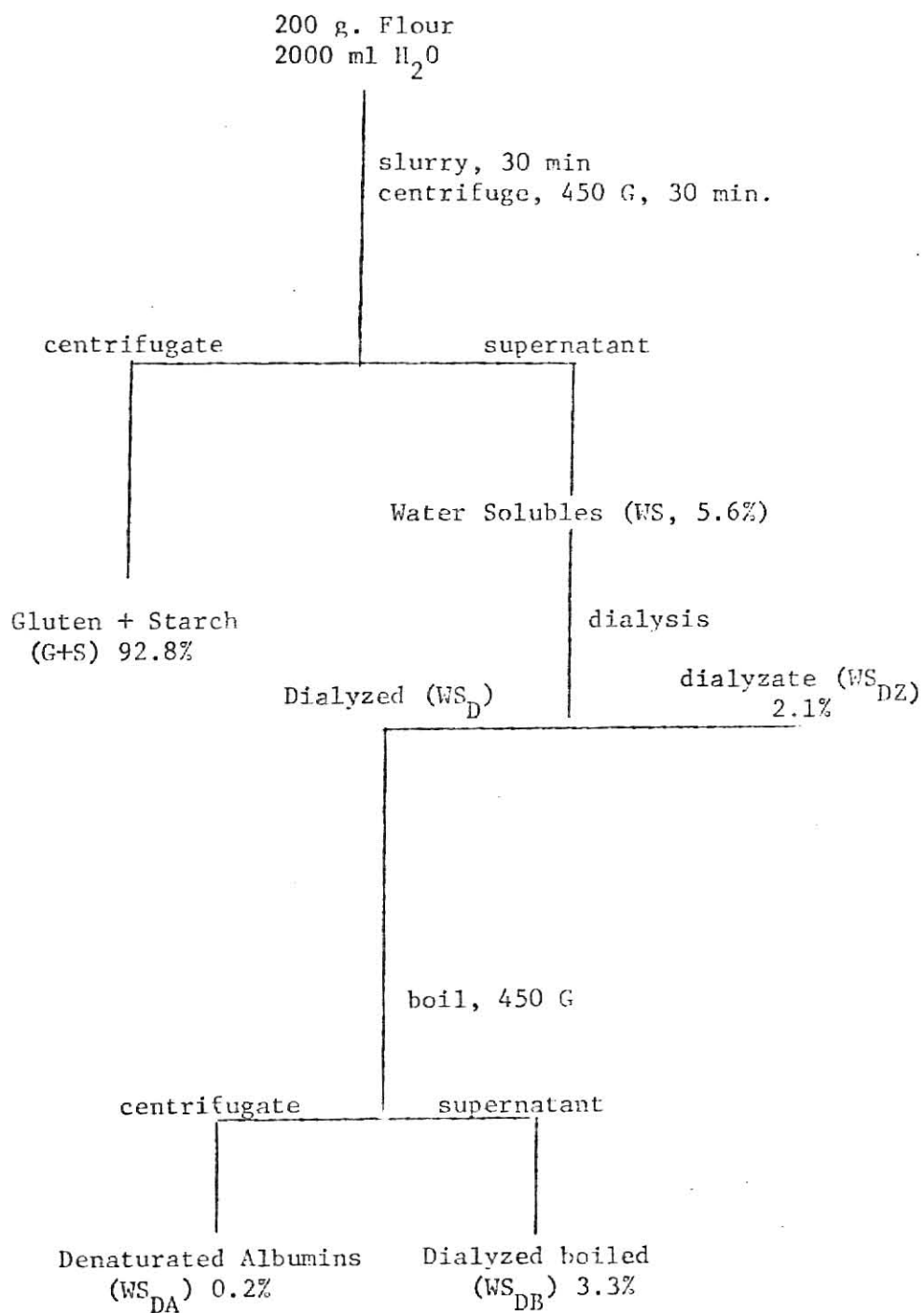


Fig. 1. Fractionation scheme employed to obtain flour fractions.

Percentages of amounts recovered are based on flour weight.

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reduced to a powder in a mortar. Figure 1 illustrates the fractionation scheme and the fraction amounts recovered based on flour weight.

Water soluble pentosans were obtained and fractionated by the procedure of Fincher and Stone (75). The amounts recovered were: crude pentosans, 1.60%; pentosans soluble in ammonium sulphate, 0.12%; and pentosans insoluble in ammonium sulphate, 0.23%.

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

Defatted flour was obtained by extraction with petroleum ether (b.p. 35°-60°C) for 16 hr. in a soxhlet type extractor.

## RESULTS AND DISCUSSION

### Effects of Oxidants, Reducing and Thiol-Blocking Substances on Dough Mixing Properties

The effects of fast-acting oxidants (potassium iodate- $\text{KIO}_3$ ) a reducing agent (cysteine), and a thiol-blocking reagent (N-ethylmaleimide, NEMI) are shown in figures 2, 3, and 4, respectively. Those substances increase the rate of dough breakdown and cause thinning of the mixogram tail. Potassium iodate does not affect the mixing time, while both cysteine and NEMI are effective in reducing the mixing time. The effect of cysteine on mixing time reduction and dough breakdown is proportional to the amount added, while iodate and NEMI appear to reach a saturation point after which further additions do not cause additional changes.

### Effect of Fumaric Acid and Related Compound on Dough Mixing Properties

The effects of fumaric acid (FA) and ferulic acid (FER) on dough mixing characteristics are shown in figures 5 and 6, respectively. Maleic acid, the cis-isomer of FA, has the same effect on dough mixing properties as does FA (Fig. 7). Figure 8 shows the known effect of pH on dough mixing properties (43-45). It also shows that FA is effective in a dough pH range from 5.0 to 8.2.

The effect of fumaric acid and related compounds on dough mixing properties are similar to those caused by NEMI except that NEMI is more effective in causing dough breakdown. We can see from the structural formula of these compounds (Fig. 9) that each one contains a

Fig. 2. Mixograms showing the effects of potassium iodate ( $\text{KIO}_3$ ),  
at the levels of 7.5, 15, and 30 ppm (flour basis).

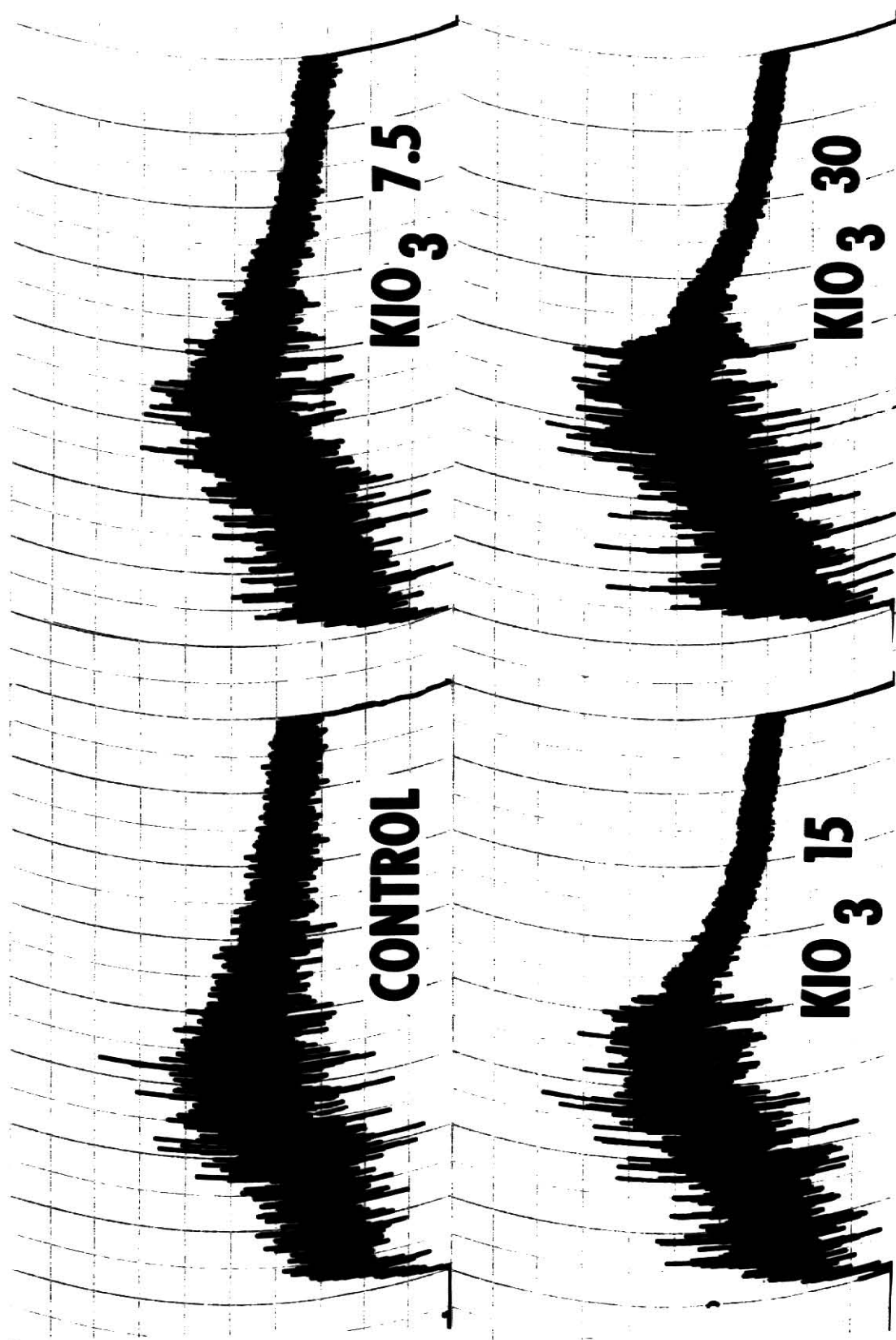


Fig. 3. Mixograms showing the effects of cysteine at levels of 30, 300, and 500 ppm (flour basis).



