STUDIES OF DOUGH DURING FERMENTATION AND OVERMIXING

by

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Major Professor

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DEDICATION

To Dean and my parents

for all of their support and faith

when the going got rough

INTRODUCTION AND LITERATURE REVIEW

When wheat flour doughs are mixed beyond their point of minimum mobility (Finney, 1984) or optimum mixing time, a number of changes occur in the physical and functional properties of those doughs. Extensibility increases and elasticity decreases at the same time as the dough surface (which was dry and smooth) becomes wet and extremely sticky. The phenomenon, commonly referred to as dough breakdown or overmixing, is easily seen in mixograms as a thinning and decrease in height of the curve after the point of optimum mixing. Dough breakdown is of much more than trivial interest since an overmixed dough is difficult to process and incapable of producing a final product of optimum quality. Further, it is known that wheat varieties differ in their ability to resist dough breakdown. This characteristic, mixing tolerance or stability, is an important quality factor in the selection of new wheat varieties for use as bread flours.

Even though overmixing is a well recognized and long studied phenomenon, the actual mechanism of dough breakdown is poorly understood. Research has identified several classes of compounds that can accelerate dough breakdown as well as conditions that can reverse or prevent it. The relationship between the actions of these compounds or conditions as well as their exact mode of action are not yet known with certainty. Such information would be valuable in

helping us to understand overmixing.

ACTIVATED DOUBLE BOND COMPOUNDS

The first class of compounds known to affect dough breakdown are molecules which possess a double bond alpha to a carbonyl group. These are known as activated double bond compounds (ADB).

Low levels of ADBs are present naturally in wheat, as confirmed by Fausch (1963) who detected ferulic acid through thin layer chromatography of the water soluble pentosans of wheat flour. Ferulic acid appeared to be covalently linked to an arabino-xylan chain in the principle glycoprotein fraction of the water soluble pentosans. In work confirming the presence of phenolic acids containing activated double bonds in cereals, Sosulski et. al. (1982) defined three classes or chemical states of wheat flour phenolic acids; free, esterified, and bound. The concentration of free phenolic acids is very low - a total of 2.3 ppm in wheat flour. Fresh wheat flour contained low but measurable quantities of free trans-ferulic, syringic, and vanillic acids. All are ADBs. These same, rheologically active compounds existed in a soluble ester form at levels of 9.1 ppm. The bulk of the phenolic acids (60ppm, or 84%) present were in the insoluble-bound form. Trans-ferulic acid was the predominant insoluble-bound phenolic acid. In fact, ferulic acid represented 90% of the total phenolic

acids present. Flour stored for 6 months contained the same phenolic acids in each fraction but the total phenolic acid content was only one-third that of fresh flour.

Yeh et.al. (1980) also found ferulic acid to be associated with only the largest molecular weight part of the arabinoxylan fraction of pentosans. Studies on the effects of mixing showed that overmixing dough in air reduced the assayable ferulic acid in the purified pentosan by 30%. Overmixing in the presence of the fast acting oxidant, potassium iodate, resulted in an additional loss of ferulic acid from the purified pentosans.

The effect of exogenous activated double bond compounds on dough mixing properties was studied with the Mixograph by Schroeder and Hoseney (1978). They found that the effect of these compounds was similar to that of the thiol blocking agent N-ethylmaleimide (NEMI). Dough breakdown was accelerated. However, different mechanisms are involved since NEMI reverses the effect of added cysteine while activated double bond compounds do not. Flour fractionation showed that mixing properties were a result of interactions between the gluten-starch fraction and the water soluble fraction. Fast acting oxidants increased the rate of breakdown of gluten/starch only when water soluble fractions were present. Activated double bond compounds reduced mixing time and stability, even in the absence of water solubles. Overall, the effects of activated double bond compounds were

reversed by lipoxygenase and by free radical scavaging antioxidants. The model proposed to account for these results suggested that activated double bond compounds acted through a free radical mechanism, possibly interacting with free radicals created on gluten proteins during mixing.

A great deal of experimental evidence lends indirect support to the model. Earlier studies by Dronzek and Bushuk (1968) supported the formation of free radicals in dough during mixing. Likewise, MacRitchie (1975) presented evidence that disulfide bonds can rupture during mixing. Danno and Hoseney (1982) extended this concept. Viscometric studies showed that overmixing and the addition of activated double bond compounds caused the viscosity of extracted proteins to be reduced, suggesting a depolymerization of the proteins had occurred. The fact that treatment with mercaptoethanol caused the viscosities of proteins extracted from optimally mixed, overmixed and ADB treated doughs to be equal, indicated that disulfide bond cleavage and reformation was involved.

Two series of studies by Sidhu and colleagues (Sidhu et. al, 1980 a,b) added support to the free radical based model. Sidhu et. al. (1980a) showed that, during mixing, cysteine residues in the higher molecular weight fraction of gluten will covalently associate with added ^{14}C Fumaric acid. Further work (Sidhu et. al. 1980b) demonstrated that ultraviolet irradiation will cause ^{14}C cysteine to bind,

covalently, with the water soluble arabinoxylan fraction of wheat flour water solubles. Since ferulic acid is known to be associated with this fraction, the data supported the conclusion the thiyl free radicals, generated by UV irradiation, were reacting with the endogenous activated double bond compound.

Finally, Jackson and Hoseney (1986b) isolated and characterized a covalent adduct between ferulic acid and cysteine present in overmixed dough. The same workers (Jackson and Hoseney, 1986a) showed that the gluten/starch fraction and not the level of ferulic acid controls dough breakdown. Even so, during overmixing, both free ferulic acid and its soluble ester form are lost from the flour fraction that initiated breakdown (the water solubles).

HYDROQUINONES

A compound present in wheat germ which condenses with glutathione was first isolated by Vuataz (1950), and later identified as methoxy-p-benzoquinone (Cosgrove et. al.,1952). The precursor of methoxy-p-benzoquinone was isolated from wheat germ and identified by Bungenberg de Jong et. al. (1953) as methoxyhydroquinone. Bungenberg de Jong and collegues demonstrated that, in the presence of oxygen, or a peroxidative enzyme and oxygen, methoxyhydroquinone is oxidized to methoxy-p-benzoquinone. Methoxy-p-benzoquinone merits our attention since its structure is

that of an activated double bond compound. Therefore, if present in flour, methoxyhydroquinone could, during mixing, oxidize to form a rheologically active compound.

LIPOXYGENASE

Another compound that affects dough breakdown is lipoxygenase. This enzyme, present at a very low concentration in wheat flour (Wallace and Wheeler, 1975) , can reverse the action of activated double bond compounds if added to a dough. In other words, lipoxygenase increases mixing tolerance. The mechanism by which lipoxygenase increases mixing tolerance and affects dough rheology remains unclear (Faubion and Hoseney, 1981). Lipoxygenases attack and oxidize most free fatty acids containing cis,cis-14-pentadiene unsaturation, but prefer linoleic acid. The enzyme's mechanism for action is well known (Faubion and Hoseney, 1981; Koch, 1956) and starts with abstraction of a hydrogen from the C-8 methylene group of the substrate to form a fatty acid free radical. This first reaction step can take place in the absence of oxygen. In oxygen's presence, a peroxide radical is formed.

The mechanism by which lipoxygenase increases mixing tolerance was studied by Hoseney et.al.(1980). Not only does lipoxygenase greatly improve mixing stability, but it also overcomes the effects of both activated double bond compounds and fast acting oxidants. In addition,

lipoxygenase overcomes the effect of both activated double bond compounds and fast acting oxidants in a nitrogen atmosphere. In other words, oxygen is not required for lipoxygenase to increase mixing stability. However, oxygen was found necessary for lipoxygenase to have its effect on the rheology of mixed dough as measured by the spread test (Hoseney et. al., 1979).

A mechanism was proposed in which lipoxygenase improves mixing tolerance by creating free radicals on unsaturated fatty acids which compete with the gluten thiyl radicals for reaction with activated double bond compounds indigeneous to flour. This theory was supported by the fact that lipoxygenase interferes with the binding of radioactive fumaric acid to gluten proteins during mixing (Hoseney et. al., 1980).

TYROSINASE

Another oxidase enzyme may be important in relation to its oxidative effects on dough. Kuninori et.al. (1976) studied the effects of tyrosinase (EC 1.10.3.1, also known as polyphenol oxidase) on dough. Addition of a mushroom extract rich in tyrosinase increased dough resistance and decreased its extensibility, mimicking the effects of some oxidants. The oxidative effect of the mushroom extract was lost by preincubation of the extract with phenylthiourea, a potent inhibitor of tyrosinase and, most importantly, by

pretreatment of flour with N-ethylmaleimide, a compound known to react with and block thiol groups.

OXIDATION

Oxidation promotes dough breakdown. A dough with added fast-acting oxidant breaks down more quickly than a dough mixed in air (Hoseney et. al., 1980). A dough mixed in the absence of oxidation (a nitrogen atmosphere) exhibits still greater tolerance to overmixing, indicating that even air has an oxidative effect. Other oxidative effects were studied by Meredith and Bushuk (1962). They noted after mixing doughs in nitrogen, air, or oxygen that the effect of oxygen was to increase the time to maximum development, increase the maximum consistency, and increase the rate of breakdown after maximum consistency was obtained. Merdith and Bushuk (1962) found, when studying systems containing iodate and NEMI, that when iodate was added first and NEMI second there was a definite additional breakdown effect due to NEMI. However, when NEMI was added first and iodate second, iodate had no additional breakdown effect.

Oxidants and reducing agents exert their effects, at least indirectly, on dough proteins. Schroeder and Hoseney (1978) found the mixing in air not only caused a decrease in thiol group content, but that mixing in air plus potassium iodate increased the loss of thiols. Disulfide content increased during mixing with iodate, indicating an oxidation

from thiol to disulfide. Tsen (1969) studied oxidizing and reducing reagents as well as NEMI. He found that although the amount of acetic acid extractable flour protein increased when dough was mixed, the change in extractibility intensified when the dough was treated with either oxidizing or reducing agents. Specifically, oxidants and NEMI increased the extractable glutenin fraction while reducing agents increased both the glutenin and the gliadin fraction, as shown by gel filtration. In the presence of oxidants, the extractibility of dough proteins continued to increase with continued mixing. However, when reducing agents were present in the dough, protein extractibility remained nearly constant, even during extended mixing.

CHARGE

Charge, specifically the charge on gluten protein molecules, is also involved in controlling dough breakdown. The accelerated breakdown effects of iodate, ferulic acid, and NEMI can be reversed by the addition of sodium chloride or sodium dodecyl sulfate (SDS), molecules capable of interacting with charged sites on proteins (Danno and Hoseney, 1982). The addition of sodium chloride alone increases the width and height of the mixogram curve and increases mixing time. Low levels of SDS increase mixing time, while higher levels progressively shorten mixing times. In addition to this effect, the mixogram curves, in

the presence of sodium chloride or SDS plus; potassium iodate, ferulic acid, or NEMI, rapidly widen and increase in height. The NaCl and/or SDS is, therefore reversing the effect of dough breakdown. In proposing a model for dough development and breakdown, the researchers speculated that

> the glutenin molecule occurs in optimally mixed dough in a macrospherical or helical structure with a hydrophobic surface and a hydrophilic interior. As a result of overmixing, a few of the disulfide bonds are broken. Adding an activated double-bond compound to the thiyl radical causes the glutenin molecule to invert and become hydrophilic on the exterior and hydrophobic on the interior. That gives the dough a large excess of water, hidden previously in the glutenin molecule. This excess results in the wet, sticky dough with no elasticity. Adding SDS or NaCl to the overmixed dough masks the positive charges, now on the exterior of the glutenin. Without the positive charges, the glutenin reverts to the original configuration of a hydrophobic exterior and a hydrophilic interior, which causes the dough to regain its dry, elastic properies.

This model, while highly speculative, answers many of the questions associated with overmixing.

Since differences in pH will also affect the net charge on proteins, pH may also affect dough breakdown. Hoseney and Brown (1983) investigated the effects of pH on dough mixing and breakdown. They found that decreasing pH from a native flour value of 6.12 decreased the mixing stability while increasing pH increased both mixing time and mixing stability. Free radical scavengers did not reverse the effects of low pH, showing that low pH affects mixing stability by a different mechanism than do activated double bond compounds. Fast acting oxidants and activated double bond compounds were most effective only at a native flour

pH, and not at lower or higher pHs. The effect of pH on the flour-water mixogram stability was reversible. These data were consistent with the previously stated model in which a conformational change occurs in gluten proteins as a result of overmixing. The relationship and interaction between these three seemingly unrelated factors (ADBs, oxidation and charge) have not yet been determined.

THIOL - DISULFIDE INTERCHANGE

The disulfide and thiol content of wheat flours and doughs has long been thought to be one piece of the puzzle of overmixing. Tsen and Anderson (1963) found, using amperometric titration, that hard wheat flour contained the highest number of disulfide bonds, and soft wheat, both the fewest disulfide bonds and the fewest thiol groups. On a protein basis, though, soft wheat contained the highest and hard wheat the lowest number of disulfide bonds. Bloksma (1963) found that thiol groups in doughs from wheat flours that have been defatted were more slowly oxidized by molecular oxygen than thiol groups in doughs from normal flours. He attributed this phenomenon to either a competition for oxygen between thiol groups and flour lipids or the removal of lipid peroxides that may oxidize thiol groups.

Bloksma (1975) developed a line of reasoning in which the stiffening of dough, induced by oxidants, cannot be due to the conversion of thiol groups into additional disulfide

crosslinks. Thiol blocking reagents (for instance NEMI), which do not form disulfide bonds, have an effect similar to that of oxidation. Therefore, the stiffening of dough was thought to be due to the removal of thiol groups. In addition, "rheologically effective" thiol groups were identified by Bloksma as most probably occurring in small peptides.

Mauritzen and Stewart (1966), also using amperometric titration, found that up to 26% of the sulfhydryl groups originally present in flour disappeared during dough formation. Doughs mixed in the presence of air or iodate showed a rapid initial, and subsequently more gradual loss of sulfhydryl groups as mixing proceeded, or as the iodate concentration increased. During overmixing, and in the presence of iodate or NEMI, the sulfhydryl groups from soluble proteins were more labile than the sulfhydryl groups of the gluten complex. In addition, the greatest proportion of thiol groups in doughs from poorer quality flours was found in the soluble fractions. Conversely, the higher quality flours had most residual thiol groups located in the gluten fraction of the dough.

Mauritzen (1967) performed further studies on thiols and disulfides in wheat flour doughs by following the incorporation of radioactive cysteine, cystine or NEMI. Cysteine incorporation into the gluten fraction of doughs varied directly with flour quality. At the same time,

isotope incorporated into the soluble protein fraction varied inversely with flour quality. Even when mixed under nitrogen, a considerable portion of the cysteine added to dough was oxidized to cystine. By following the addition of labeled cysteine with NEMI, Mauritzen found that the incorporation of cysteine into gluten was rapid and occurred during mixing, whereas its incorporation into soluble protein was slower and occurred on standing. If swamping the system with labelled NEMI was followed by the addition of labelled cystine, very little radioactive sulfur was incorporated into soluble proteins, while considerable incorporation into the gluten fraction was still observed, again suggesting that thiol blocking and disulfide-sulfhydryl interchange in gluten occurs mainly as a direct result of mixing. The results "support the belief that, in untreated doughs, rheologically important disulfide-sulfhydryl interchange occurs mainly between diffusible sulfhydryl or disulfide compounds on the one hand, and gluten on the other, and further, that such interchange reactions occur most rapidly during actual mixing of the dough."

CHANGES IN GLUTEN PROTEINS DUE TO MIXING

Danno and Hoseney (1982) used changes in protein extractibility and gel filtration chromatography to study changes in flour proteins during dough mixing. Dough mixing

increased protein extractibility from 72.6% to 95%. The additional extractable protein consisted, primarily, of glutenin. SDS extracts of doughs mixed with rheologically active compounds such as NEMI, potassium iodate, and/or activated double bond compounds had elution profiles similar to those from optimally mixed doughs. The increase in protein extractibility by SDS during dough mixing was not related to a decrease in protein size. A great reduction in the molecular weight of glutenin after being reduced with mercuric chloride or 2-mercaptoethanol strongly suggests the presence of intermolecular disulfide bonds.

RHEOLOGICAL EFFECTS

Although changes occur in a dough during overmixing, these changes are measurably different than the rheological changes that occur when an optimally mixed dough is fermented. While the two systems may be related, they are separate and should not be in any way confused. While overmixed doughs can be studied with the mixograph, rheological changes in an optimally mixed dough can be determined, after mixing and fermentation, with the spread test (Hoseney et. al., 1979). Increasing spread ratios (w/h) indicate viscous-flow characteristics, while decreasing spread ratios indicate more elastic characteristics. Both yeast and oxidants decrease the spread ratio, while reducing agents caused an increase, indicating that the

spread ratio was sensitive to physical changes in the fermenting dough.

The effect of yeast was seen clearly when a flour/water dough was compared to a yeasted flour/water dough. Yeast appeared to have a rheological effect on the dough, strengthening it as does an oxidant. This effect was due to yeast and not to fermentation as shown by a dough without sugar. Although the fermentation was much less than that in the dough with sugar, the spread ratios were equal. Therefore, yeast itself seems to have a rheological effect on the dough system.

MATERIALS AND METHODS

MATERIALS

Starch. Prime wheat starch was obtained from Midwest Grain Processors, Atchison, Ks.

Protein. Vital Wheat Gluten (65.33% protein, N x 5.7) was obtained from Midwest Grain Processors, Atchison, Ks.

Enzyme Active Soy Flour. Obtained from Farmland Soy Processors, St. Joseph, Mo.

Flours. Two commercially milled hard wheat flours were obtained from Ross Industries, Inc., Wichita, Ks., and designated March 1986 (10.5% protein (N x 5.7), 0.516% ash), and July 1986 (11.5% protein (N x 5,7), 0.47% ash).

High Gluten Hard Red Winter flour (12.65% protein, 0.523% ash) was obtained from Midwest Grain Processors, Atchison, Ks., and designated strong flour. Siouxland Hard Red Winter flour (11.34% protein, 0.563% ash), a gift from Dr. R. C. Hoseney, was designated weak flour.

Organic Solvents. Petroleum Ether (bp 38-58°C) and Ethyl Acetate (ACS Certified) were obtained from Fisher Scientific Company, Fair Lawn, NJ. 2-Propanol was obtained from J.T. Baker Chemical Company, Phillipsburg, NJ. Benzene, Propionic acid, and formic acid were obtained from Fisher Scientific Company, Fair Lawn, NJ.

Yeast. Instant dry yeast was obtained from Fermipan, Delft, Holland. Red Star compressed yeast was obtained from Universal Foods, Milwaukee, WI.

Reagents. The following is a list of reagents used and their suppliers. All were reagent grade.

Ascorbic Acid. Sigma Chemical Company, St. Louis, Mo.

Arbutin. Sigma Chemical Company. St. Louis, Mo.

L-Cysteine. Sigma Chemical Company, St. Louis, Mo.

Ferulic Acid. ICN Pharaceuticals, Inc. Plainview, NY.

Fumaric Acid. Sigma Chemical Company, St. Louis, MO.

Hydroquinone. Eastman Kodak Company, Rochester, NY.

Lactic Acid. MCB Manufacturing Chemists, Inc., Cincinnati, Ohio.

Phosphomolybdic Acid. Alltech Associates, Deerfield, Il.

Potassium Bromate. Aldrich Chemical Company, Inc., Milwaukee, WI.

Potassium Iodate. Matheson Coleman & Bell. Manufacturing Chemists, Norwood, OH.

Sodium Bicarbonate. Arm & Hammer, Old Fort, OH.

Tenox-4. Eastman Chemical Products, Inc. Kingport, TN.

METHODS

Moisture Determinations. Flour moistures were determined according to AACC method #44-19.

Dough pH. Ten grams (10g) of dough was blended with 100 ml. of distilled water for one min. in an Osterizer blender set on high. Three drops of octanol were added to disperse any foam that formed during blending. A stir bar magnet was used to keep the solution dispersed while the pH was taken using a Corning 125 pH meter.

Dough Mixing Under Nitrogen. A large rubber stopper (#15) with one inlet for nitrogen and one outlet to pull a vacuum was placed over the mixogram bowl containing 10g of flour. The atmosphere over the flour was evacuated and flushed with nitrogen three times. The inlet and outlets were then sealed to maintain the vacuum and the bowl placed in the large plastic bag that also contained the mixograph. Also placed in the large bag was a sealed bottle of water which had been boiled to degas and flushed with nitrogen. The large bag was then sealed, evacuated and flushed with nitrogen three times. Degassed water was then added and the dough was mixed.

Spread Test. The spread test was performed according to the technique detailed by Hoseney et. al. (1979). Dough formulas depended upon the experiment being performed.

Isolation of Water Solubles. Flour(250g):Water(750 ml.) was shaken until the lumps were dispersed. Centrifugation at 1000g for 20 min. pelleted the gluten/starch fraction. The water soluble fraction (supernatant) was decanted away.

Lipid Extraction. Flour samples (500g) were extracted with petroleum ether (Soxhlet) as solvent for 24 hours to remove lipids. Heating rate was adjusted so that a complete solvent change occurred every 30 min. Defatted flour was air dried until all traces of solvent odor were gone.

Fermentation of Water Solubles. After separation from the gluten/starch fraction (see above), water solubles were fermented with 2% compressed yeast (based on original flour weight). Fermentation was carried out for 3 hours in a covered flask at 86°F, 90% R.H., with stirring every 30 min. After fermentation, yeast was removed by centrifugation (10 min., 1000g). Fermented water solubles were then frozen and lyophilized.

Flour Extraction Techniques.

Graveland Method A: Flour and water (25g:600 ml.) were mixed for 30 min. under nitrogen (Graveland, 1984). After extraction, the gluten/starch fraction was separated from water solubles by centrifugation at 1000g for 20 min. Gluten/starch was frozen and lyophilized.

Graveland Method A Without Nitrogen: Flour and distilled water (25g:600 ml.) was mixed for 30 min. in air atmosphere. The gluten/starch was separated from water solubles by centrifugation for 20 min. at 1000g and lyophilized.

Regular Method (long): Flour and distilled water (250g:750g) were mixed for 30 min in an air atmosphere. The gluten/starch fraction was separated from water solubles by

centrifugation for 20 min. at 1000g after which it was lyophilized.

Graveland Method B: Flour:water (25g:600 ml.) were shaken until lumps were dispersed. The gluten/starch was separated from water solubles by centrifugation for 20 min. at 1000g.

Regular Method (short): In the standard extraction technique, flour and water (250g:750ml) were shaken until lumps were dispersed. The gluten/starch fraction was separated from water solubles by centrifugation for 20 min. at 1000g.

Propanol Extraction: 100g of flour was mixed with 300 ml. 2-propanol:water (80:20). The extraction was performed under nitrogen three times, 30 min. each time.

Ethyl Acetate Extraction: Flour (100g) was mixed with 300 ml. of ethyl acetate. The extraction was performed three times under nitrogen, (30 min./extraction). The gluten/starch and water soluble fractions were isolated as described above.

RESULTS AND DISCUSSION

CHAPTER I - RHEOLOGY OF FERMENTING DOUGH

Lipoxygenase. The oxidative enzyme, lipoxygenase, is known to affect the mixing stability of doughs (Hoseney et. al., 1980). However, its effects after mixing is complete remained unclear. Therefore, the spread test was used to help determine the rheological effect of lipoxygenase upon an optimally mixed dough. All spread test data are averages of duplicates.

The first studies were designed to assess the speed and effectiveness of lipoxygenase during dough mixing. Since the spread ratio of flour/water doughs containing 0.5% enzyme active soy flour did not increase over fermentation periods from 0 to 60 minutes before molding (Figure 1), it was concluded that lipoxygenase must act immediately after or during mixing. To determine when during mixing lipoxygenase acted, enzyme active soy flour was added to dough at different points during mixing, thereby controlling the enzyme's reaction time. All the resulting flour/water/soy flour doughs were rested only 15 minutes before moulding. The results presented in Figure 2 show that if lipoxygenase was present initially, it had the full rheological effect on the spread ratio of the dough. However, as the reaction time decreased, the subsequent rheological effect decreased. Note that 'lipoxygenase

Figure 1. Effect of Lipoxygenase on Spread Ratios of Doughs over Fermentation Times. Standard Deviation = $\pm$0.05

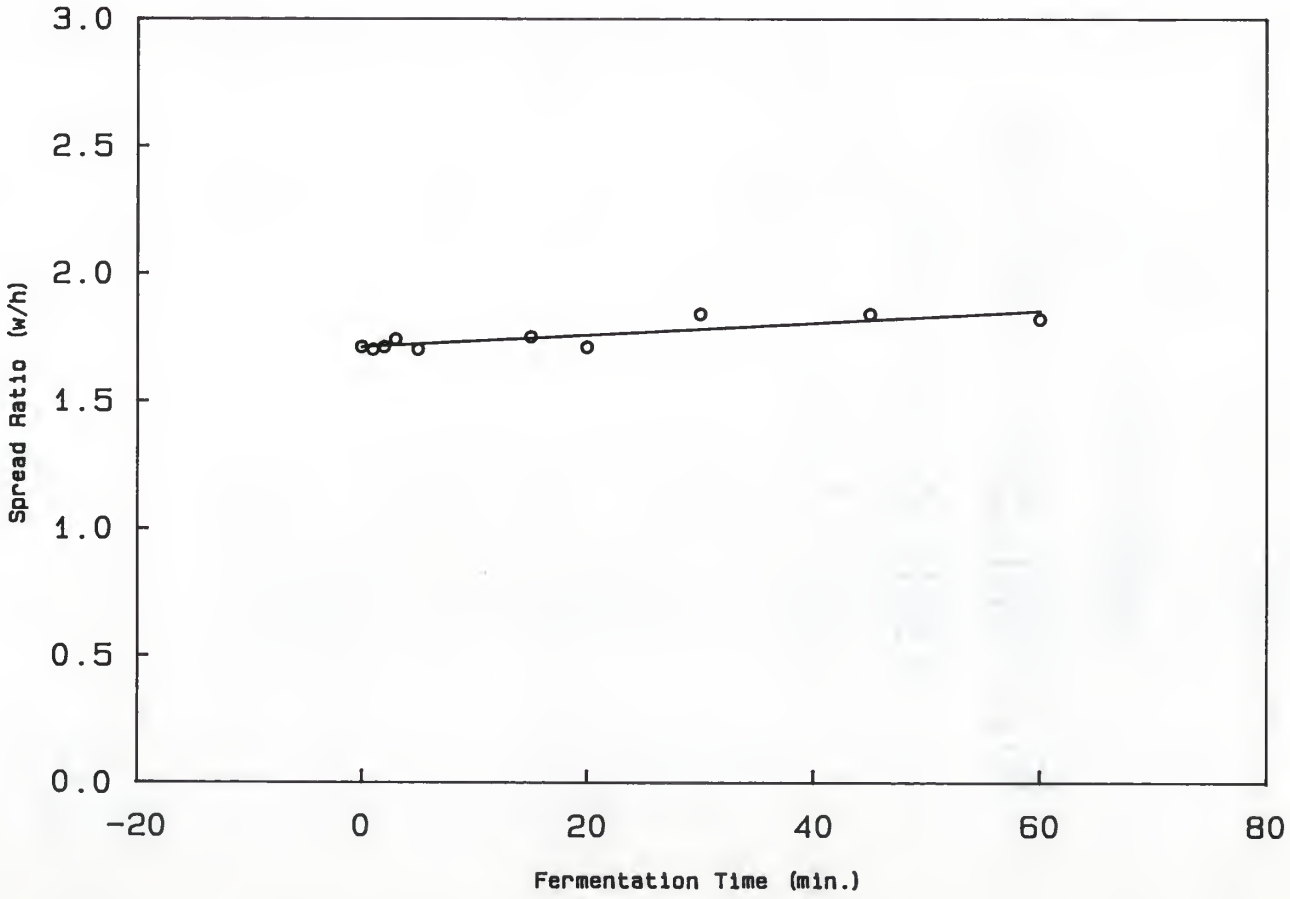
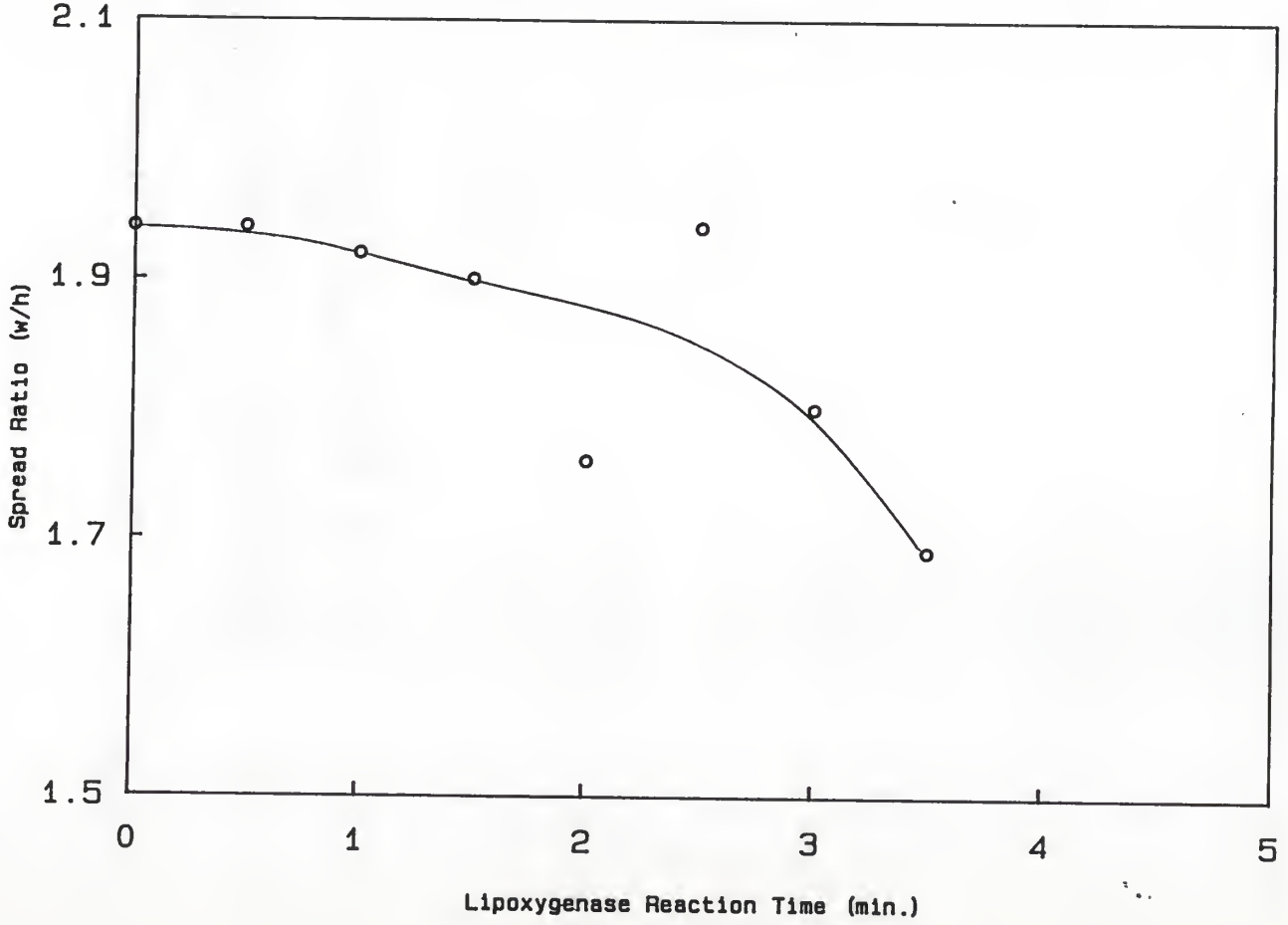

3.0
2.5
2.0
1.5
1.0
0.5
0.0
Spread Ratio (w/h)
-20
0
20
40
60
80
Fermentation Time (min.)

Figure 2. Rheological Effects of Adding Lipoxygenase at Different Points During Mixing.
Standard Deviation = $\pm$0.06


2.1
1.9
1.7
1.5
Spread Ratio (w/h)
0
1
2
3
4
5
Lipoxygenase Reaction Time (min.)

reaction time' in Figure 2 signifies the amount of time that lipoxygenase was present during mixing. A value of 3.5 min. implies that lipoxygenase was present during the full mixing period. A value of 2 min., however, implies that lipoxygenase was only present during the last 2 min. of mixing. Spread ratio values of doughs with lipoxygenase present only one minute or less during mixing were similar to those of doughs with no added soy flour. Apparently, lipoxygenase must act immediately to have its full rheological effect.

In an attempt to limit the action of lipoxygenase, different amounts of soy flour (thus different amounts of lipoxygenase) were added to doughs. The goal was to determine at what concentration lipoxygenase needed to be present to produce a significant rheological effect. Lipoxygenase appears to mimic the effect of yeast. However, it is unclear whether lipoxygenase has the same action as yeast, or whether the effect is in addition to that of yeast. Therefore, yeast was incorporated to study the interactions of lipoxygenase and yeast. Flour/water/yeast doughs with increasing concentrations (0.01-0.5% (w/w)) of soy flour were rested 180 minutes before moulding. No difference was apparent among the doughs with different concentrations of soy flour.