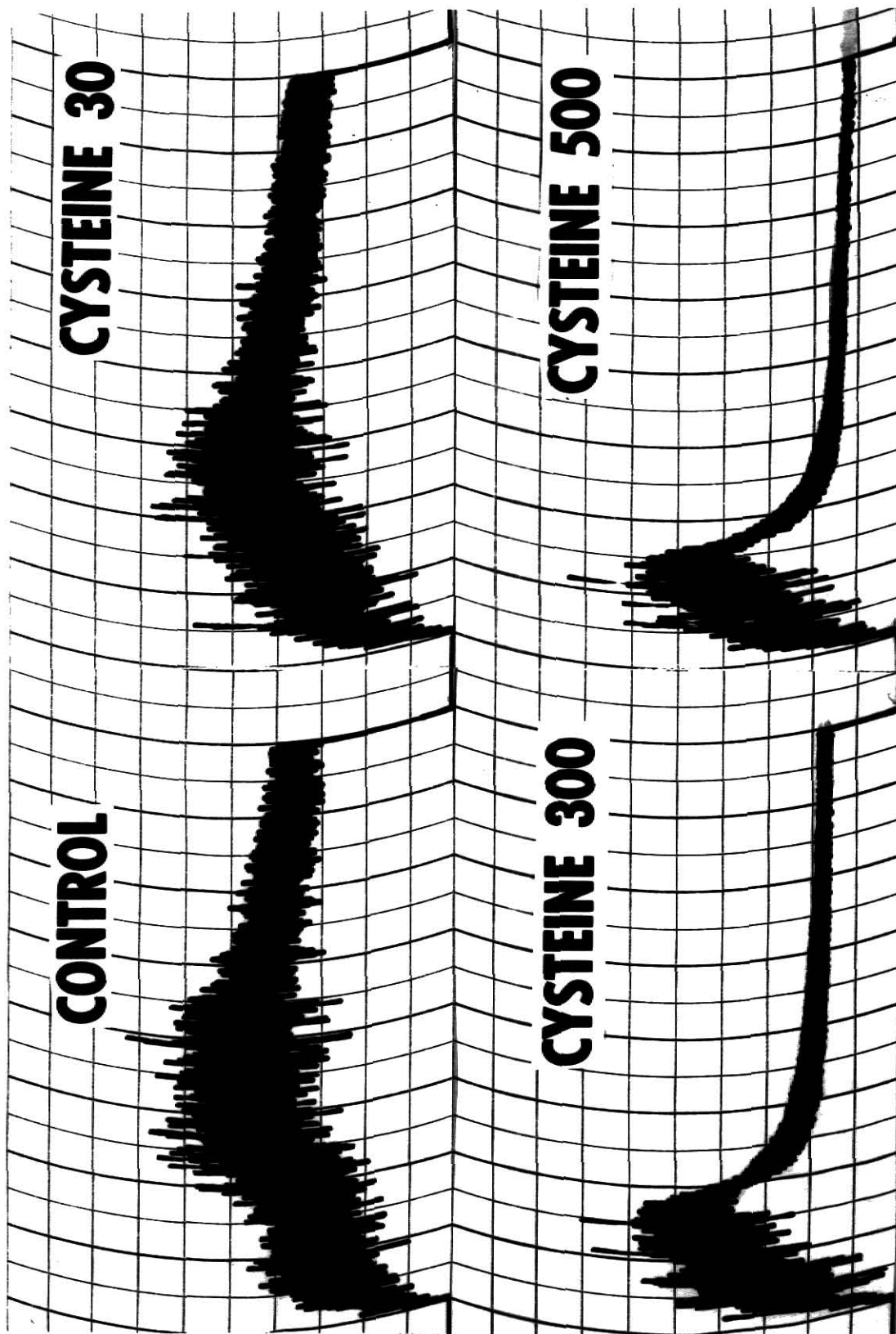


Fig. 4. Mixograms showing the effects of N-ethylmaleimide (NEMI) at the levels of 500, 1000, and 2000 ppm (flour basis).

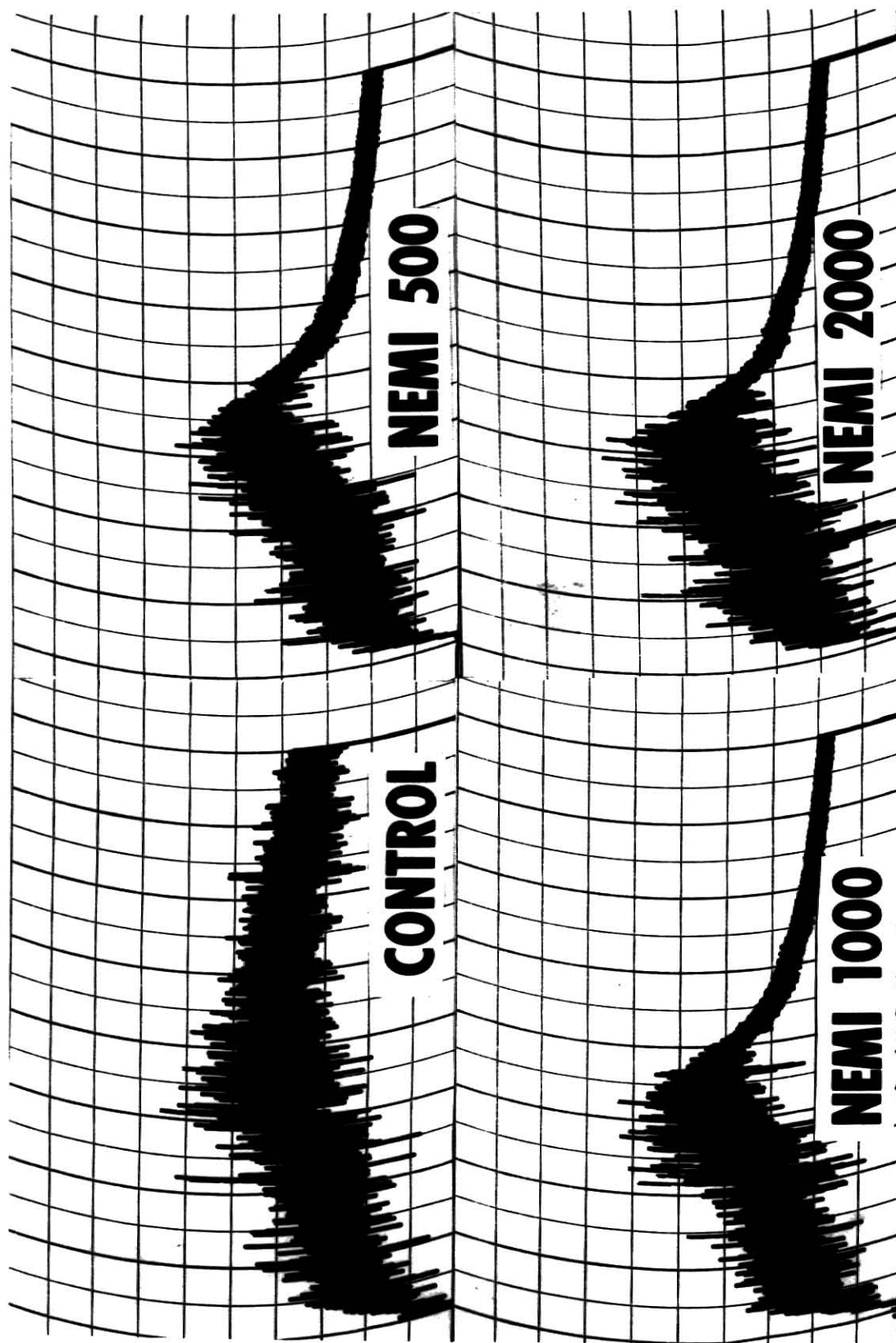


Fig. 5. Mixograms showing the effects of fumaric acid (FA) at the levels of 500 (FA 5), 1000 (FA 10) and 2000 (FA 20) ppm (flour basis).

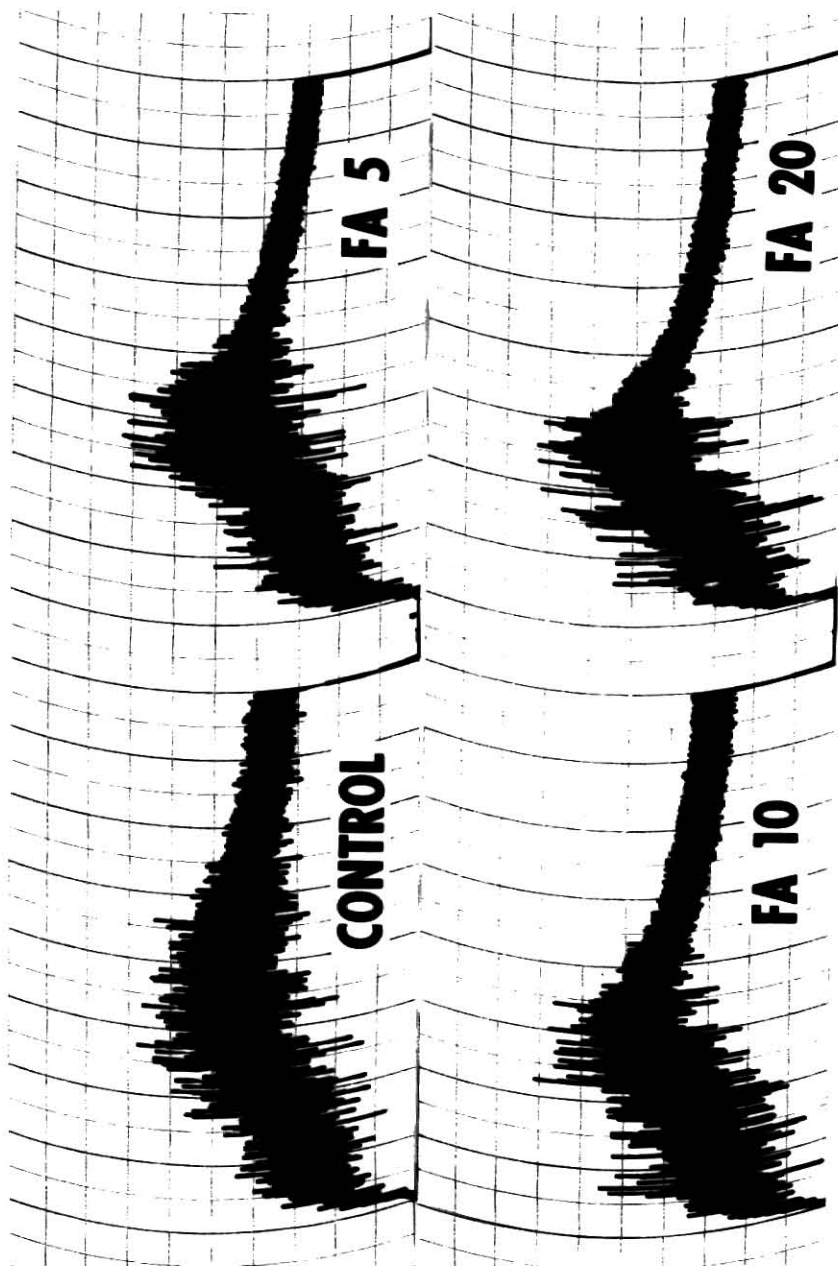


Fig. 6. Mixograms showing the effects of ferulic acid (FER) at levels of 33.75, 67.5, and 125 ppm (flour basis).