Since yeast and lipoxygenase compete for oxygen in a wholemeal dough during mixing (Galliard, 1986), it was

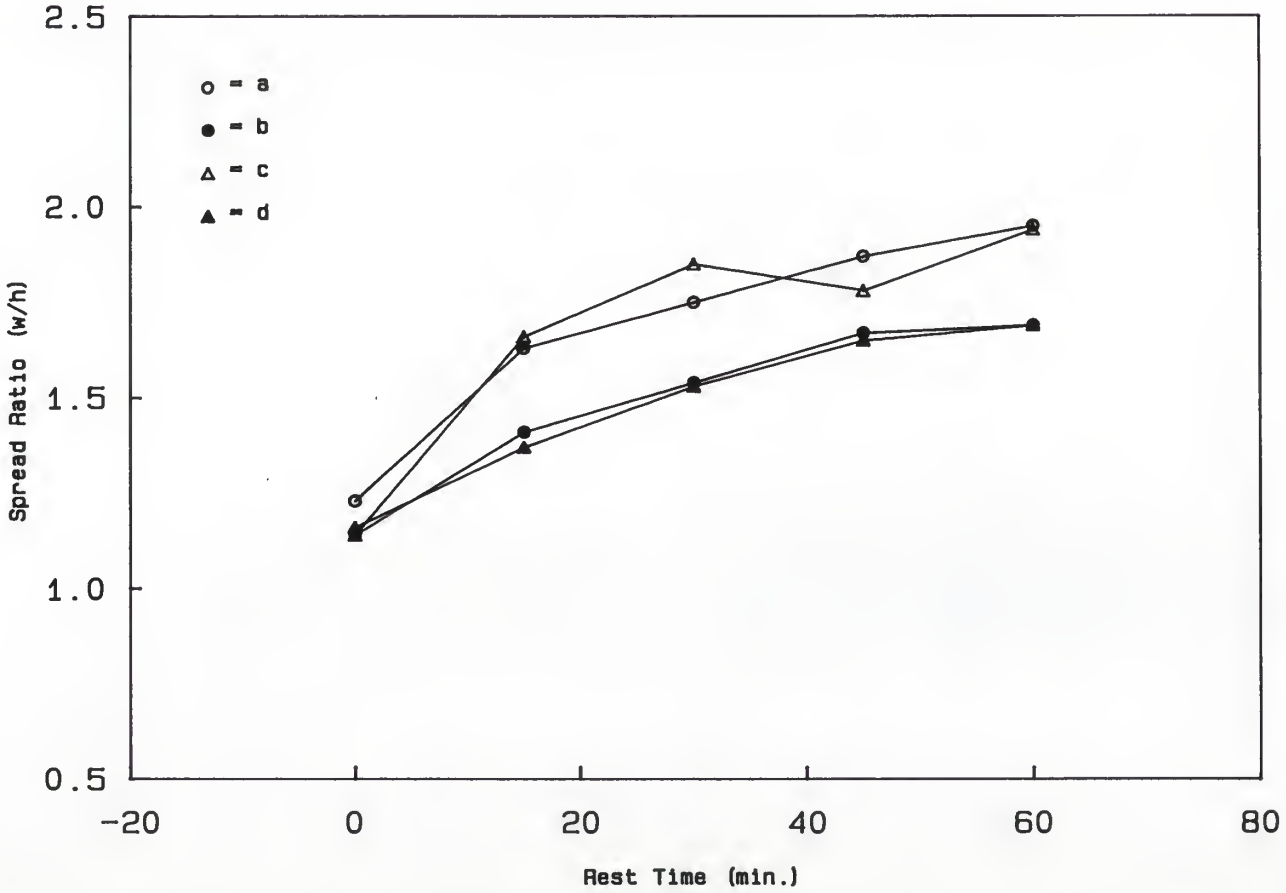
possible that yeast was making the doughs anaerobic and by depleting oxygen, limiting the enzyme's activity. If oxygen was the factor limiting lipoxygenase action, then increasing the available oxygen should increase the effect of the enzyme. To test this, additional oxygen was incorporated into the dough by remixing. Flour/water/enzyme active soy flour doughs were fermented 30 minutes before remixing for 1.5 minutes and then fermented 30 minutes more before moulding. The spread ratios of remixed doughs were no different than those of doughs that had not been remixed (Figure 3).

Since the above attempts to modify the action of lipoxygenase were unsuccessful, two further possibilities were considered. Either the attempts to incorporate more oxygen into the system had been unsuccessful or oxygen was not a limiting factor in the rheological activity of lipoxygenase. It may be that the lipid free radicals created by the enzyme have the same function as the subsequently formed lipid peroxide radicals. However, for oxygen to be unnecessary for the action of lipoxygenase seems unlikely since Hoseney et. al. (1980) found that oxygen was required for enzyme active soy flour to improve the rheological (spread test) properties of dough.

Oxidation. In order to differentiate the effects of oxidants on yeasted doughs from the effect of lipoxygenase,

Figure 3. Effect of Lipoxygenase in Remixed Doughs.

a = Remixed flour/water
b = Remixed flour/water/0.5% enzyme active soy flour
c = flour/water
d = flour/water/0.5% enzyme active soy flour
Standard Deviation = $\pm$0.05


2.5
2.0
1.5
1.0
0.5
Spread Ratio (w/h)
o = a
● = b
△ = c
▲ = d
-20
0
20
40
60
80
Rest Time (min.)

the second series of spread test studies examined the effects of oxidants on yeasted doughs with and without added soy flour. In a yeasted dough fermented for 180 minutes prior to molding, all three oxidants; 20 ppm potassium bromate, 15 ppm potassium iodate, and 50 ppm ascorbic acid, had approximately the same effect. Their presence further reduced spread if lipoxygenase was present (as 0.5% enzyme active soy flour). However, in the presence of soy flour, each oxidized dough behaved differently (Graph 4a & b). The enzyme and the oxidants, in general, had an additive effect in reducing the spread ratio. This was maintained throughout the rest time. However, the additional effect of lipoxygenase was not apparent in the presence of ascorbic acid. The dough containing both 50 ppm ascorbic acid and 0.5% enzyme active soy flour exhibited characteristics similar to those of a dough treated with ascorbic acid alone. This result could be explained if ascorbic acid was acting as a free radical scavenger and eliminating the free lipid radicals created by lipoxygenase, thereby neutralizing the effect of the enzyme.

In next set of experiments the spread test was used to study whether or not the oxidative effect of mixing in air was occurring solely via lipoxygenase action. If air oxidation were occurring only through the lipoxygenase-oxygen system, then removing the enzyme's primary substrate by defatting the flour should result in doughs with

Figure 4a. Effect of Oxidation on Yeasted Doughs.

a = flour/water/yeast
b = 15 ppm potassium iodate
c = 50 ppm ascorbic acid
d = 20 ppm potassium bromate
Standard Deviation = $\pm$0.05

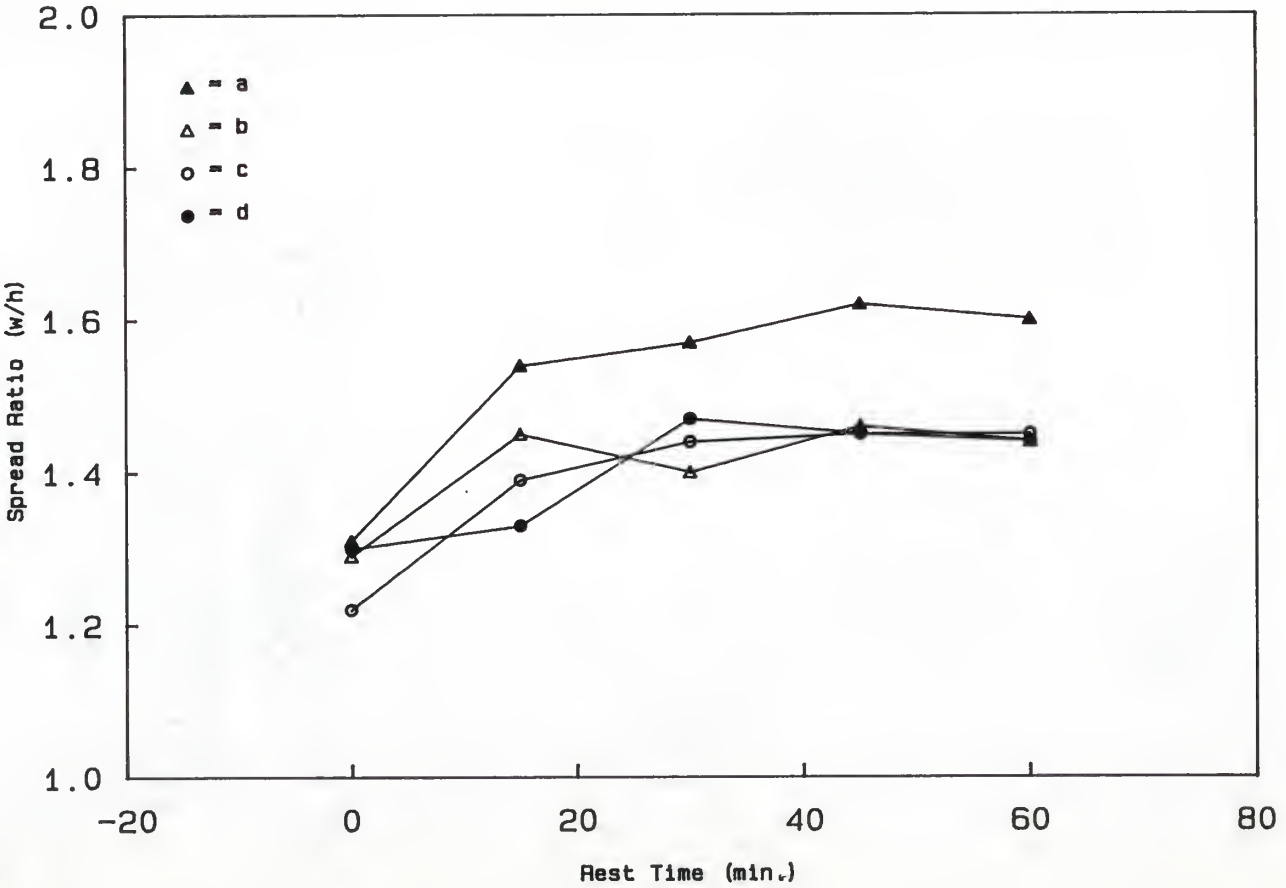
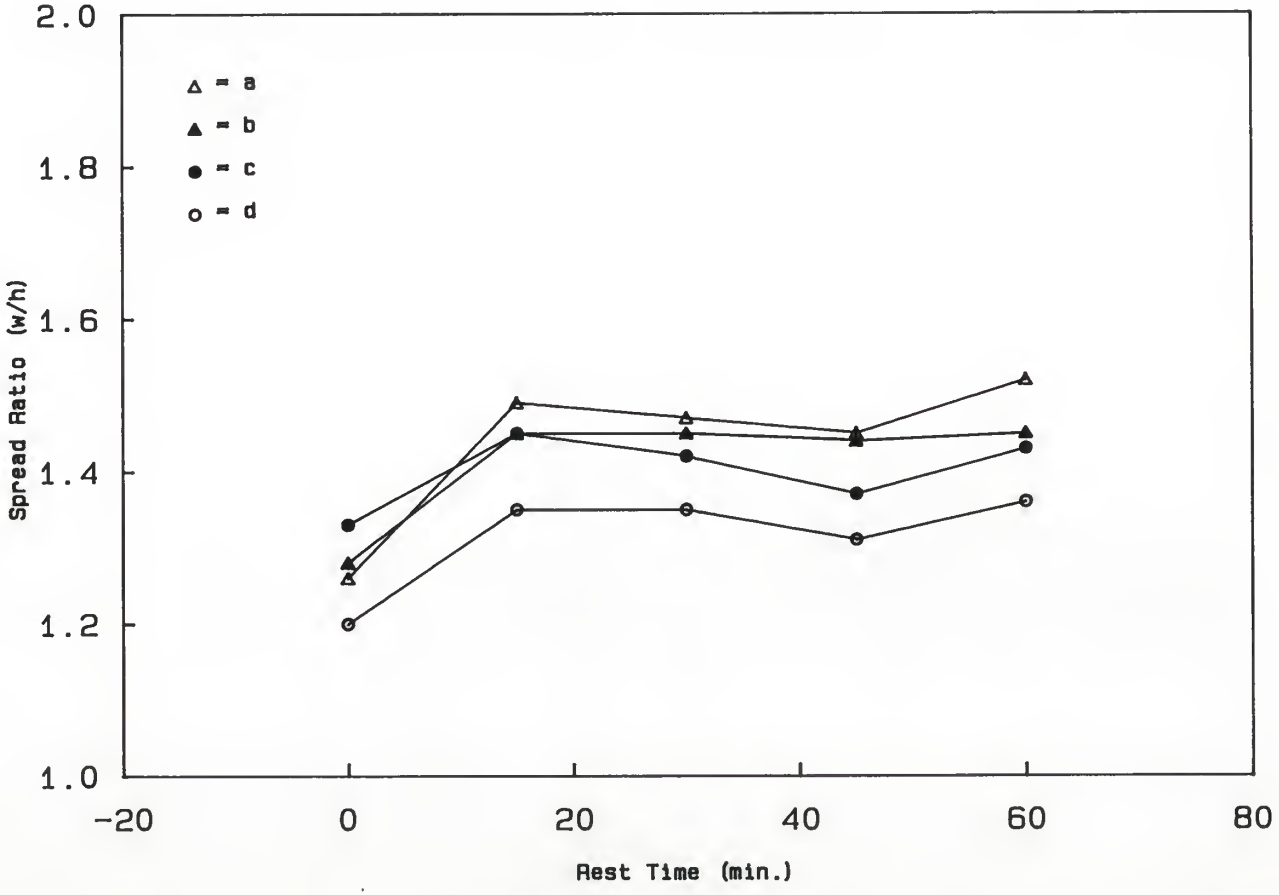

2.0
1.8
1.6
1.4
1.2
1.0
Spread Ratio (w/h)
▲ = a
△ = b
o = c
● = d
-20
0
20
40
60
80
Rest Time (min.)

Figure 4b. Effect of Oxidation plus Lipoxygenase on Yeasted Doughs.

a = flour/water/yeast/0.5% enzyme active soy flour
b = 50 ppm ascorbic acid
c = 15 ppm potassium iodate
d = 20 ppm potassium bromate
Standard Deviation = $\pm$0.05


2.0
1.8
1.6
1.4
1.2
1.0
Spread Ratio (w/h)
△ = a
▲ = b
● = c
○ = d
-20
0
20
40
60
80
Rest Time (min.)

identical spread characteristics when mixed in either a nitrogen or an air atmosphere. Both defatted flour/water and defatted flour/water/yeast doughs responded to oxygen. The doughs produced were more elastic (lower spread ratio) when mixed in an air atmosphere than when mixed in a nitrogen atmosphere (Figure 5). This is consistent with air's known oxidizing effect. We can, therefore, conclude that the oxidative effect of mixing in air is not occurring solely through the action of lipoxygenase. It may be, then, that air is oxidizing some compound endogenous to flour to produce the observed result.

Yeast. Yeast is known to affect dough rheology. These effects were also studied with the spread test. Previous spread test studies (see above) with lipoxygenase indicated that lipoxygenase produced an elastic dough immediately upon mixing. This is in contrast to yeast, which has its rheological effect over time (Figure 6). When doughs were fermented only 15 minutes before moulding, yeasted doughs had a much greater spread ratio than flour/water doughs. However, after 180 minutes fermentation, this effect was reversed and yeasted doughs were more elastic than flour/water doughs. Yeast and lipoxygenase in combination had an additive effect in making the doughs more elastic after 180 minutes fermentation (Figure 7).

It has been reported that yeast rapidly depletes a

Figure 5. Defatted Flour Mixed in Nitrogen and Air.

a = defatted flour/water, mixed in nitrogen
b = yeasted, mixed in nitrogen
c = defatted flour/water, mixed in air
d = yeasted, mixed in air
Standard Deviation = $\pm$0.08

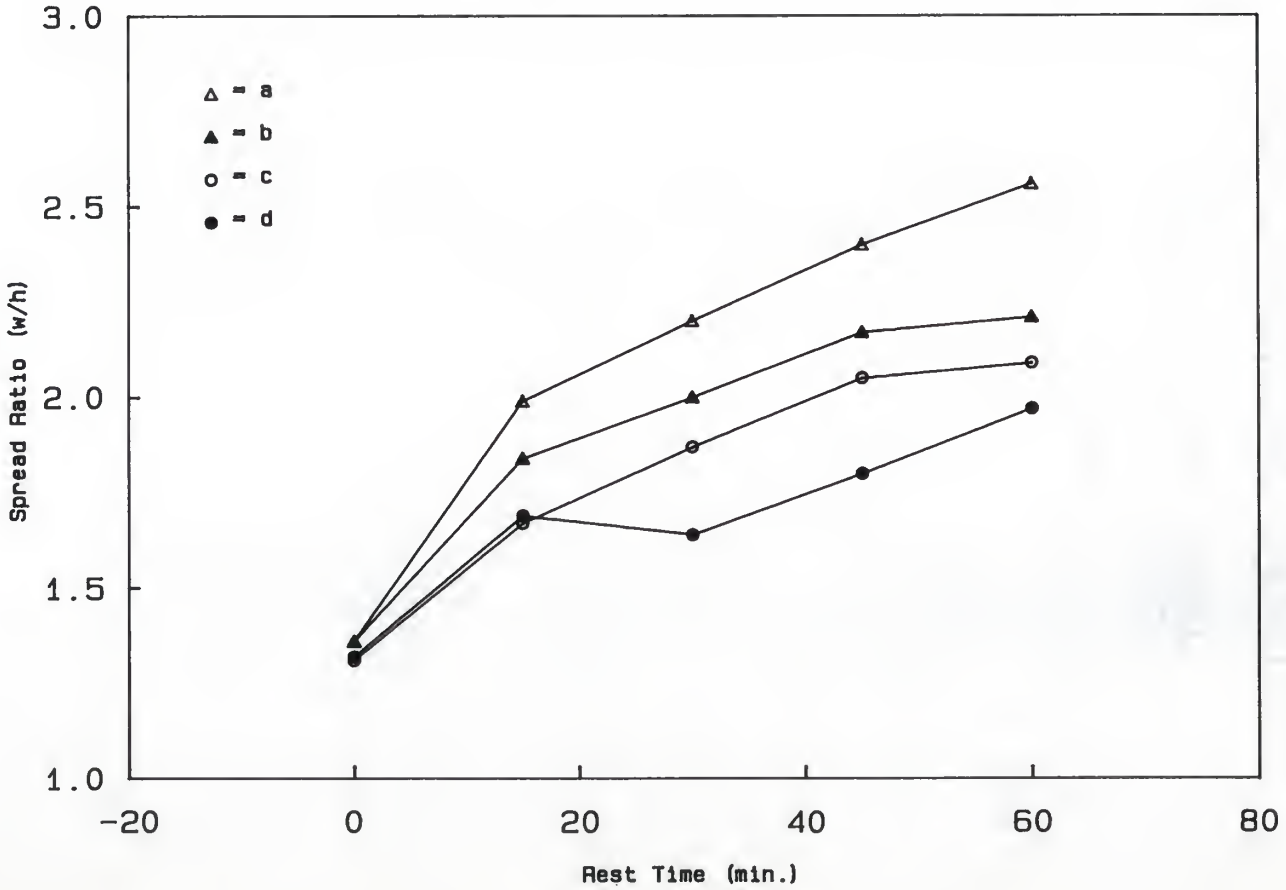

3.0
2.5
2.0
1.5
1.0
Spread Ratio (w/h)
△ = a
▲ = b
○ = c
● = d
-20
0
20
40
60
80
Rest Time (min.)

Figure 6. Rheological Effect of Yeast

Over Fermentation Time

a = yeasted, fermented 15 minutes
b = flour/water, fermented 180 minutes
c = flour/water, fermented 15 minutes
d = yeasted, fermented 180 minutes
Standard Deviation = $\pm$0.05

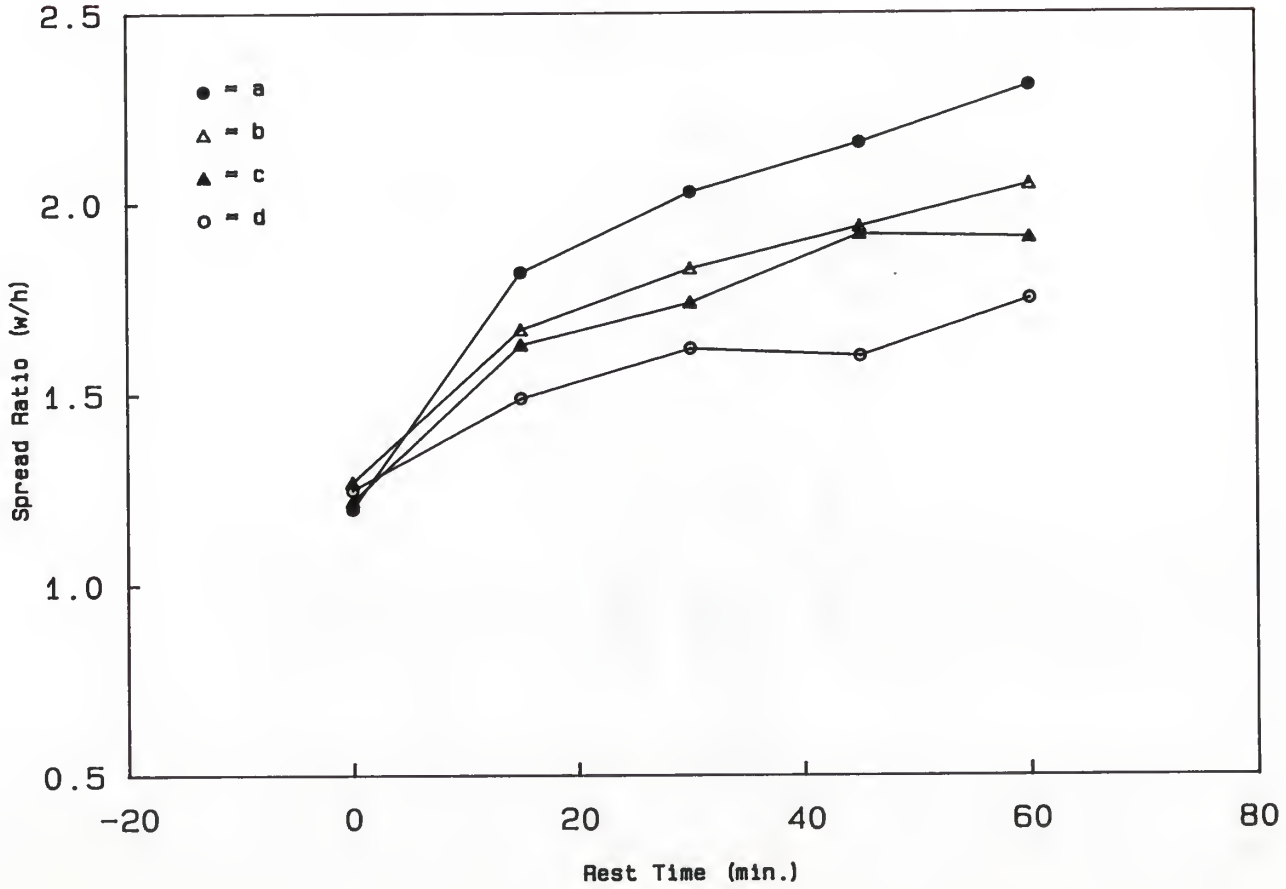
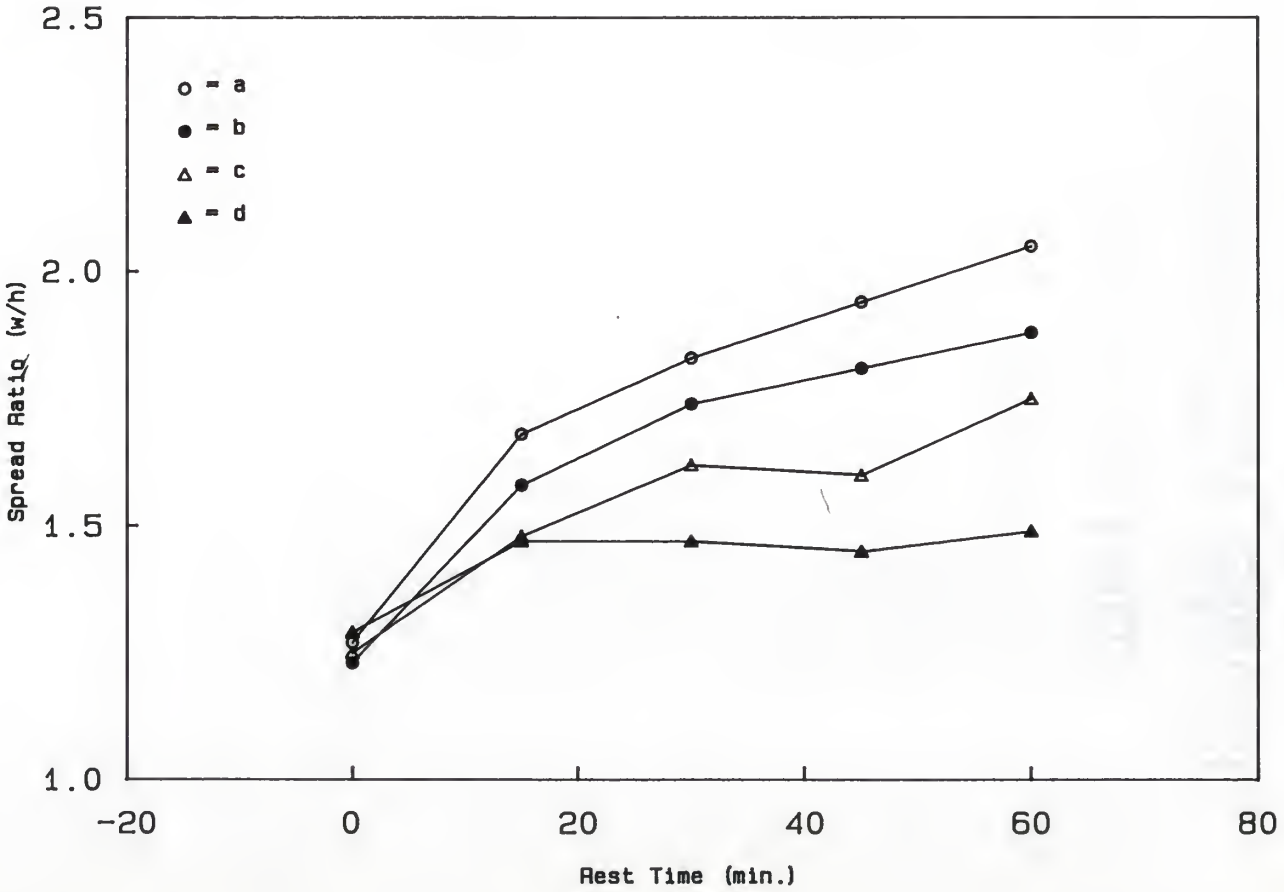

2.5
2.0
1.5
1.0
0.5
Spread Ratio (w/h)
● = a
△ = b
▲ = c
o = d
-20
0
20
40
60
80
Rest Time (min.)

Figure 7. Rheological Effect of Yeast and Lipoxygenase After 180 minutes Fermentation.

a = flour/water
b = flour/water/0.5% enzyme active soy flour
c = flour/water/yeast
d = flour/water/yeast/0.5% enzyme active soy flour
Standard Deviation = $\pm$0.04


2.5
2.0
1.5
1.0
o = a
● = b
△ = c
▲ = d
Spread Ratio (w/h)
-20
0
20
40
60
80
Rest Time (min.)

dough of oxygen with the effect occurring even during mixing (Dr. R. C. Hoseney, private communication). Therefore, it should follow that a yeasted dough rapidly becomes an anaerobic system. If yeast creates an anaerobic system during mixing, then yeasted doughs should have the same rheological properties as doughs mixed in a nitrogen atmosphere. However, yeasted doughs exhibit different characteristics than doughs mixed in a nitrogen atmosphere (Figure 8), as witnessed by the fact that yeasted doughs overmix while doughs mixed under nitrogen do not. While yeast may deplete a dough of oxygen, some oxidation still occurs when mixing in an air atmosphere.

The next set of experiments was designed to study how yeast acts in dough. One possibility is that the products or intermediates of fermentation are rheologically active compounds. If so, a metabolically inactive yeast should have no rheological effect. Initial attempts to starve yeast by using unmalted flour were unsuccessful. To remove all fermentables, doughs produced from commercial gluten and starch were employed in the spread test. Results are shown in Figure 9. Yeasted gluten/starch doughs and yeasted gluten/starch doughs with sugar added to aid fermentation showed a difference only initially (when fermentation time was zero). The two curves then became parallel. Since both of these doughs lacked water solubles, this strongly suggests that yeast requires the water soluble fraction of

Figure 8. Rheological Responses of Yeasted and Unyeasted Doughs Mixed in Air and Nitrogen.

a = flour/water, mixed in nitrogen
b = yeasted, mixed in nitrogen
c = flour/water, mixed in air
d = yeasted, mixed in air
Standard Deviation = $\pm$0.04

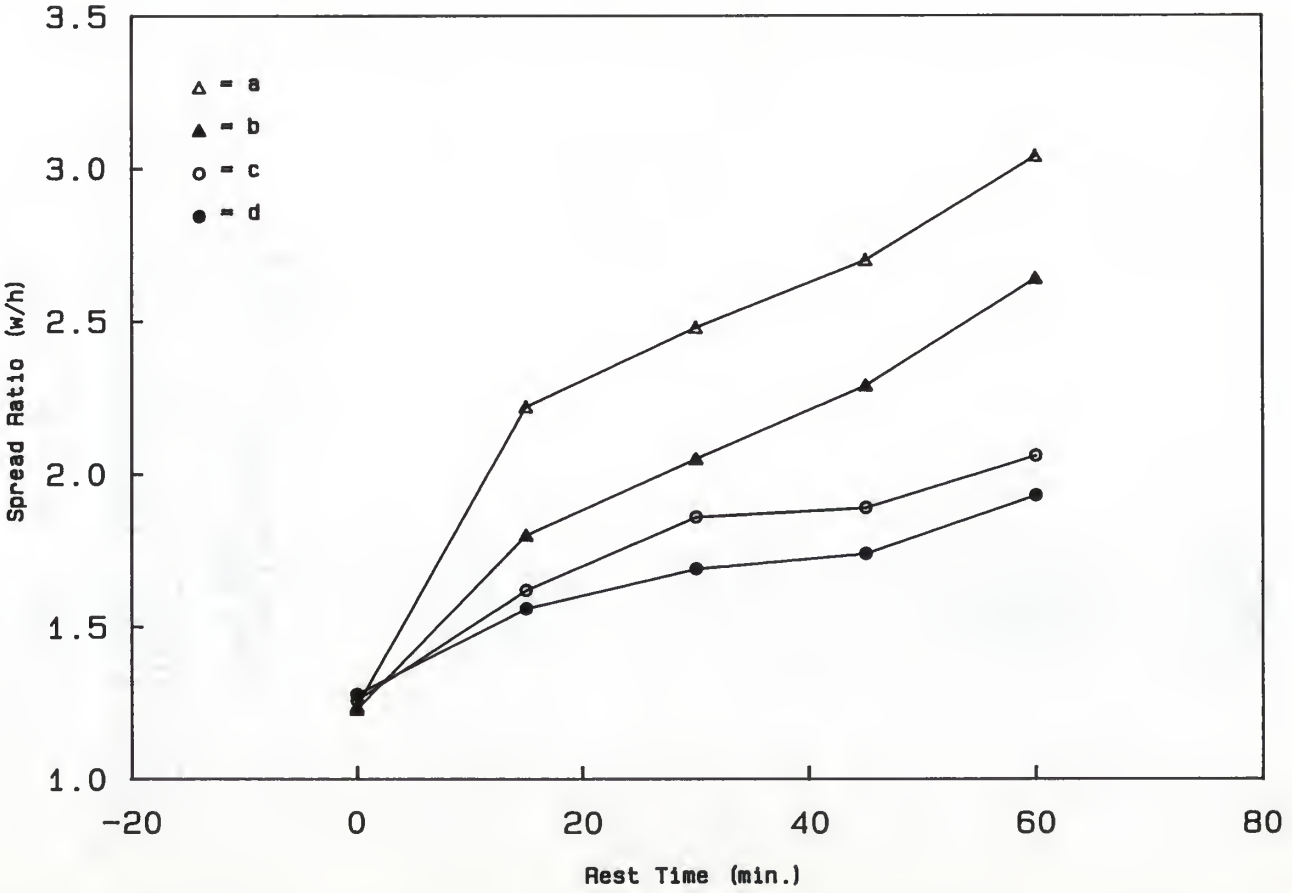
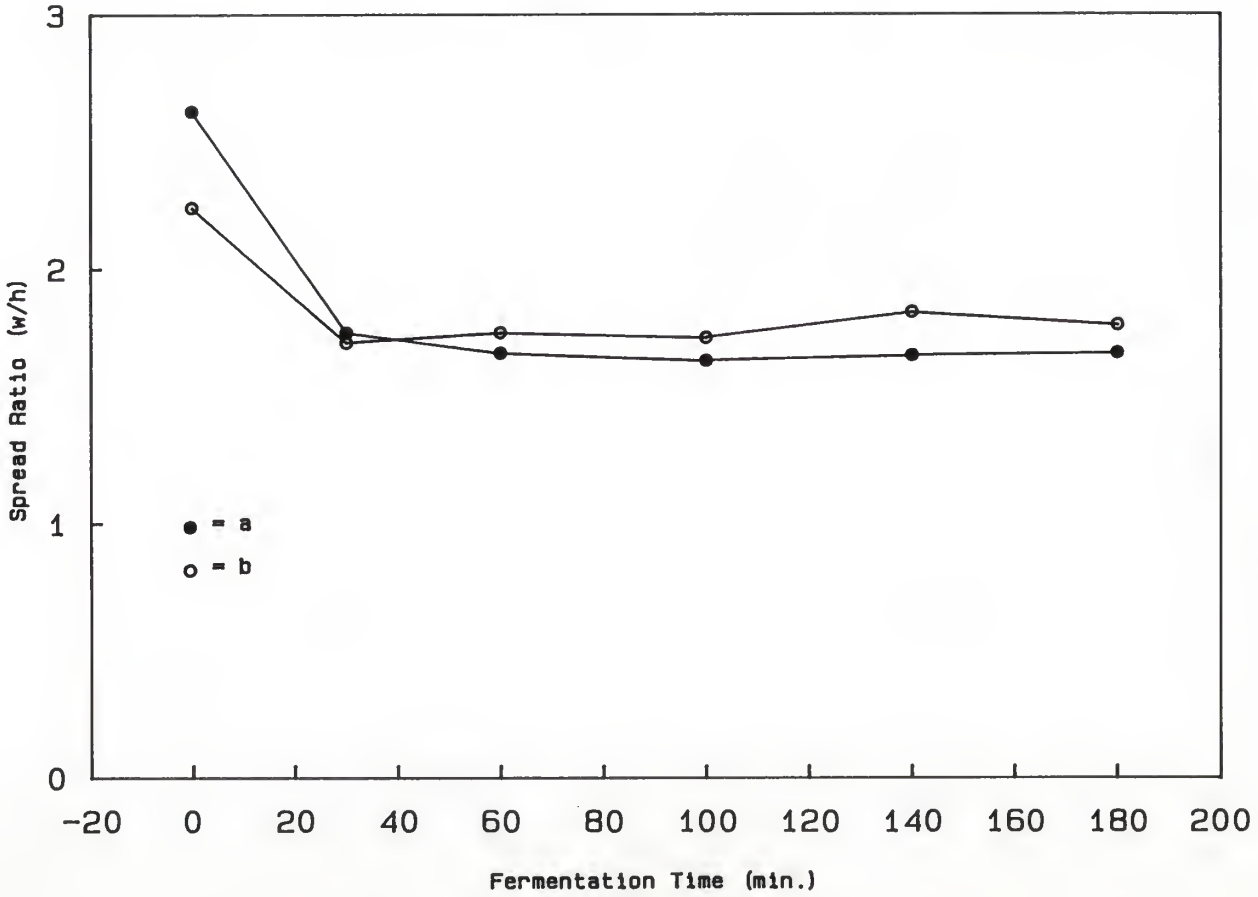

△ = a
▲ = b
o = c
● = d
Spread Ratio (w/h)
3.5
3.0
2.5
2.0
1.5
1.0
-20
0
20
40
60
80
Rest Time (min.)