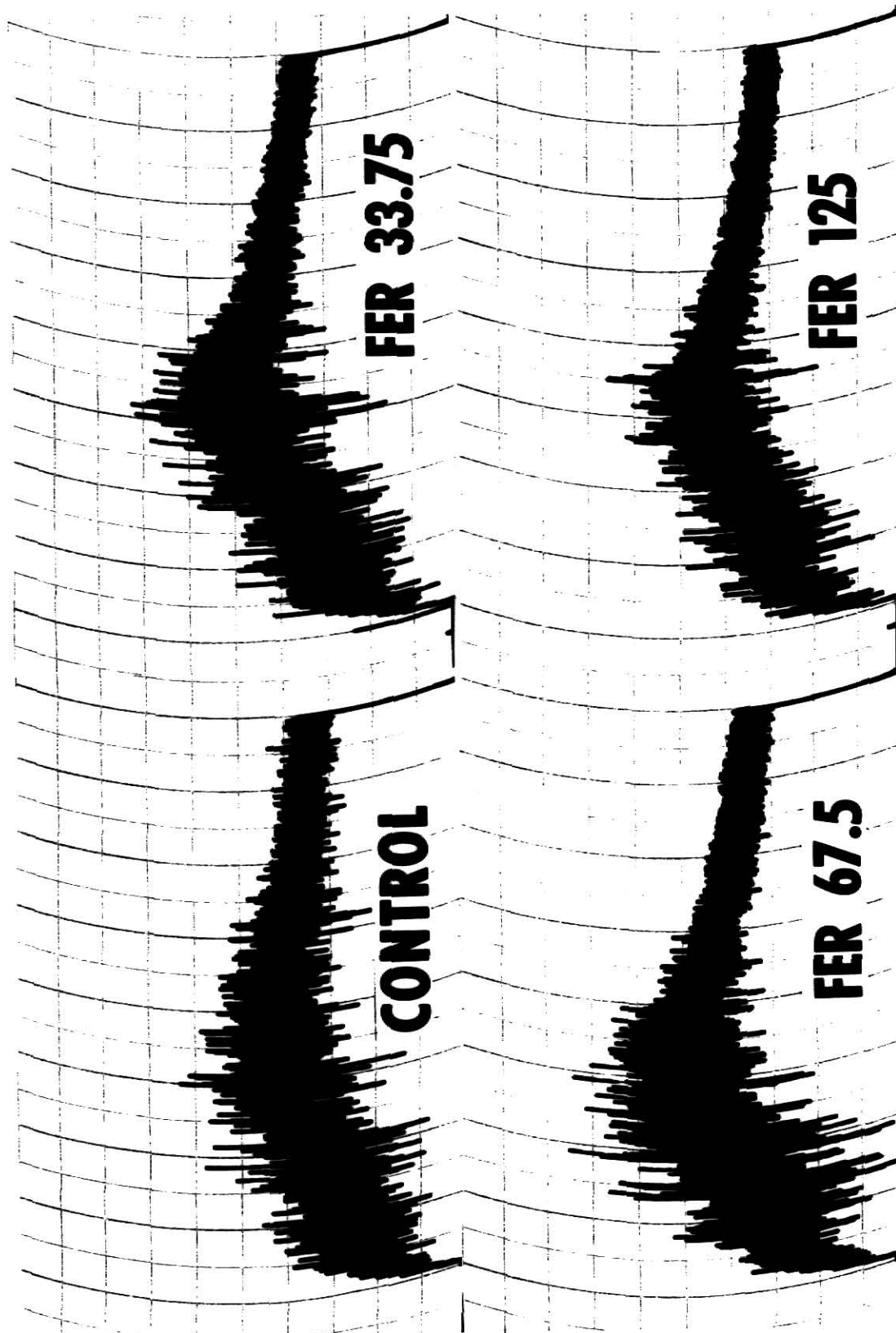


Fig. 7. Mixograms showing the combination of maleic acid (MA - 2000 ppm) with cysteine (20 ppm).



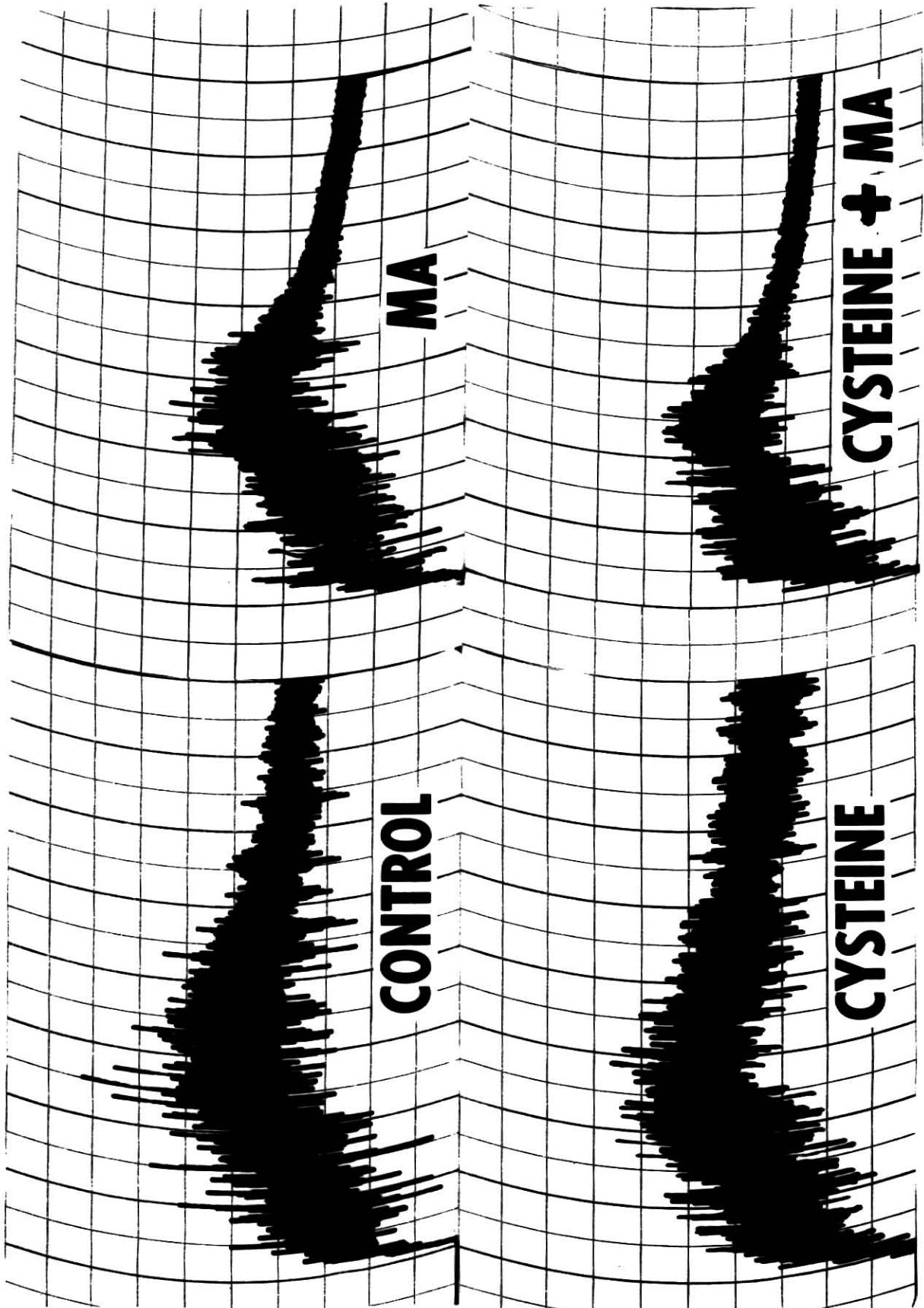


Fig. 8. Mixograms showing the effects of pH and fumaric acid (FA) at level of 2000 ppm (flour basis).

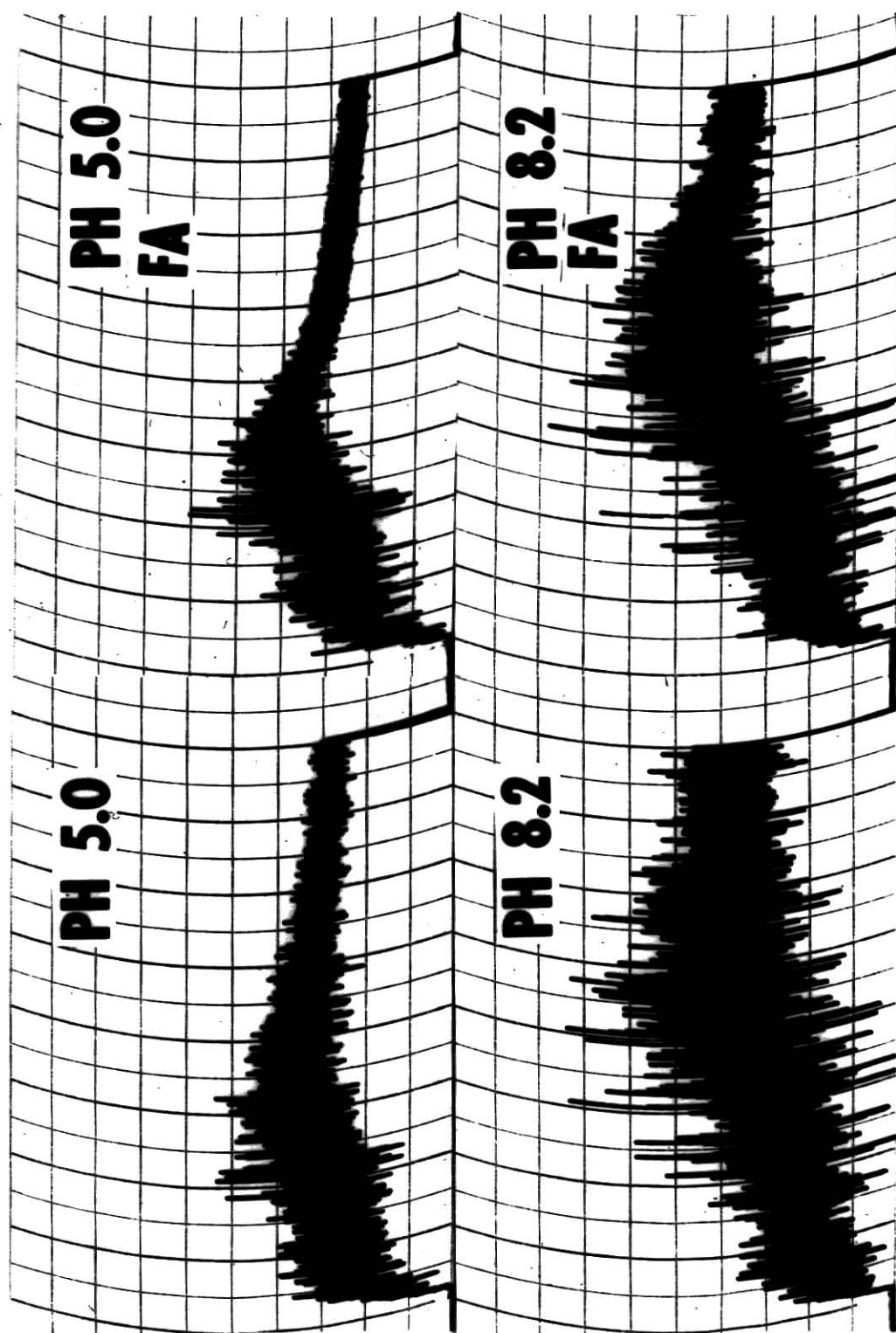
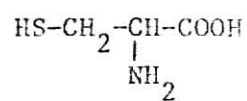
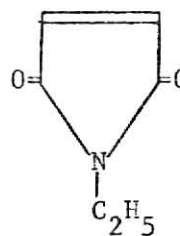
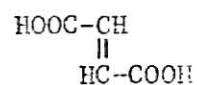
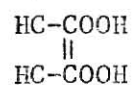
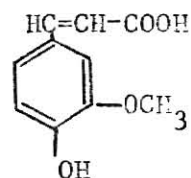


Fig. 9. Structural formula of the compounds used in the study.