Figure 9. Rheological Effect of Fermentation on Gluten + Starch Doughs.

a = gluten/starch/yeast/sugar
b = gluten/starch/yeast
Standard Deviation = $\pm$0.02


3
2
1
0
Spread Ratio (w/h)
● = a
○ = b
-20
0
20
40
60
80
100
120
140
160
180
200
Fermentation Time (min.)

the flour to exert its rheological effect.

One component of flour water solubles thought to have an effect on dough behavior is low molecular weight thiol compounds. Therefore, cysteine (40 ppm) was added to the gluten and starch doughs to mimic the presence of low molecular weight thiols and test the hypothesis that their presence was required for yeast to have an effect. However, rather than the increased spread ratio seen over time in a cysteine-treated flour/water dough (Hoseney et. al., 1979), the cysteine-treated gluten + starch dough exhibited a high initial spread ratio that decreased with time (Figure 10). The effect of the cysteine seemed to disappear over time in the gluten + starch dough, unlike in the flour/water dough. Although we do not understand this phenomenon, it should be pointed out that the difference between the two systems is the water soluble fraction of the flour. When a flour/water/yeast dough was compared with a flour/water/yeast/15 ppm cysteine dough, the cysteine treated doughs had a greater spread ratio initially, but after 100 minutes of fermentation its spread ratio was no greater than that of the flour/water yeast dough (Figure 11).

The rheological effects of yeast during fermentation can be reversed by the addition of a free radical scavenger. Tenox (BHA and BHT dispersed in corn oil) added at 2% (w/w) reversed the effect of yeast on malted flour doughs (Figure

Figure 10. Rheological Effect of Yeast on Cysteine in Gluten + Starch Doughs.

a = gluten/starch/40 ppm cysteine
b = gluten/starch/40 ppm cysteine/yeast
c = gluten/starch/yeast
d = gluten/starch
Standard Deviation = $\pm$0.06

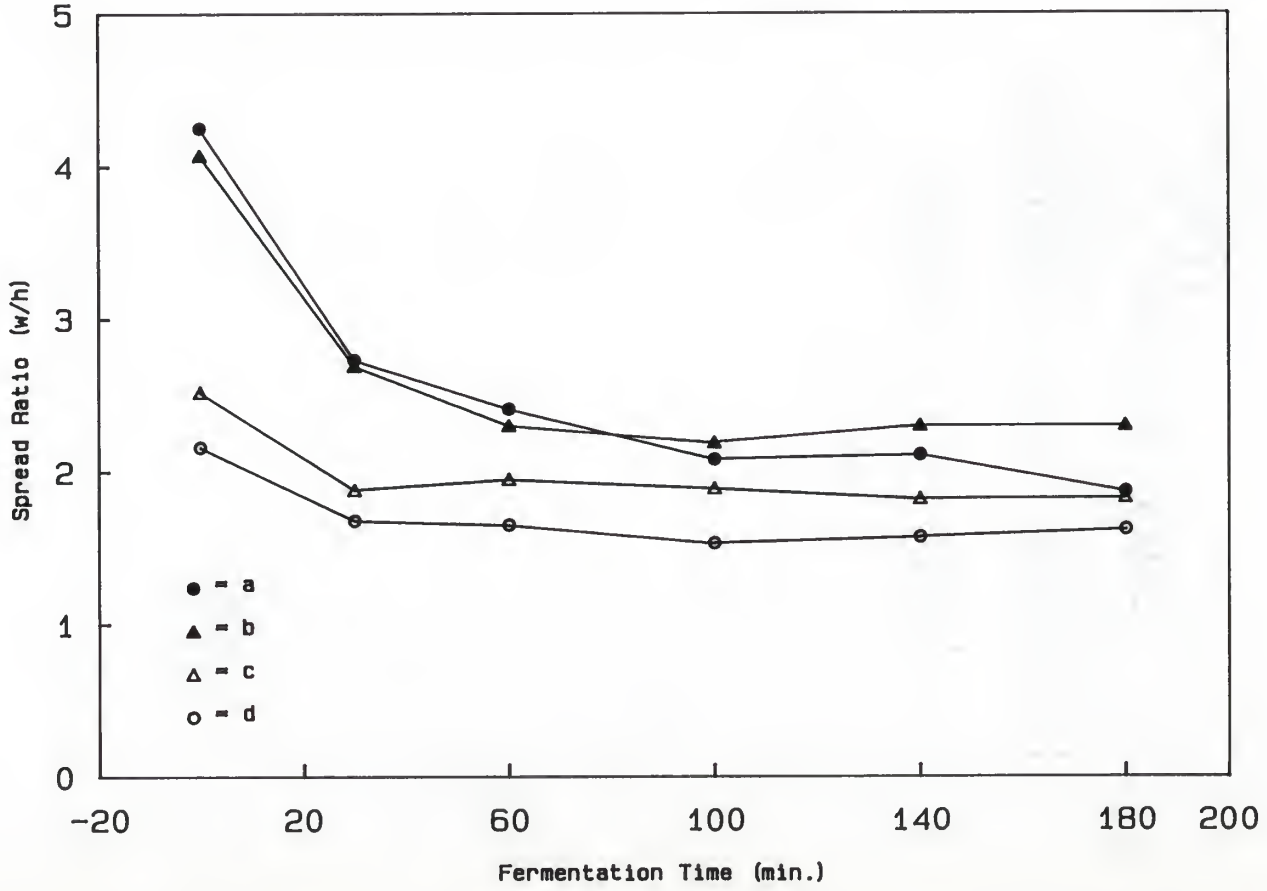
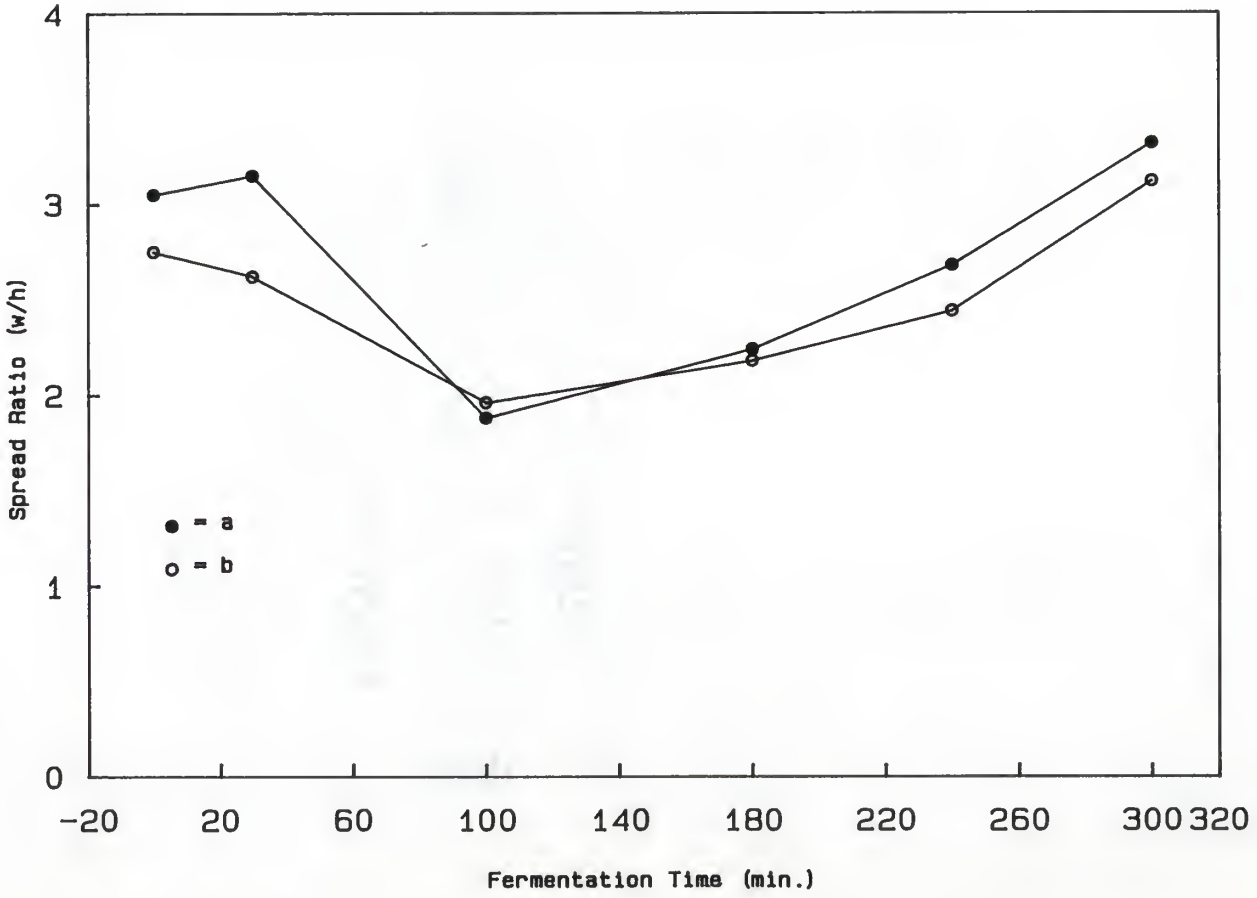

5
4
3
2
1
0
Spread Ratio (w/h)
● = a
▲ = b
△ = c
○ = d
-20
20
60
100
140
180
200
Fermentation Time (min.)

Figure 11. Effect of Cysteine on Yeasted Dough.

a = flour/yeast/15 ppm cysteine
b = flour/yeast
Standard Deviation = $\pm$0.08


4
3
2
1
0
Spread Ratio (w/h)
● = a
o = b
-20
20
60
100
140
180
220
260
300
320
Fermentation Time (min.)

12). Addition of the free radical scavengers created a dough that progressively gained more viscous flow characteristics during fermentation.

When 2% Tenox is added to an unyeasted gluten/starch dough, it creates a more elastic system (Figure 13). However, in a cysteine treated gluten/starch dough, Tenox again reversed the effect of yeast, giving the dough greater viscous flow character. The reversal of the effects of yeast by a free radical scavenger suggest that yeast operates through a free radical mechanism.

The question remained as to what yeast was doing to the water soluble fraction of the flour. One possibility is that it is removing an active compound. Indications that yeast was actively affecting the water soluble portion of the flour led to studies employing flour fractionation. Water solubles were separated from the gluten/starch fraction, which was freeze dried. The water solubles were fermented for three hours (86^{o}F, 90% R.H.) with 2% compressed yeast (based on original flour weight). The water solubles were then centrifuged to remove yeast, and lyophilized. After lyophilization, the gluten/starch and femented water soluble fractions were mixed to a dough and tested via the spread test. If yeast was acting on a component in the water solubles to remove or inactivate it, the dough containing fermented water solubles should exhibit the same spread ratio characteristics as gluten/starch.

Figure 12. Effect of a Free Radical Scavenger on Yeast's Rheological Activity in Dough.

a = malted flour/yeast
b = malted flour/yeast/2% Tenox
Standard Deviation = $\pm$0.06

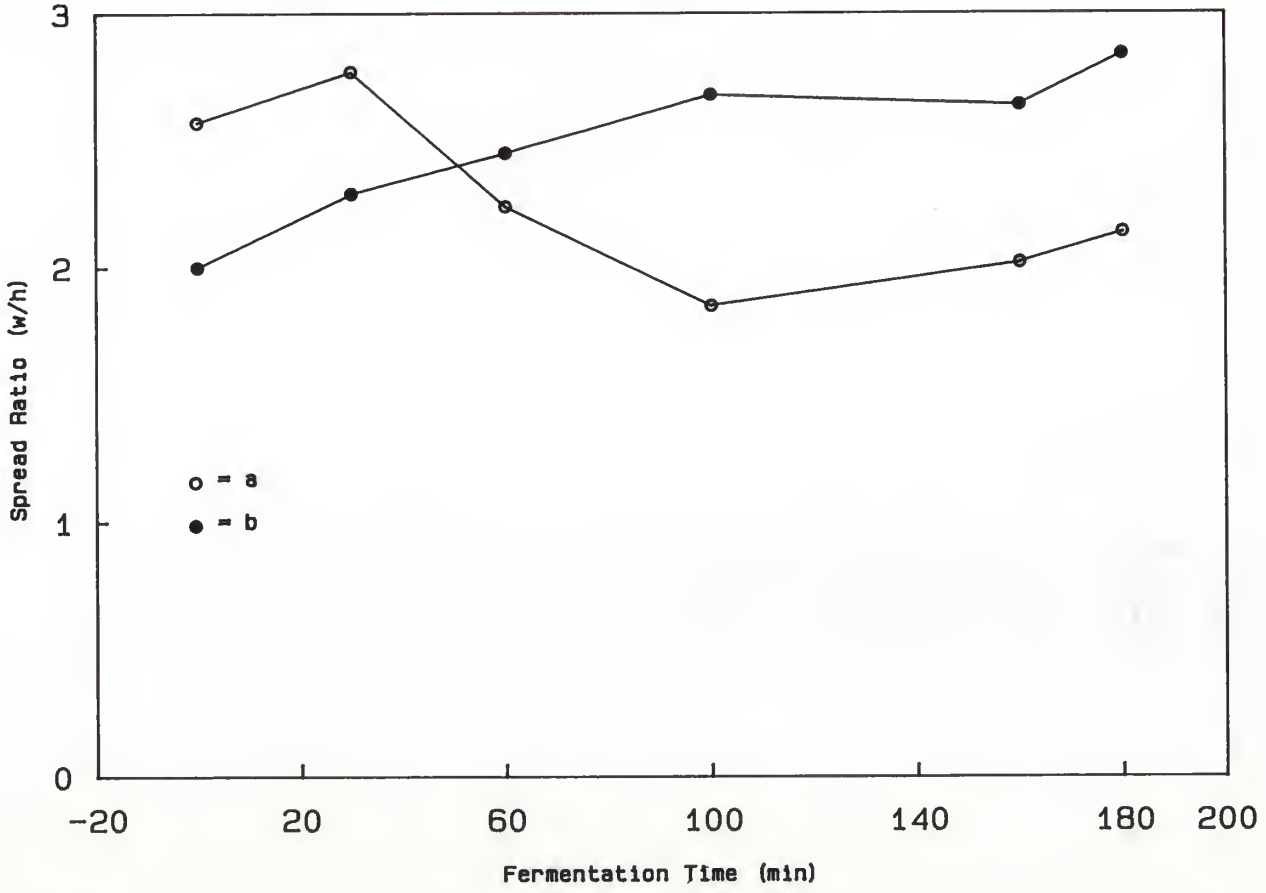
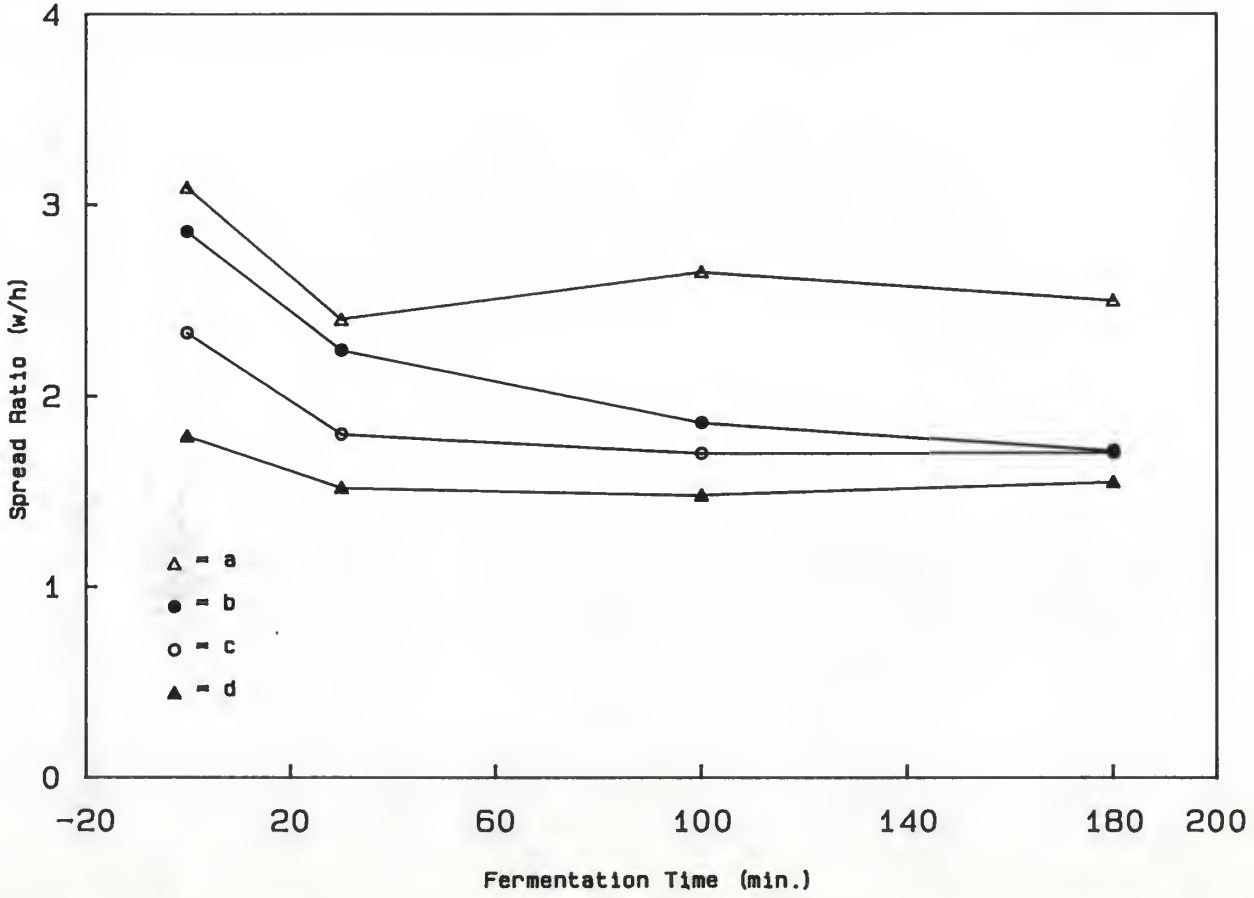

3
2
1
0
Spread Ratio (w/h)
o = a
● = b
-20
20
60
100
140
180
200
Fermentation Time (min)

Figure 13. Effects of a Free Radical Scavenger and/or Cysteine on Gluten + Starch Doughs.

a = gluten/starch/2% Tenox/40 ppm cysteine/yeast
b = gluten/starch/2% Tenox/40 ppm cysteine
c = gluten/starch
d = gluten/starch/2% Tenox
Standard Deviation = $\pm$0.07


4
3
2
1
0
Spread Ratio (w/h)
△ = a
● = b
○ = c
▲ = d
-20
20
60
100
140
180
200
Fermentation Time (min.)