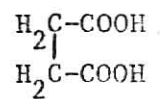
Potassium Iodate



Cysteine

N-ethylmaleimide  
NEMIFumaric Acid  
FAMaleic Acid  
MAFerulic Acid  
FER

Succinic Acid



carbon-carbon double bond ( $-C=C-$ ) activated by an adjacent carbonyl group ( $-C(=O)-$ ). These substances, classified as  $\alpha$ ,  $\beta$ -unsaturated carbonyl compounds, have properties that are characteristic to both ( $-C=C-$ ) and ( $-C(=O)-$ ) functional groups (77). The functional group important to the dough mixing properties is the  $-C=C-$ , because the saturated homolog of fumaric acid, succinic acid, does not show any effect on dough mixing properties. The action of NEMI has been explained by the reaction between its carbon-carbon double bond with  $-SH$  groups of the wheat protein (equation 4, p 4). Thus, NEMI reverses the action of cysteine during dough mixing. If fumaric acid and related compounds have similar properties, they should also interact with cysteine.

#### Combination of Compounds with Active Double Bond with Cysteine

The effects of cysteine (50 ppm), fumaric acid (2000 ppm), N-ethylmaleimide (500 ppm) and certain of their combination during dough mixing are shown in fig. 10. The effect of these compounds and of their combination on mixing time are summarized in Table 1.

These results show clearly that NEMI and cysteine interact during dough mixing while FA and cysteine do not. The combination of FA and cysteine showed an additive effect in reducing mixing time and in causing dough breakdown after the peak. Maleic acid also did not interact with cysteine during dough mixing (Fig. 7). A similar study showed that ferulic acid behaved identical to fumaric and maleic acids.

The results of these studies indicated that fumaric acid and its related compounds do not exert their effects on dough mixing properties

Fig. 10. Mixograms showing the effects of fumaric acid (FA - 2000 ppm), N-ethylmaleimide (NEMI - 500 ppm), cysteine (50 ppm) and the combinations of cysteine - FA and cysteine - NEMI.

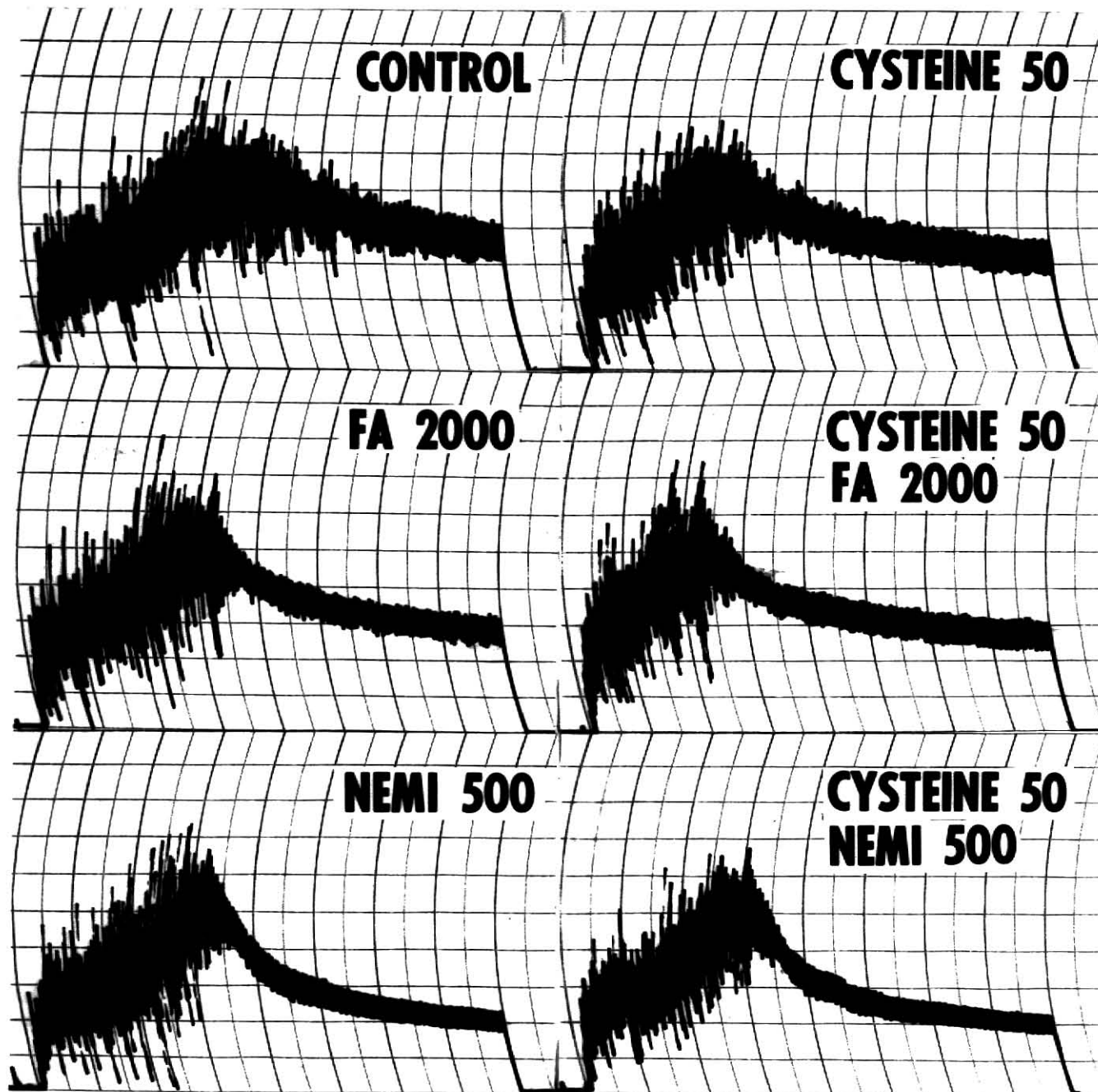




Table 1. Effect of Cysteine, N-Ethylmaleimide and Fumaric Acid on  
Dough Mixing Time.

Additive	Concentration ppm	Mixing Time min.
Control	---	3½
Fumaric Acid - FA	2000	3¼
N-Ethylmaleimide - NEMI	500	3¼
Cysteine	50	2½
Cysteine plus Fu	50	2¼
Fumaric Acid	2000	
Cysteine plus	50	3¼
NEMI	500	

by the same -SH reaction scheme as proposed for NEMI (5, 15). Those results, however, do not eliminate the possibility that the action of fumaric acid and related compounds is due to their reaction with some specific -SH groups present in a dough system. There is also a possibility that these compounds exert their effects via reactions with disulfide groups. If any of those hypotheses are true,  $\alpha$ ,  $\beta$ -unsaturated carbonyl compounds should cause a decrease in -SH and/or disulfide groups of the dough.

Thiol and Disulfide Determination. Effects of Mixing and Addition of Fumaric Acid, Iodate and N-Ethylmaleimide

The results of the thiol and disulfide groups determinations are shown in Table 2. The control flour results are mean values of 6 determinations. The standard deviation for the determination were 0.03 and 0.12 micro-equivalents per gram for thiol (-SH) and disulfide, respectively. For the other samples, results are the average of at least 2 determinations. Values shown in Table 2 as "changes" 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 the sample mixed to the peak was greater than that of the sample mixed 1 min. Mixing the sample with  $\text{KIO}_3$  increased the loss of -SH. At a  $\text{KIO}_3$  level of 60 ppm and mixed to the peak, almost all the -SH disappeared.

Mixing a dough containing NEMI caused a total loss of SH groups. However, doughs mixed with FA gave -SH values essentially equal to

Table 2. Thiol and Disulfide Determination of Flour and Dough at Different Mixing Times. Effect of Fumaric Acid, Iodate and NEMI.

Treatment	SH - $\mu\text{eq./g}^*$		SS - $\mu\text{eq./g}^*$	
	Found	Change	Found	Change
Control Flour	0.58	--	11.10	--
<u>1 min. mixing</u>				
Control Dough	0.44	-0.14	11.26	+0.16
+ 2000 ppm FA	0.41	-0.17	11.17	+0.07
+ 30 ppm $\text{KIO}_3$	0.22	-0.36	11.26	+0.16
<u>Mixed to peak</u>				
Control Dough	0.32	-0.26	11.20	+0.10
+ 2000 ppm FA	0.31	-0.27	11.08	-0.02
+ 4000 ppm FA	0.38	-0.20	11.16	+0.06
+ 30 ppm $\text{KIO}_3$	0.14	-0.44	11.42	+0.32
+ 60 ppm $\text{KIO}_3$	0.05	-0.53	11.58	+0.48
+ 2000 FA + 30 $\text{KIO}_3$	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	

\*Flour dry basis.

samples mixed without FA. Those results show that FA does not react with the -SH groups of the flour.

The values for -SS groups represent those originally present in the flour plus any generated during mixing. The only values that are significantly different from the control are those for doughs that were mixed to the peak with  $\text{KIO}_3$ . 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 for those 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, suggests that in this case, 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 11 shows that the water insoluble fraction of flour, gluten plus starch (G+S), has a mixing time of 8 min. and a greater dough stability than the original flour. When the water soluble fraction (WS) was added back to the G+S, the mixing properties of the reconstituted flour were similar to the original flour. These results agree with previous reports that the water fractionation technique used does not change the flour properties (65-69), and that the dough mixing properties are due to an interaction of the water insoluble and water soluble fractions (66, 69, 72). The longer mixing time

Fig. 11. Mixograms of gluten plus starch fraction (G&S) and G&S plus water solubles (WS).

**CONTROL**

**G & S**

**G & S & WS**

required by reconstituted flour compared to the control flour can be attributed to the fractionating techniques and to the mixing required to re-establish the protein association and interaction that existed in the original flour (72).

Figure 12 shows potassium iodate has no effect on the mixogram of the gluten plus starch fraction, however fumaric acid was effective in shortening mixing time and increasing the rate of dough breakdown. When a combination of potassium iodate and fumaric acid were added to G+S, the mixogram showed a much greater degree of dough breakdown. Similar results were obtained when NEMI or ferulic acid was substituted for fumaric acid. Thus it appears that fast-acting oxidants, like potassium iodate, need the water solubles fraction or compounds with an activated double bond system to have their effect.

To determine what in the water solubles was responsible for the reduction in mixing time and the increased rate of dough breakdown, the water solubles was fractionated by the scheme shown in Fig. 1. The fractions and their combinations were added back to gluten plus starch and mixograms determined in the presence of 30 ppm of potassium iodate (Fig. 13). The most effective fraction in reducing mixing time was the nondialyzable and heat-stable ( $WS_{DB}$ ). This fraction has been reported to contain ferulic acid. The dialyzable fraction of water solubles ( $WS_{DZ}$ ) had less effect on mixing time, while the water solubles denaturated albumins ( $WS_{DA}$ ) did not effect either the mixing time or the dough stability. Dough breakdown appears to be due to an interaction of  $WS_{DB}$  and  $WS_{DZ}$ , because the fully reconstituted dough

Fig. 12. Mixograms showing the effects of potassium iodate ( $\text{KIO}_3$  - 30 ppm) fumaric acid (FA - 2000 ppm) and their combination on gluten plus starch (G & S).



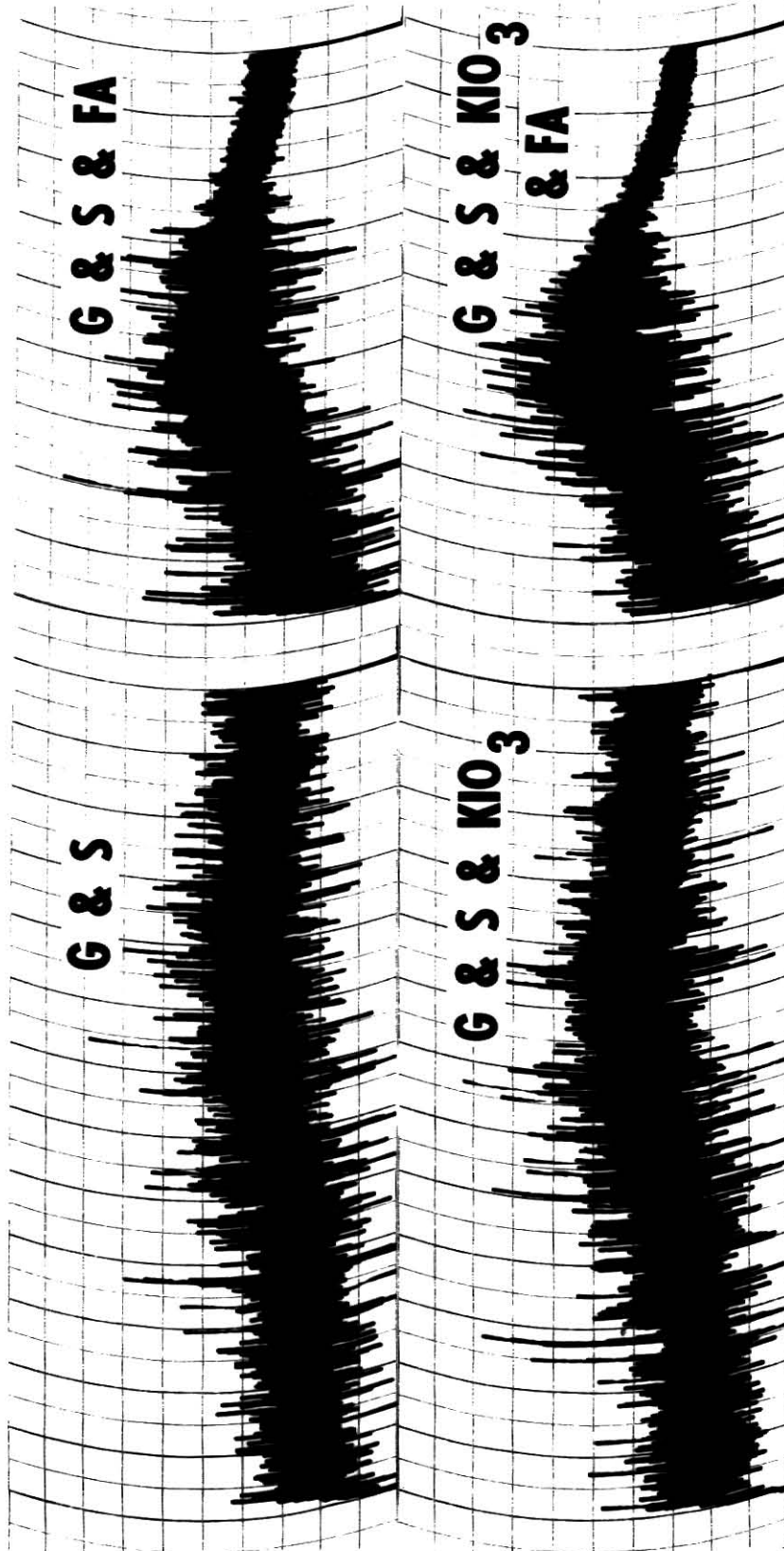
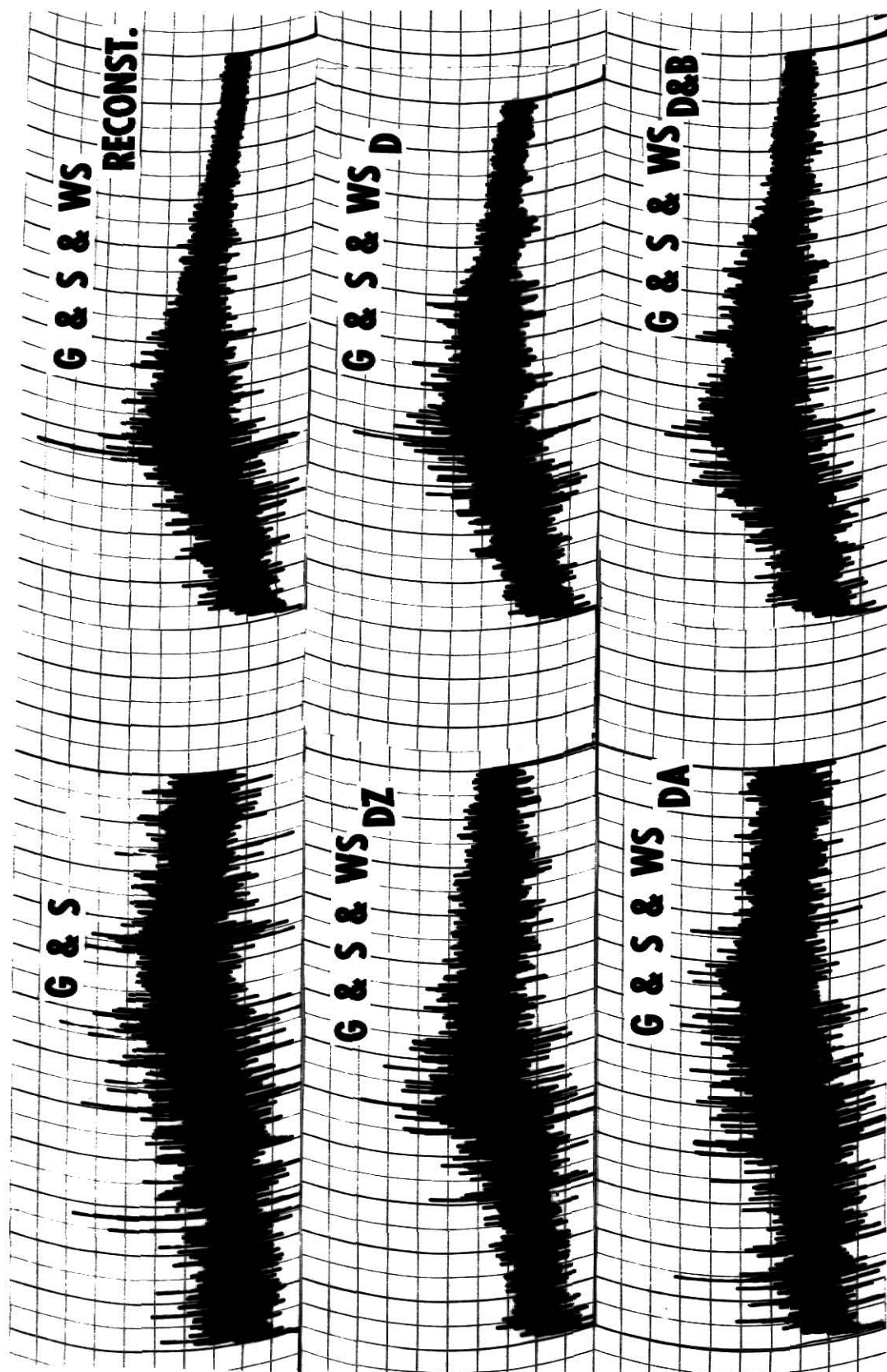


Fig. 13. Mixograms showing the effects of water solubles fractions on gluten plus starch (G & S). Amounts added were the same than recovered in fractionation.  $WS_{DZ}$  water soluble dialyzate (2.1%)  $WS_{DB}$  water soluble dialyzed and boiled (3.3%); WS reconst. total water solubles (5.6%).



showed a greater rate of breakdown than either of those fractions separately.

The  $WS_{DB}$  fraction is known to contain the water soluble pentosans. The effects of isolated pentosans on the mixing properties of gluten plus starch are shown in Fig. 14. The crude pentosans fraction shortened the mixing time and increased the rate of dough breakdown. Purified pentosan fractions obtained by ammonium sulfate fractionation were less effective, indicating that during the purification process something was lost or altered or that the effect was because of an interaction of both fractions. The degree of breakdown was increased when  $WS_{DZ}$  was added to the system (Fig. 15), indicating that this fraction has a synergetic effect on dough stability.

The results shown in Figure 12 raised some doubts that the effects of oxidants and NEMI are because of their reaction with -SH groups of the flour protein as has been suggested (5-9, 13, 15, 18, 19). If the gluten plus starch fraction has good mixing stability because of -SH groups effective in thiol-disulfide interchange, then why does potassium iodate not cause rapid rate of dough breakdown? If gluten plus starch contain no -SH groups effective in thiol-disulfide interchange why are compounds with active double bonds still effective?

The results obtained suggest that the effects of those compounds with activated double bonds on the mixing time reduction and dough breakdown are because of their interaction with some group or radical

Fig. 14. Mixograms showing the effects of pentosans on gluten plus starch (G & S). CRUDE PENT.-crude pentosans (added 1.6%, flour basis); SOL. PENT.-pentosans solubles in ammonium sulphate (added 0.4%, flour basis); INS. PENT.-pentosans insolubles in ammonium sulphate (added 0.2%, flour basis).