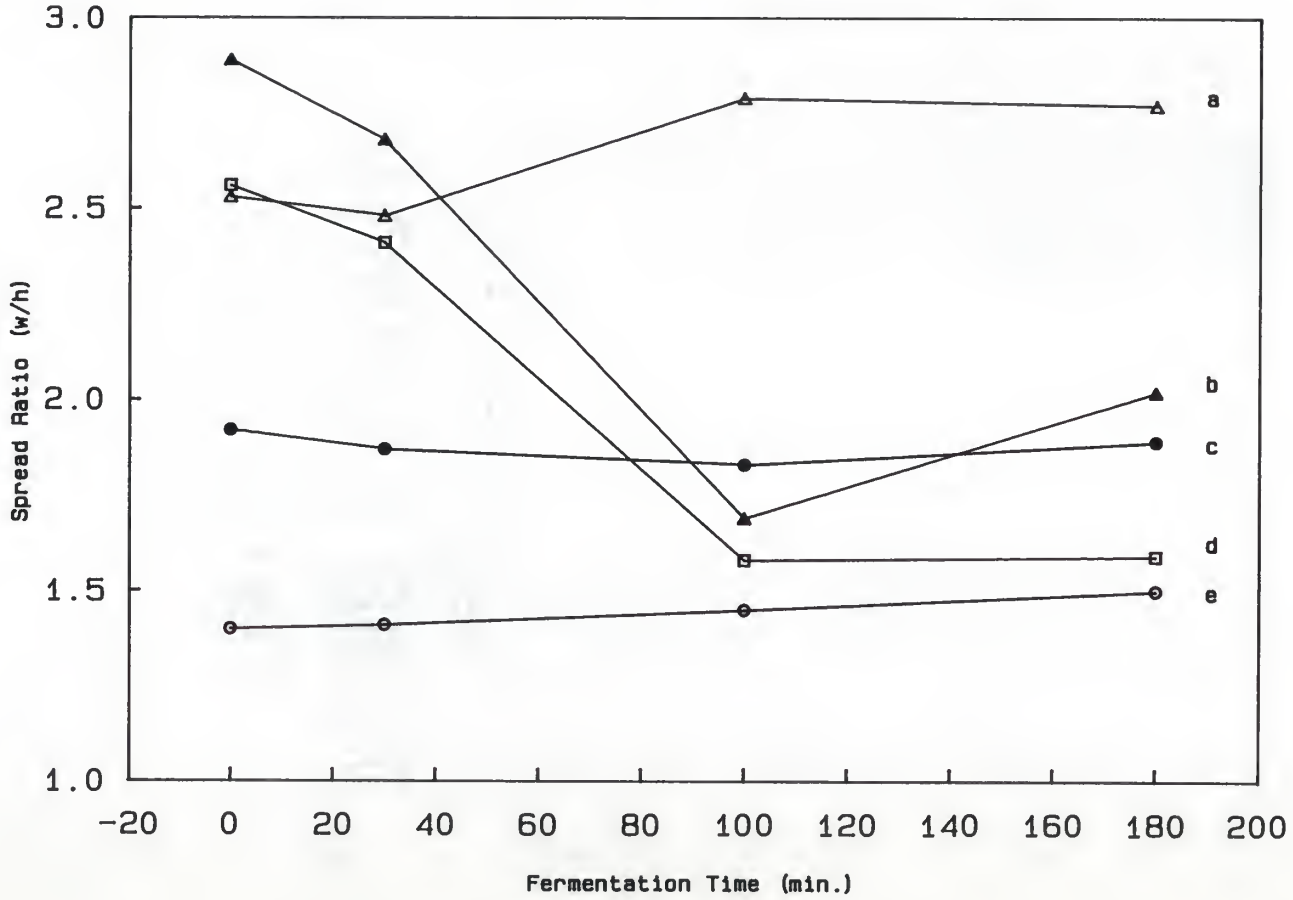
Indeed, this was the case, as the gluten/starch and gluten/starch/fermented water soluble doughs both exhibited level spread ratios (Figure 14). It is also apparent that yeast contains a reducing compound as the curve containing yeast was higher at zero time than its control. In addition, these results suggest that yeast has its effect by acting on cysteine or some other rheologically active thiol compounds. The cysteine-treated dough was affected during the course of fermentation so that its spread ratio was now equivalent to that of its control. During fermentation of the control dough (reconstituted with non-fermented water solubles), the yeast modified the dough to a spread ratio similar to that of the dough containing the fermented water solubles. Apparently, yeast is removing the low molecular weight thiol compounds from the system or at least making them increasingly susceptible to reactions allowing increased spread (flow).

CHAPTER II - DOUGH BREAKDOWN

As described in the literature review, dough breakdown is affected by a number of seemingly unrelated factors. To understand dough breakdown, a three component model involving these factors (oxidation, ADB compounds and charge) was developed to investigate their interactions. All three of these factors affect dough breakdown, and depending upon the treatment, dough breakdown may be

Figure 14. Rheological Effect of Cysteine and Yeast on Doughs Containing Fermented Water Solubles.

a = gluten/starch/fermented water solubles/cysteine
b = gluten/starch/fermented water solubles/cysteine/yeast
c = gluten/starch/fermented water solubles
d = gluten/starch/water solubles/yeast
e = gluten/starch
Standard Deviation = $\pm$0.06


3.0
2.5
2.0
1.5
1.0
Spread Ratio (w/h)
-20
0
20
40
60
80
100
120
140
160
180
200
Fermentation Time (min.)
a
b
c
d
e

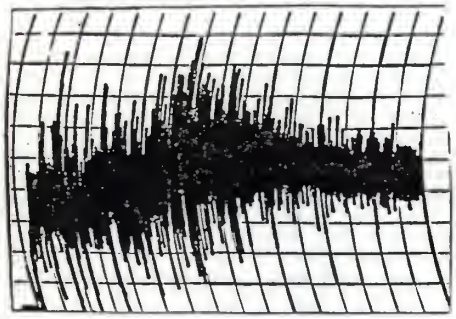
accelerated or reversed.

Single Factor Studies: Activated Double Bond Compounds. Most, if not all previous studies on the action of ADBs in dough have used flour/water doughs as the test system. Thus, the models proposed to explain the role of ADBs in aiding or abetting dough breakdown have been based on the simplified flour/water system. It has remained an open question as to whether the phenomenon (and, by extension, the model) is applicable to fully formulated bread doughs. The additional compounds present in a full formula dough might be expected to cancel, or at least modify the effects seen with flour/water doughs. To test this, the first studies undertaken with the mixograph examined the effects of a single factor, ADB compounds, on dough breakdown. Fumaric and ferulic acid solutions were prepared in increasing concentrations (fumaric acid, 1000-4000 ppm; ferulic acid, 100-250 ppm, acid dissolved by adjustment of the solution to pH 7), and the effect of these two ADB compounds tested in both flour/water and full formula doughs (Table 1). Although the accelerated breakdown due to the ADB compounds was much more pronounced in the flour/water doughs than in the full formula doughs, there was still a definite effect on full formula dough stability that increased with increased ADB concentration (Figure 15). Thus, ADBs still have their effects when present in full formula doughs. Unyeasted full formula doughs exhibited the

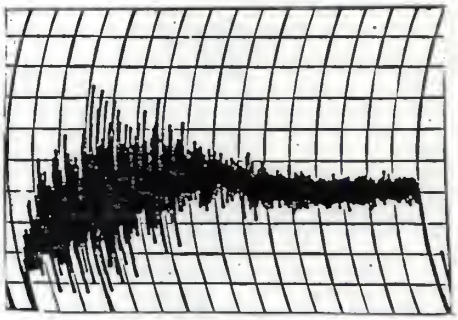
FULL FORMULA DOUGH	
Ingredient	Baker's %
Flour	100.0
Sucrose	6.0
Sodium Chloride	1.5
Shortening	3.0
NFDM	4.0
Active Dry Yeast	0.76

Figure 15. Effect of Activated Double Bond Compounds on Mixograms of Flour/Water and Full Formula Doughs.

FLOUR/WATER

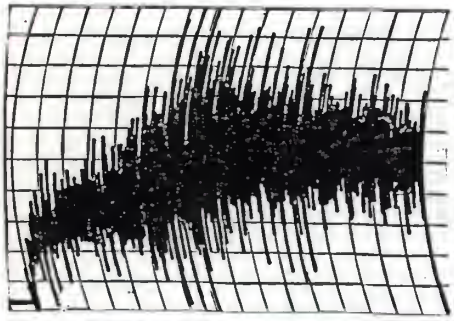


Control

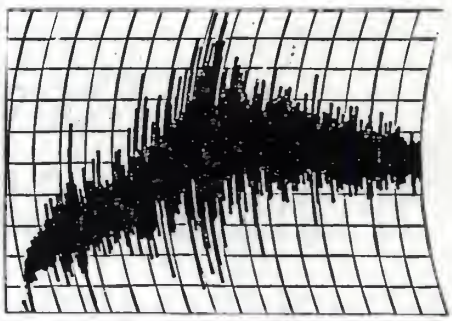


250 ppm Ferulic Acid

FULL FORMULA



Control



250 ppm Ferulic Acid

same response as yeasted (and therefore more anaerobic) full formula doughs. These results indicate that oxygen was not a factor limiting activity of the ADB compounds when mixed in an air atmosphere. When ADB compounds were added to a full formula dough in the presence of 10 ppm potassium iodate, a fast acting oxidant, the dough breakdown was still more pronounced than in its absence. The ADB and oxidant appeared to have additive effects.

Another study with the mixograph resolved whether or not lipoxygenase could act directly on ADB compounds in dough rather than through its lipid substrate. The fatty acid radical created by lipoxygenase is thought to compete with the thiyl radicals created on gluten for reaction with ADB compounds. The result of this competition would be to strengthen the dough. Oxidants such as potassium iodate are thought to increase the stress in the dough system by removing thiols from the system, thereby forcing the creation of more gluten thiyl radicals and contributing to dough breakdown. Therefore, if lipoxygenase were to reverse the effect of iodate in defatted flour, as it does in native flour, it would indicate that lipoxygenase was affecting ADB compounds directly. However, results clearly demonstrated that lipoxygenase does not reverse the effect of iodate in defatted flour, indicating that lipoxygenase does not act directly on ADB compounds.

Single Factor Studies: Oxidation. The effects of

oxidants were studied in a yeasted dough as well as a yeasted dough containing 0.5% enzyme active soy flour. Soy flour is known to increase mixing tolerance, while yeast does not affect the mixing curve. Three oxidants were studied: potassium bromate (20 ppm), potassium iodate (15 ppm), and ascorbic acid (50 ppm). Bromate is a slow acting oxidant, and had no effect on the mixogram (Figure 16b). The accelerated dough breakdown caused by potassium iodate was reversed to a great extent by lipoxygenase (Figure 16a). The reversal was not complete as a small amount of breakdown was still visible, possibly a simple matter of level. Ascorbic acid alone had no visible effect on the mixogram. When both ascorbic acid and enzyme active soy flour were present (Figure 16c), however, the effect of the lipoxygenase was masked. These data are consistent with the action of lipoxygenase in spread tests (Figures 4a & b). Perhaps ascorbic acid is acting as a free radical scavenger, eliminating the fatty acid radical and negating its effect.

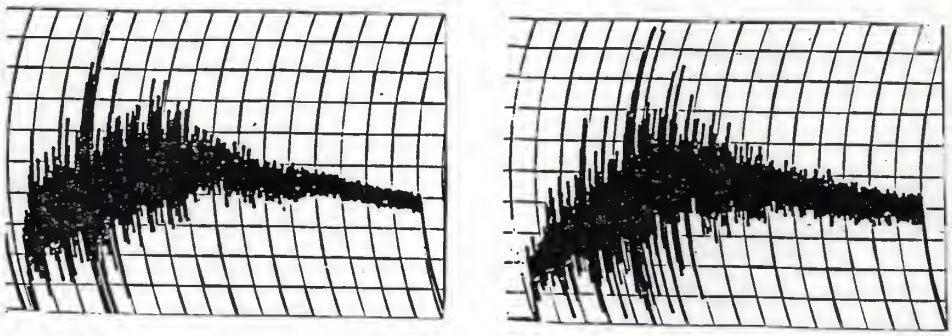
The "classical" oxidation theory holds that small molecular weight thiols in the water soluble portion of the flour are oxidized to disulfides by chemical oxidants such as potassium iodate. Thiols are thought to react with thiyl free radicals formed during mixing as a result of stress created by the entanglement of large gluten proteins. By oxidizing these compounds to disulfides, they are prevented from reacting, allowing the creation of more free radicals

Figure 16. Combined Effects of Oxidation and Lipoxygenase on Dough Mixing Characteristics.

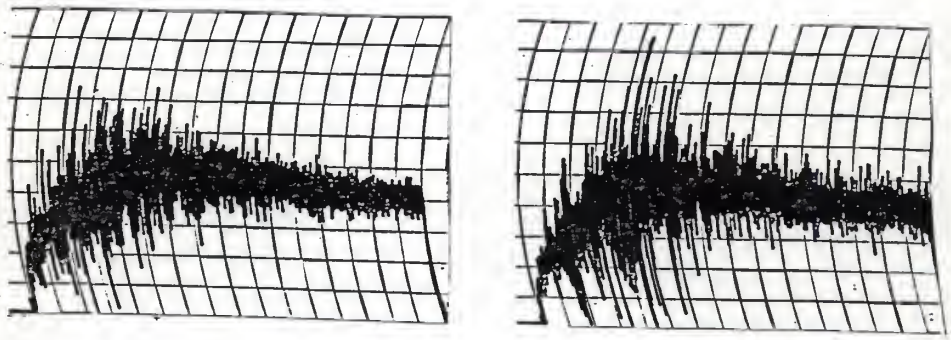
A) 15 ppm KIO_3
B) 20 ppm $KBrO_3$
C) 50 ppm Ascorbic Acid

Oxidant only

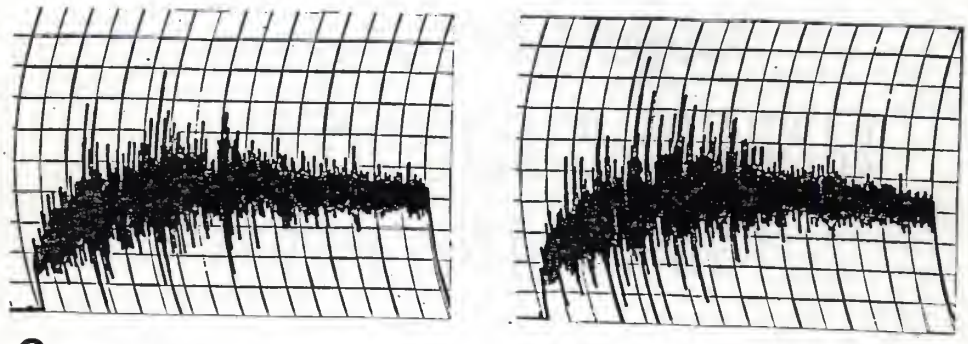
+ lipoxygenase



A



B



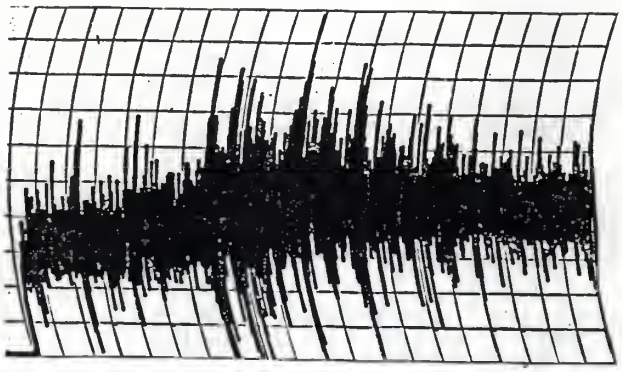
C

which can react with endogenous ADBs to cause greater dough breakdown.