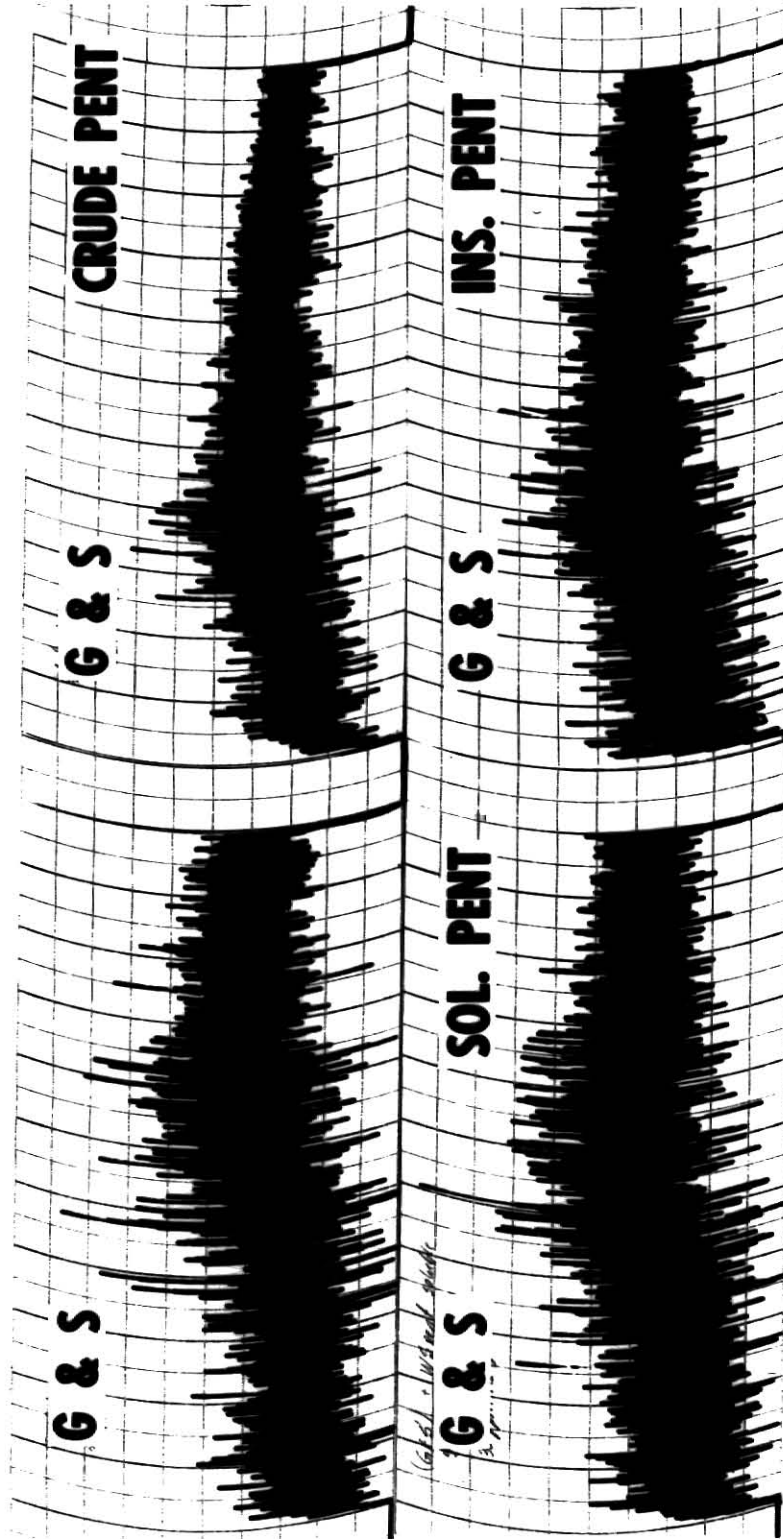
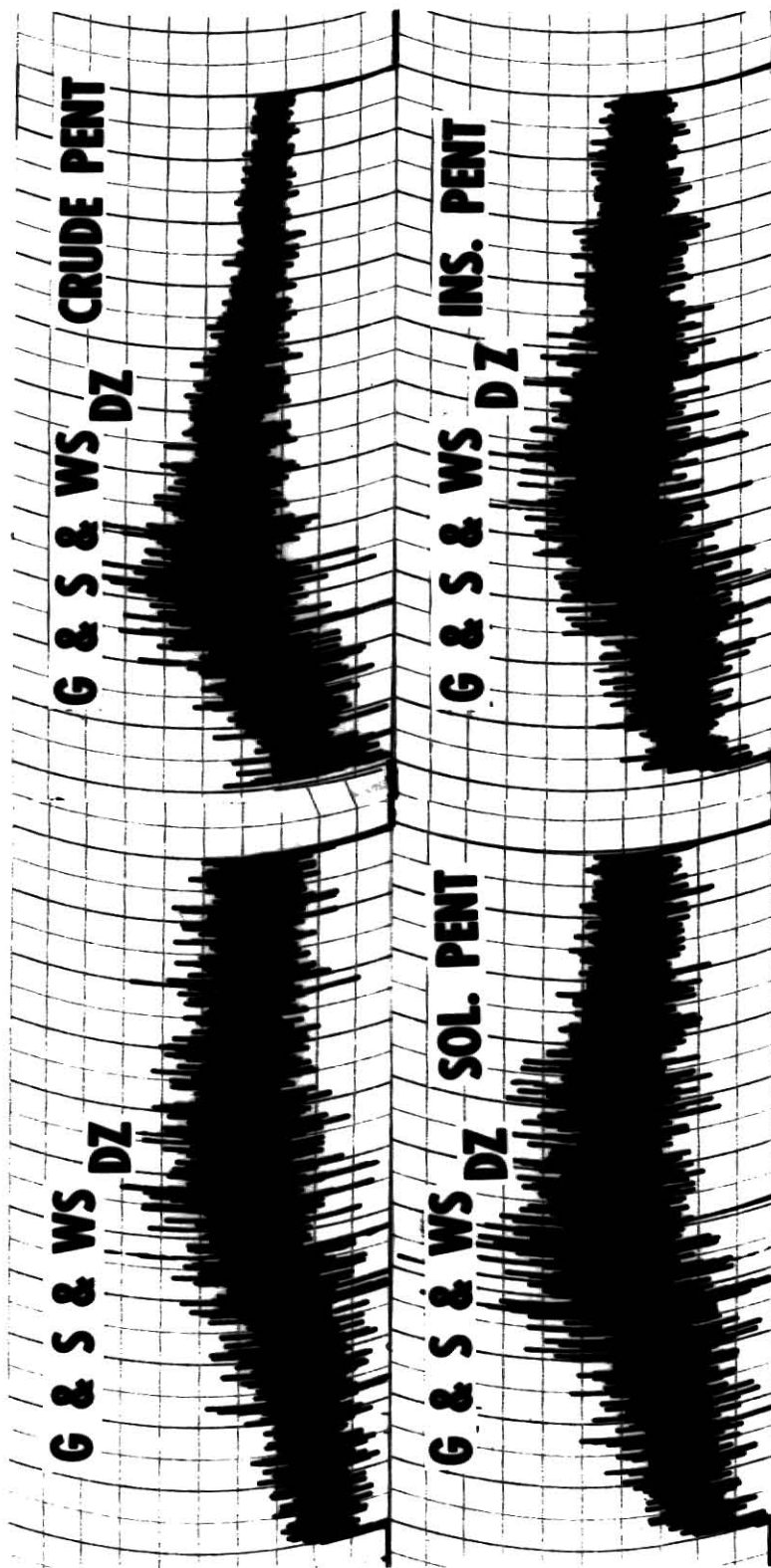


Fig. 15. Mixograms showing the effects of pentosans on gluten plus starch (G & S) plus water solubles dialyzate ( $WS_{DZ}$ , 2.1%). Amount added the same than in Fig. 14.



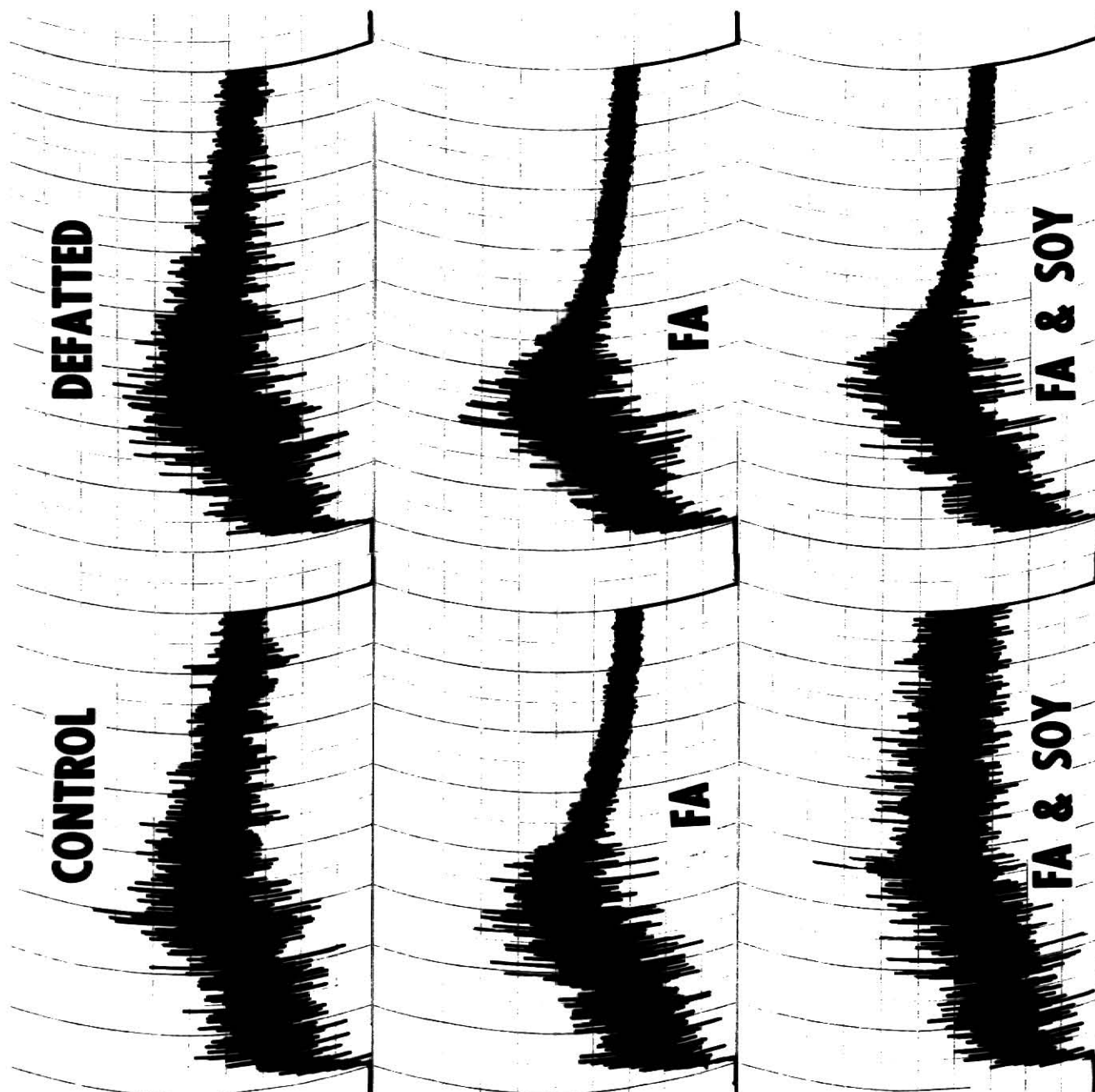


created in the gluten plus starch fraction of flour during dough mixing. The fact that fast-acting oxidants increase dough breakdown only when those activated double bond compounds are present in the system, indicates that the action of the oxidant is to create a functional group or radical which will interact with the added or indigenous activated double bond compounds. There is evidence of formation of free radicals during dough mixing (78), and that activated double bond compounds can undergo one-electron reduction by radicals (79). Therefore during dough mixing certain reactions may be caused 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 purposed that increased stability is because of the interaction of free radicals, formed during peroxidation of lipids, with certain flour constituents (34). Results in the previous section showed that compounds with activated double bonds were involved in more rapid dough breakdown. If free radicals formed by the soy enzymes acting on lipids interact with activated double bond compounds during dough mixing, then the effect of FA should be reversed. Figure 16 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. Therefore FA is

Fig. 16. Interaction of fumaric acid (FA - 2000 ppm) and enzyme active soy flour (SOY, 2% flour basis) in normal flour (left column) and in defatted flour (right column).