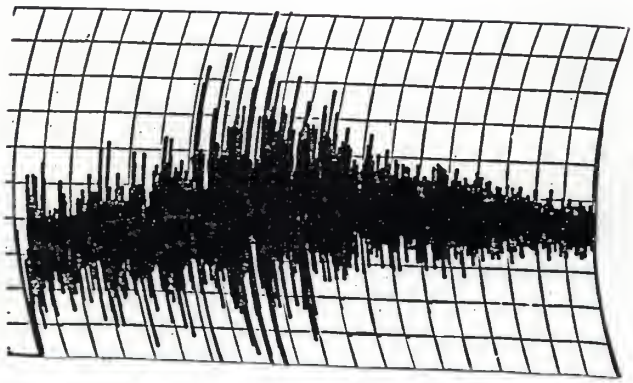
When the water soluble fraction of flour is removed, oxidation still affects the gluten/starch fraction of the flour (Figure 17). To ensure that the action of oxidants was not to release bound ferulic acid from the water insoluble pentosans present in flour, this same experiment was repeated with commericial gluten/prime starch. The water insoluble pentosan fraction is not present in prime satrch. However, oxidation by potassium iodate was found to still accelerate breakdown during overmixing when compared to its control (Figure 18). This suggested that oxidation was either affecting the gluten/starch fraction directly or affecting a compound bound in the gluten/starch fraction.

Possibily, a compound exists in flour that is susceptible to oxidation, producing a molecule that is active in accelerating dough breakdown in a manner similar to that seen with ADBs. The question existed as to what that precursor compound could be. Graveland et. al. (1984) identified a hydroquinone glycoside (B-D-Glc-p-(1-4)-B-D-Glc-p-(1-4)-B-D-Glc-p-(1-1')-3-methoxyhydroquinone) as a compound present in wheat flour. Graveland further suggested that this molecule was active in affecting dough rheology and protein solublility, specifically the SDS-insoluble glutenin I fraction. The 'active' compound (Figure 19) is structurally related to arbutin, a simple

Figure 17. Effect of Oxidation on the Mixing Characteristics of Gluten/Starch.

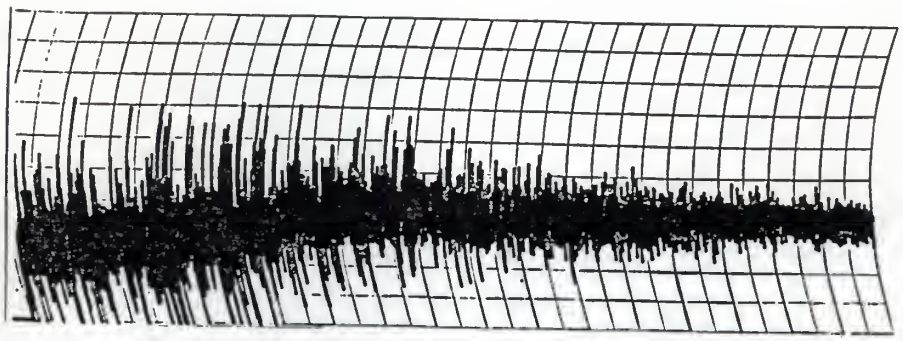


Control

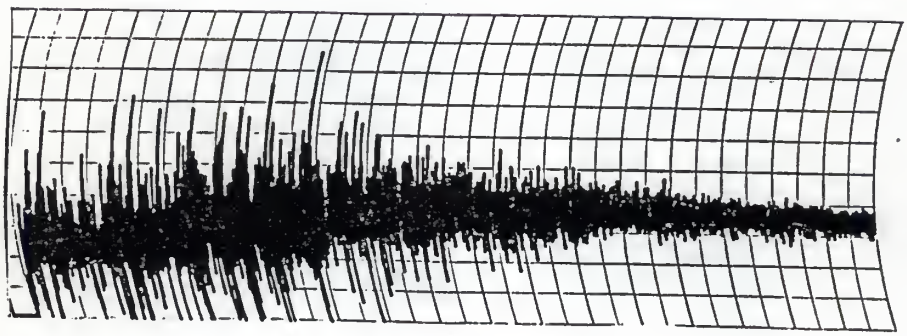


15 ppm KIO_3

Figure 18. Effect of Oxidation on the Mixing Characteristics of Commercial Gluten + Starch.



control



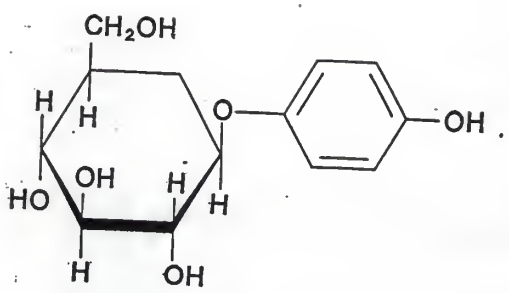
15 ppm KIO_3

Figure 19. Structure of

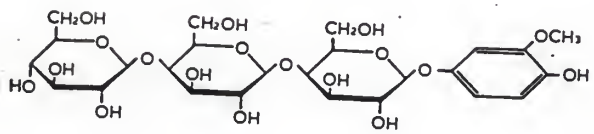
A) Arbutin

B) B-D-Glc-p-(1-4)-B-D-Glc-p-(1-4)-B-D-Glc-p-(1-1')-3-methoxyhydroquinone.

(Graveland et.al., 1984)


CH2OH
H
H
O
OH
HO
OH
H
H
H
OH

A


CH2OH
CH2OH
CH2OH
OCH3
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OH

B

phenolic mono-D-glucoside found in plants. However, addition of 200 ppm arbutin to a dough did not result in accelerated breakdown as would be expected if this was the rheologically active compound. Even in the presence of 15 ppm potassium iodate, no accelerated breakdown was noted beyond that caused by the oxidant itself. Thus, it appeared that the compound isolated and characterized by Graveland is not active in affecting dough breakdown or responsive to oxidants. This is not surprising since the site of glucosylation would prevent the compound's being oxidized to the ADB quinone form. However, if the compound were to exist unglycosylated, it could conceivably be active.

Graveland et. al. (1984) extracted the glycosylated hydroquinone from flour by mixing 25g of defatted flour in 600 ml. of water for 30 minutes under nitrogen. This procedure was followed in subsequent studies, but with non-defatted flour (referred to as Long,grv in Materials and Methods). After lyophilization and subsequent mixing in the mixograph, the dough did not develop. Instead it retained a pie dough-like consistency throughout four hours of mixing. Several other extraction procedures were performed and the extracted flours then studied in the mixograph to assess the effect of a fast acting oxidant (potassium iodate). When the same extraction procedure was performed only in an air atmosphere (Long,grv, no nitrogen) the dough did develop and breakdown (slightly). However, it showed no accelerated

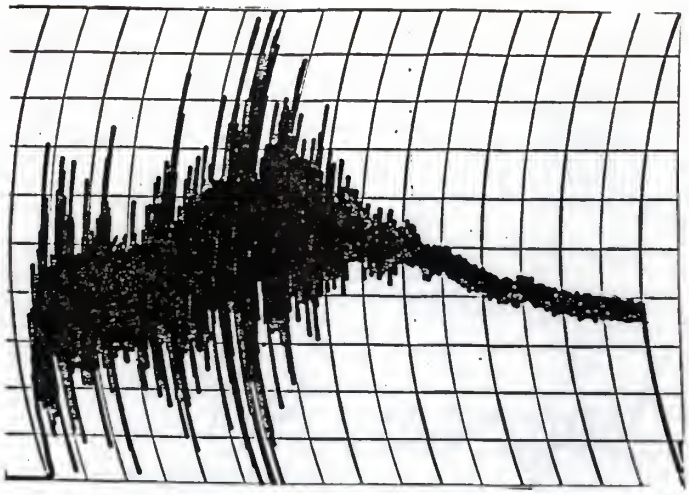
breakdown due to 15 ppm potassium iodate. All other extraction techniques (long, reg; short, grv; short, reg) clearly showed the accelerated breakdown characteristic of 15 ppm potassium iodate, suggesting that active compounds had not yet been completely removed.

To determine if a compound susceptible to oxidation was bound or extracted in the lipid fraction of the flour, defatted flour was treated with 15 ppm potassium iodate. Indeed, accelerated breakdown occurred with oxidation, indicating that the lipid fraction of the flour is not responsible for the oxidant's effect.

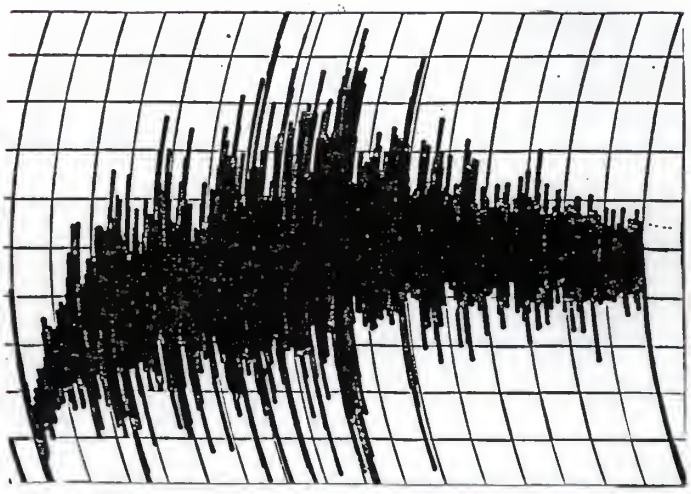
Hydroquinones have been found to be important in the bran and germ of the wheat (Vuataz, 1950). Although not yet reported to be present in flour, hydroquinones are quite easily oxidized and become highly reactive ADB compounds upon oxidation (Bungenberg de Jong et. al.,1953). When hydroquinone itself (not an ADB) is added to flour (100 ppm), greatly accelerated breakdown results. However, in a nitrogen atmosphere, the activity of the hydroquinone is greatly reduced, indicating that even air oxidation can create the reactive quinone (Figure 20).

Because hydroquinones are very soluble in ethyl acetate, defatted flour was extracted (1:3) with ethyl acetate under nitrogen, three times for 30 min. each. When this ethyl acetate extracted flour was tested in the mixograph, 15 ppm potassium iodate was found to accelerate

Figure 20. Effect of Mixing with Hydroquinone (100 ppm) in Air and Nitrogen.



Air

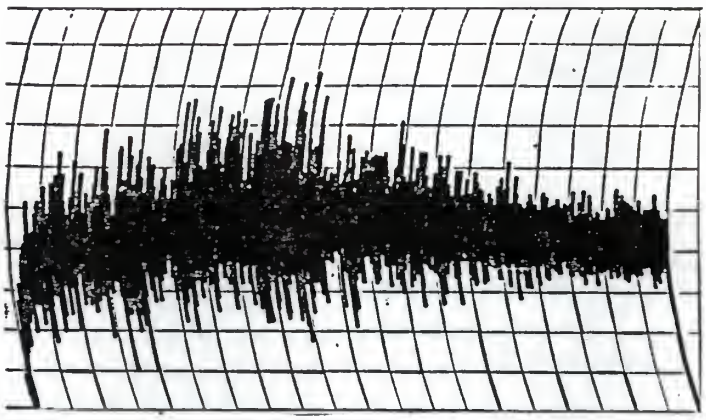


Nitrogen

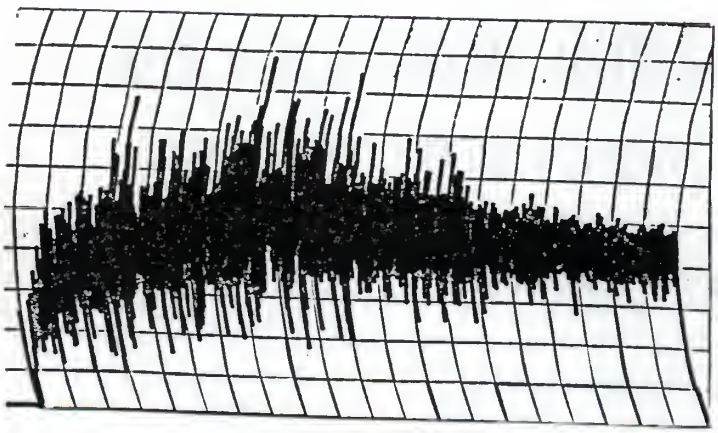
breakdown during overmixing. However, when the water soluble fraction was removed from the ethyl acetate extracted flour, 15 ppm potassium iodate did not accelerate breakdown during overmixing of the gluten/starch fraction (Figure 21). This is significant for it demonstrates that oxidation is not only affecting the flour's water soluble fraction, but also an ethyl acetate soluble compound present in the gluten/starch fraction. As shown previously, fast acting oxidants create accelerated breakdown in the gluten/starch fraction of the flour. Therefore, fast acting oxidants do not only affect the water soluble fraction, but also affect the gluten/starch fraction of the flour. If the ethyl acetate soluble fraction of the flour (solvent removed by rotary evaporation) is reincorporated into the gluten/starch fraction, fast acting oxidants once again cause accelerated breakdown.

Thin layer chromatography of the ethyl acetate extract was performed to determine if hydroquinone or methoxyhydroquinone were present. The spray reagent phosphomolybdic acid detected several phenolic compounds. However, neither hydroquinone nor methoxyhydroquinone were found in the extract when studied with two solvent systems (Figures 22, 23). Detection with sulfuric acid gave identical results. Therefore, all compounds that are present are also detectable with phosphomolybdic acid, indicating that they are easily oxidizable.

Figure 21. Effect of Oxidation on Ethyl Acetate Extracted Gluten/Starch.



control



15 ppm KIO_3

Figure 22. TLC of Ethyl Acetate Extract.

Solvent: Benzene:Methanol:Acetic Acid (90:16:8)
Plate: Silica Gel 60F-254, 250 thick, 20x20 cm
(EM Reagents, Cincinnati, OH)
Detection: Phosphomolybdic Acid (Alltech
Associates, Deerfield, IL)
Order of spots (left to right):

1) Ferulic Acid
2) Methoxyhydroquinone
3) Flour Ethyl Acetate Extract
4) Hydroquinone

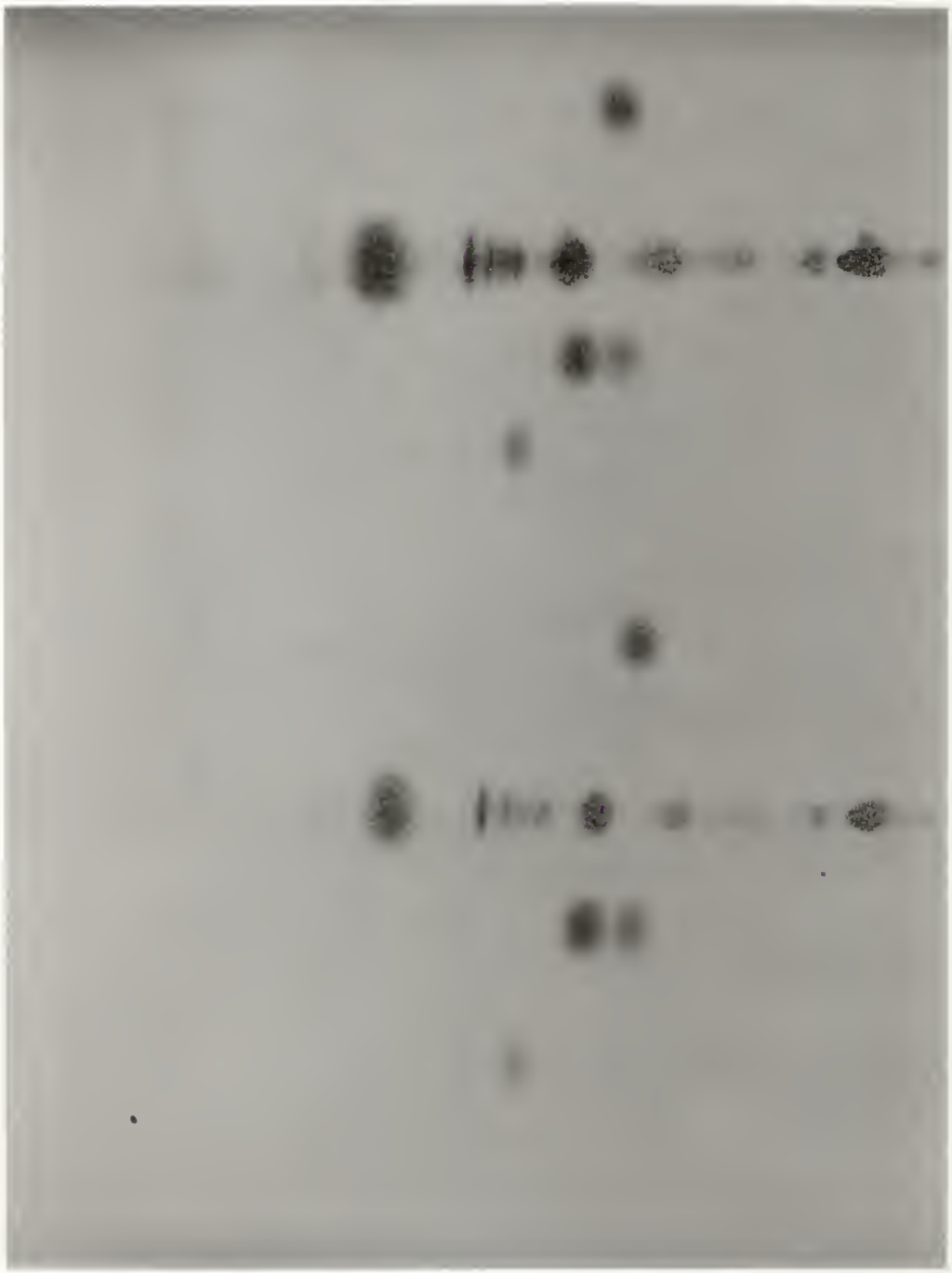


Figure 23. TLC of Ethyl Acetate Extract

Solvent: Benzene:Methanol:Acetic Acid (94:4:2)
Plate: Silica Gel 60F-254, 250 thick, 20x20 cm
(EM Reagents, Cincinnati, OH)
Detection: Phosphomolybdic Acid (Alltech
Associates, Deerfield, IL)
Order of spots (left to right):