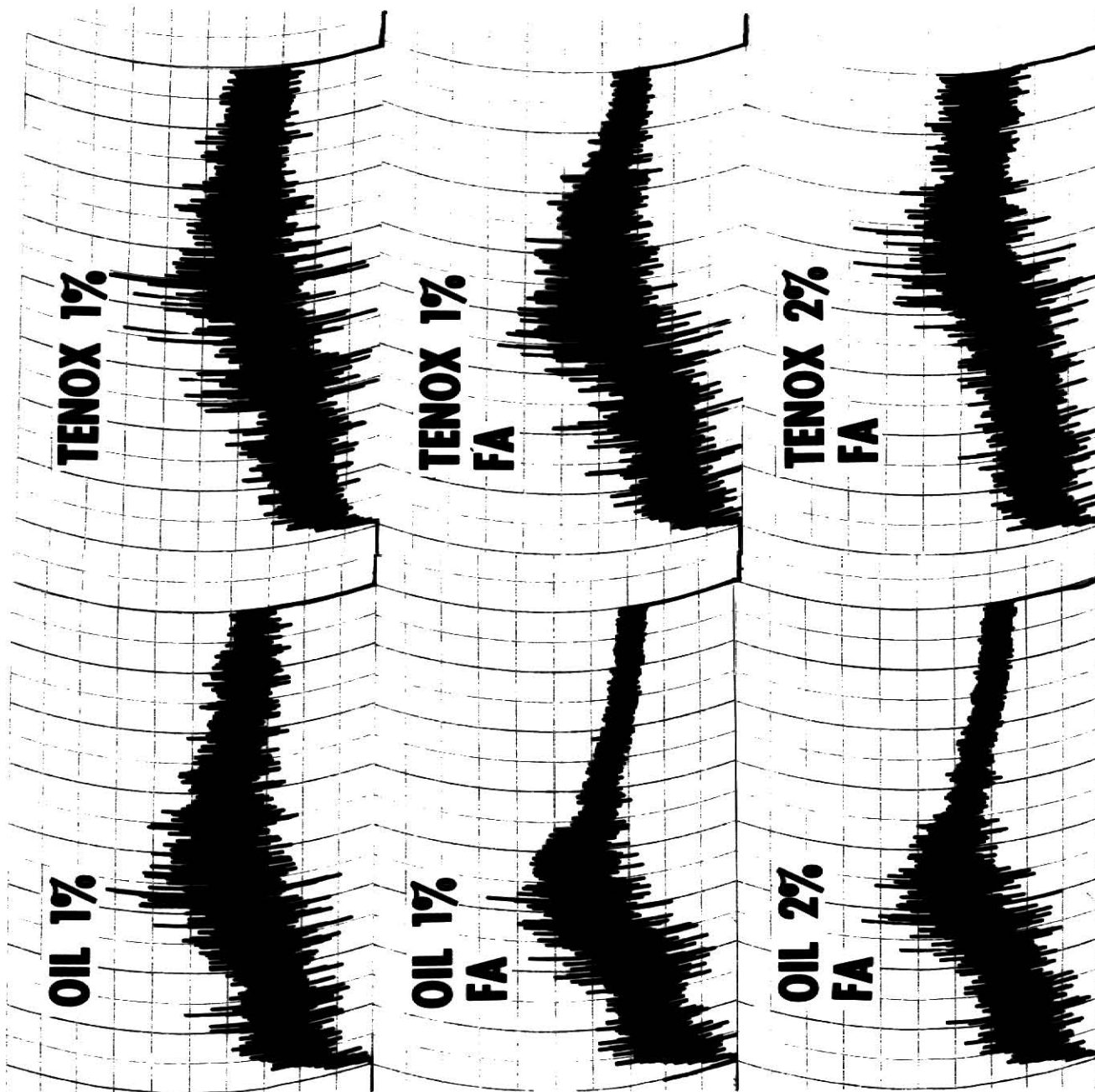


available to react with the radical or group of the flour to cause the rapid dough breakdown.

If compounds with active double bonds interact with flour constituents by a free radical mechanism, the use of antioxidants which are free radical scavengers should reverse the effect of those compounds. Figure 17 shows that the effect of fumaric acid is reversed by the presence of BHT and BHA (active ingredients of Tenox) 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 cause rapid dough breakdown, but also increased the mixing time. That suggests that a free radical mechanism may also be involved in the dough development process.

One possible mechanism that would explain the effect of compounds with activated double bonds is that during dough mixing certain disulfide 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 disulfide groups (Table 2) does not support that hypothesis. The presence of fumaric acid did not cause significant decreases in disulfide groups. It should be noted that when FA was present in the system the values found for disulfide groups were lower than those found for samples without FA. However, the differences 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 (49), it would be difficult to find

Fig. 17. Mixograms showing the effects of TENOX and corn oil (1 and 2% flour basis) and their interaction with fumaric acid (FA - 2000 ppm).



significant differences because the error involved in the determination is of the same magnitude. The use of more accurate techniques will be required to clarify the question.

The effects of fumaric acid, ascorbic acid (AA), soy, and their interactions during dough mixing are shown in Figs. 18 and 19. With a combination of soy and ascorbic acid, there is a slight decrease in dough stability compared to soy alone. Fumaric acid accentuates the effect. Apparently ascorbic acid reduces the ability of soy to increase dough stability. The action of AA and lipoxidase both involve an uptake of oxygen (32, 33). Enzyme action may be reduced due to competition of AA for the oxygen present in the dough. Figure 20 shows the effect of different levels of ascorbic acid on dough mixing properties. Dough stability was increased when AA was added at levels of 50 and 100 ppm.

#### Importance of the Results Found

The results of this research work appear to be of both theoretical and practical significance. NEMI has been used as a representative of a class of compounds that change rheological properties of dough because of its ability to block the -SH groups of the gluten protein (5, 15). Up to a certain limit, there is a correlation between the disappearance of -SH groups and changes in the dough property (5, 15). But a good correlation, by itself does not prove that the change in -SH group concentration is the cause of changes in dough properties. Other workers have suggested that the effect of NEMI is not due to

Fig. 18. Mixograms showing the effect of 1% enzyme active soy flour (SOY) and its combination with fumaric acid (FA - 2000 ppm) and ascorbic acid (AA - 100 ppm).



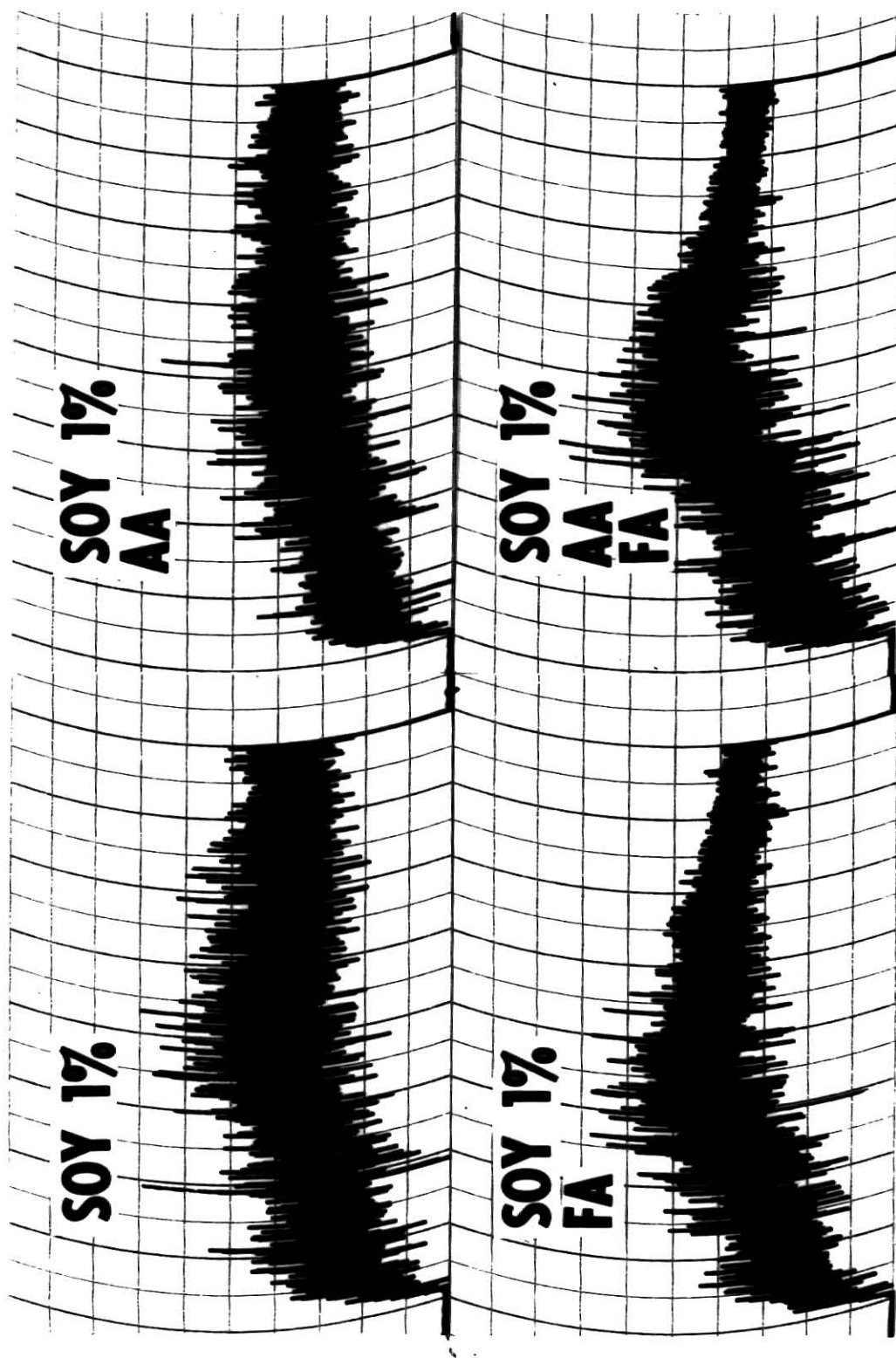


Fig. 19. Mixograms showing the effects of 2% enzyme active soy flour (SOY) and its combination with fumaric acid (FA - 2000 ppm) and ascorbic acid (AA - 100 ppm).

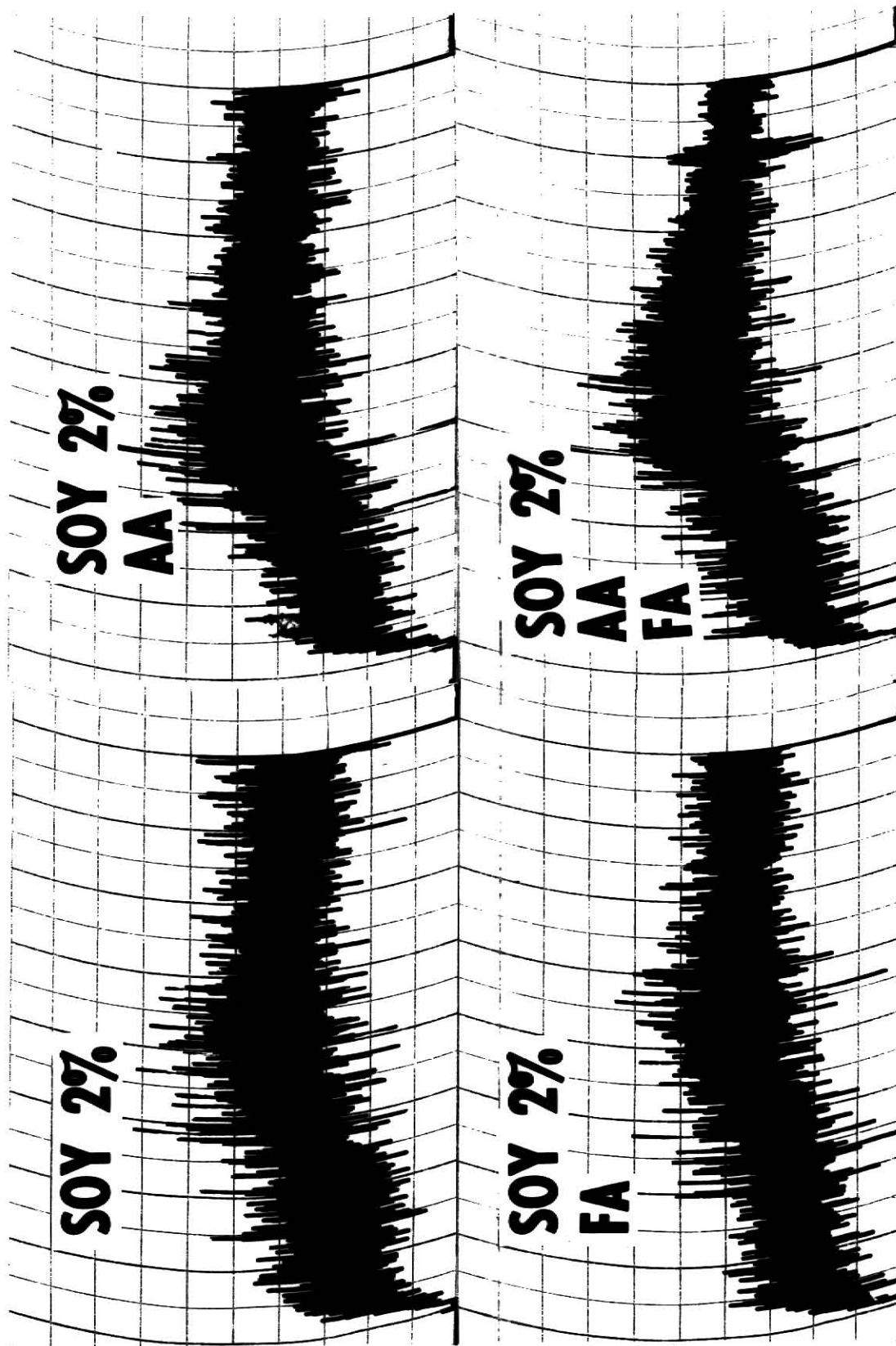
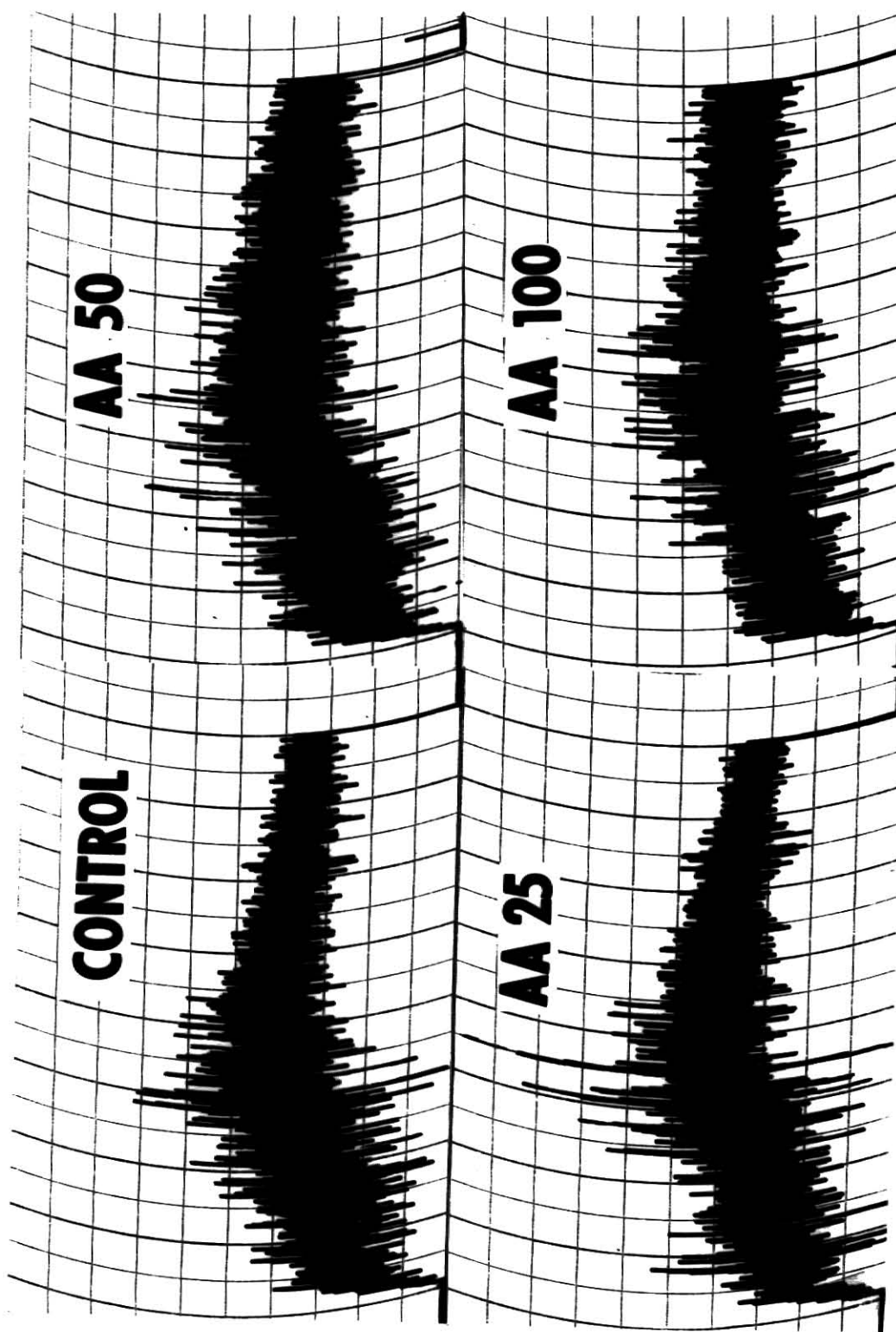


Fig. 20. Mixograms showing the effects of ascorbic acid at levels of 25, 50, and 100 ppm, flour basis.



its ability to block -SH groups (15, 69). The results reported here show that fumaric acid and related compounds have effects similar to NEMI on dough mixing properties, but do not react with the -SH groups of the flour. Thus, it appears likely that NEMI's effect on dough properties is 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 point of view, fumaric acid 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 which is associated with the reduction of mixing time. The lypoxidase system present in enzyme active soy flour appears to overcome the problem of rapid dough breakdown, however it also lengthens the mixing time.

The baking industry needs flour additives that will produce a dough with low mixing requirement and good mixing stability. The results of this study suggested that this goal can be achieved if most factors affecting dough mixing property could be well understood.

## ACKNOWLEDGMENTS

I would like to express my gratitude to my major professor, Dr. R. C. Hoseney for his able guidance during the research and his assistance in preparing this manuscript. I am also grateful for the guidance and assistance provided by the supervisory committee, Dr. Paul A. Seib and Dr. Lynn Bates. I wish to express special thanks to Kenneth Hsu for his technical assistance, Y. F. Yeh for the pentosans preparation and Gene Keltner for the amino acid analyses.

I wish to express my appreciation to my wife, Sandra, and family for their encouragement and understanding.

I also gratefully acknowledge the financial support of the Federação das Cooperativas Brasileiras de Trigo e Soja Ltda.-FECOTRIGO e Empresa Brasileira de Pesquisas Agropecuárias-EMBRAPA.

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EFFECT OF ACTIVATED DOUBLE BOND  
COMPOUNDS ON DOUGH MIXING PROPERTIES

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AN ABSTRACT OF A MASTERS' THESIS

submitted in partial fulfillment of the  
requirements for the degree

MASTER IN SCIENCE

Department of Grain Science and Industry

Kansas State University

Manhattan, Kansas

1976



The effects of  $\alpha$ ,  $\beta$ -unsaturated carbonyl compounds such as fumaric acid (FA), maleic acid (MA), and ferulic acid (FER), on dough mixing properties are similar to that of the SH-blocking reagent N-ethylmaleimide (NEMI). However, NEMI and cysteine are shown to interact during dough mixing, while cysteine and FA do not.

The thiol and disulfide determination showed that the mixing action in air caused a decrease in -SH groups. The addition of potassium iodate and NEMI increased the loss of -SH, while FA caused no change in the loss of -SH groups. No significant changes in the disulfide content of dough was found due to mixing action and/or addition of FA and NEMI. These results indicates that the effects of FA and related compounds can not 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 are due an interaction of the insoluble fraction, gluten plus starch (G+S), and water soluble fractions (WS). Further fractionations of the WS indicate that the most effective fraction in reducing the mixing time of G+S was the nondialyzable and heat-stable ( $WS_{DB}$ ). A fraction reported to contain ferulic acid. The dialyzable fraction of water solubles ( $WS_{DZ}$ ) had less effect on mixing time, while the water solubles denatured albumins ( $WS_{DA}$ ) did not effect either the mixing time or dough stability. The dough breakdown appear to be due an interaction of  $WS_{DB}$  and  $WS_{DZ}$ . The pentosans fraction separated of  $WS_{DB}$  was effective in shortening the mixing time and increasing the rate of breakdown of G+S. Fast-acting

oxidant only increased the rate of dough breakdown of G+S only when the WS were present in the system or when compounds containing activated double bonds were added.

The effects of  $\alpha$ ,  $\beta$ -unsaturated compound were reversed by lipoxidase present in enzyme active soybean flour and antioxidants which inhibit free radicals reactions. Thus, the effects of these compounds appears to be due to their reaction with free radicals created in the gluten proteins during dough mixing.