1) Ferulic Acid
2) Methoxyhydroquinone
3) Flour Ethyl Acetate Extract
4) Hydroquinone

Multiple Factor Studies: Charge. Charge is the final factor in the three dimensional interaction of factors affecting dough breakdown during overmixing. The effect of charge on overmixing has been well documented (Danno and Hoseney, 1982; Hoseney and Brown, 1983). The focus of the studies described below is the way these three factors; charge, oxidation, and ADB compounds interacted to affect a dough. Therefore, studies of several factors at different pH's were conducted. The effect of each factor or combination on dough strength was quantitated by measuring the tail width of the mixogram 3 min. after the peak. This measurement proved to give the best reflection of the trends in dough breakdown. Standard deviations were $\pm$ 1mm for tail widths < 30mm, and $\pm$ 2mm for tail widths > 30mm.

The control curve of flour stability as a function of pH provides a great deal of information. Roughly sigmoidal in shape, it illustrates both the known strengthening effect of high pH and the weakening effect of acid. Also noteworthy is the fact that the curve has a region in which stability is relatively unaffected by changes in pH. This level or flat phase of the curve corresponds roughly to the range of normally encountered flour or flour/water pH.

The control curve also suggests the ways in which a flour's stability might be altered. A change in the curve's vertical or horizontal position represents a change in the dough's tolerance to overmixing. In addition, a change in

the slope of the curve, as well as a change in the length of the level region of the curve, reflect upon a flour's stability.

Free radical scavengers were studied by inclusion of Tenox (2%) to flours of various pHs. When compared to the control, the Tenox-treated dough was stronger (greater tail width) at any given pH. This was reflected by a vertical shift in the curve (Figure 24). Tenox is thought to disperse gluten thiyl radicals so that ADB compounds can no longer react with them. With a gluten/starch dough, on the other hand, the ADB compounds have been removed from the system. Yet, the response mimics the response of the dough with added Tenox. This indicates that the same effect has been achieved through different mechanisms.

Oxidation was also studied over a range of flour pHs. Mixing in nitrogen (lack of oxidation) shifted the doughs response to pH (Figure 25). Mixing in nitrogen also eliminated the level region of the response curve and made the curve linear (over the pH's tested), making the dough stronger. It appears that this effect is most prominant at pH 6 and above. The presence of iodate (15 ppm) resulted in a different response. Specifically, it shifted the curve down while maintaining the relatively level region so that the dough was weaker (smaller tail width) at any given pH. The effect was most pronounced at those pHs corresponding to the flat region of the curve.

Figure 24. Effect of pH on the Gluten/Starch Fraction and on Doughs Treated with Free Radical Scavengers.

a = flour/water
b = gluten/starch
c = flour/water/2% Tenox

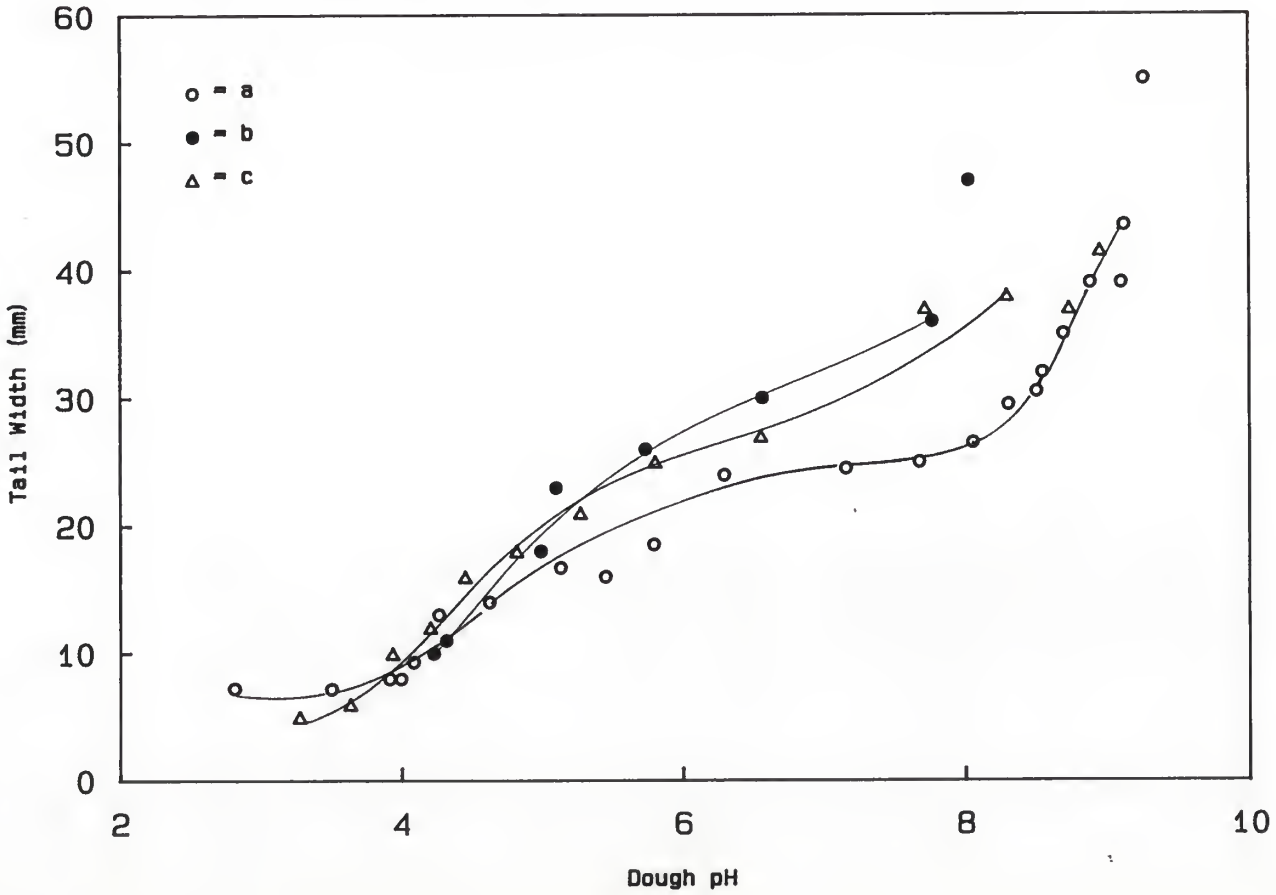
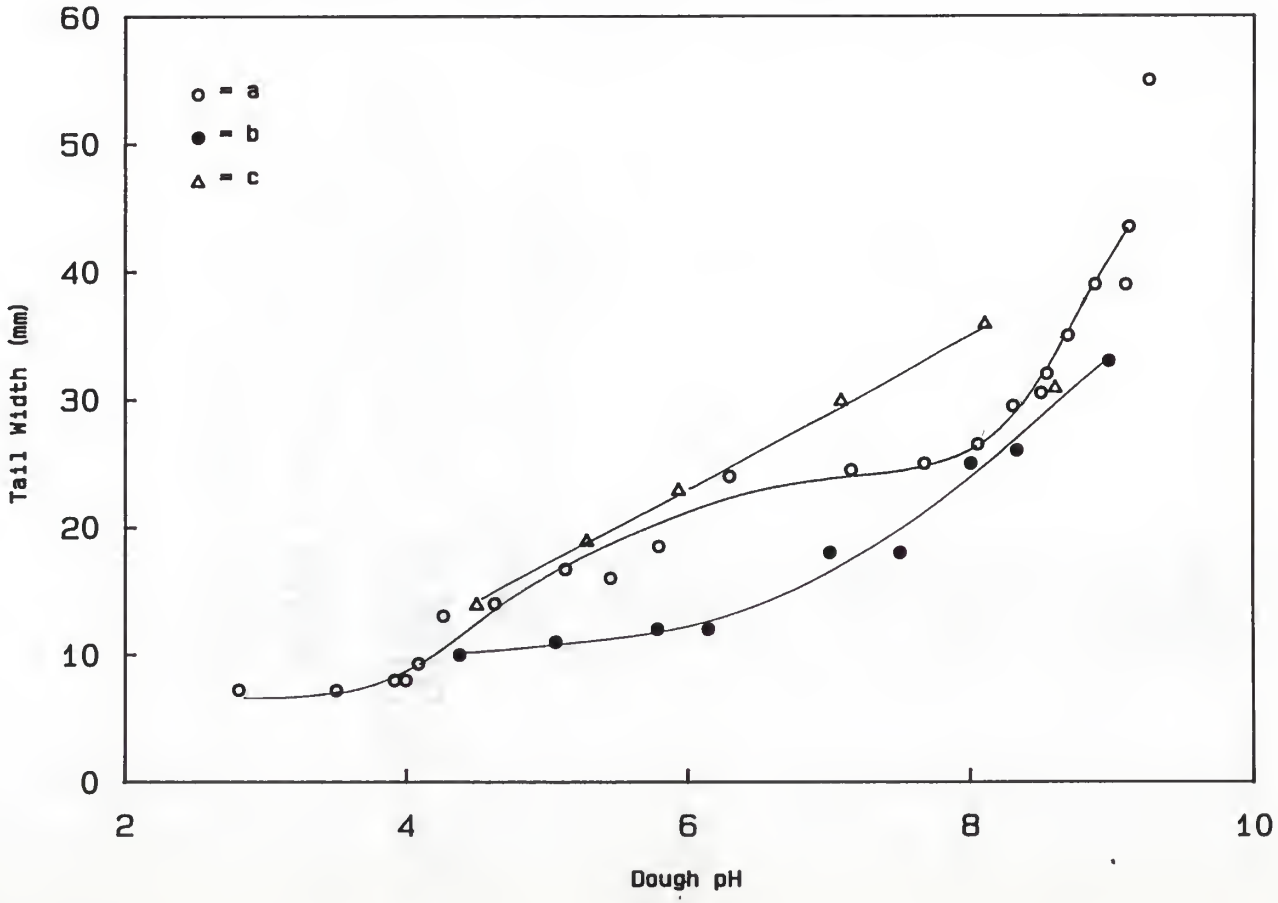

60
50
40
30
20
10
0
2
4
6
8
10
Tail Width (mm)
Dough pH
○ = a
● = b
△ = c

Figure 25. Effect of pH on Response to Oxidation.

a = flour/water
b = 15 ppm potassium iodate
c = mixed in nitrogen


60
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0
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4
6
8
10
Tail Width (mm)
Dough pH
○ = a
● = b
△ = c

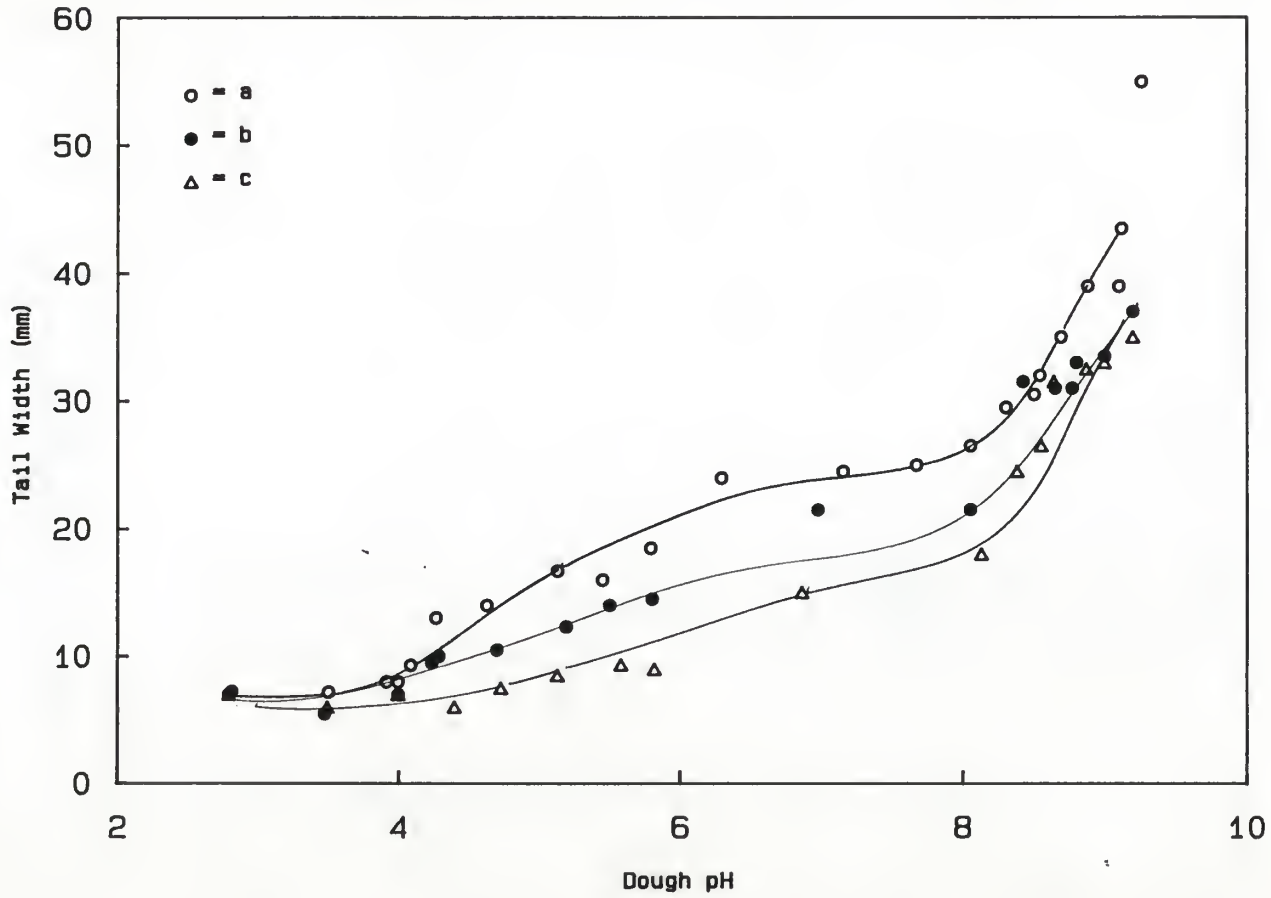
When ADB compounds (100 ppm ferulic acid) were added to a dough, the curve retained a shape similar to the control, but was shifted both down and to the right (Figure 26). Oxidation plus ADB compounds shifted the curve still further down, but little to left or right. Although ADB compounds and oxidants appeared to have an additive effect in shifting the flours response to pH, oxidants appear to shift the curves primarily up or down, while ADB compounds shift the curves both vertically and horizontally.

As pH was reduced below pH 6, differences in tail width were reduced. Below pH 4, no difference was apparent between treatments. This suggests that at high hydrogen ion concentration, charge is the primary factor affecting dough stability. Again, effects of oxidants and ADB compounds were most apparent in the flat or level region of the curve.

After observing the above phenomena, flours of different known strengths were tested. We found that flours of different strengths have shifted responses to pH (Figure 27). Indeed, a strong HRW flour is stronger at any given pH than the control HRW, as indicated by a curve that has been shifted up and to the left. In addition, the level region of the curve is no longer evident. On the other hand, a weak HRW produced a curve that was shifted down, giving a smaller tail width (less tolerance to overmixing) at any given pH. The level region of the curve has been greatly extended, resulting in little response to any change in pH.

Figure 26. Effect of pH on Ferulic Acid and Potassium Iodate Treated Doughs.

a = flour/water
b = 100 ppm ferulic acid
c = 100 ppm ferulic acid + 15 ppm potassium iodate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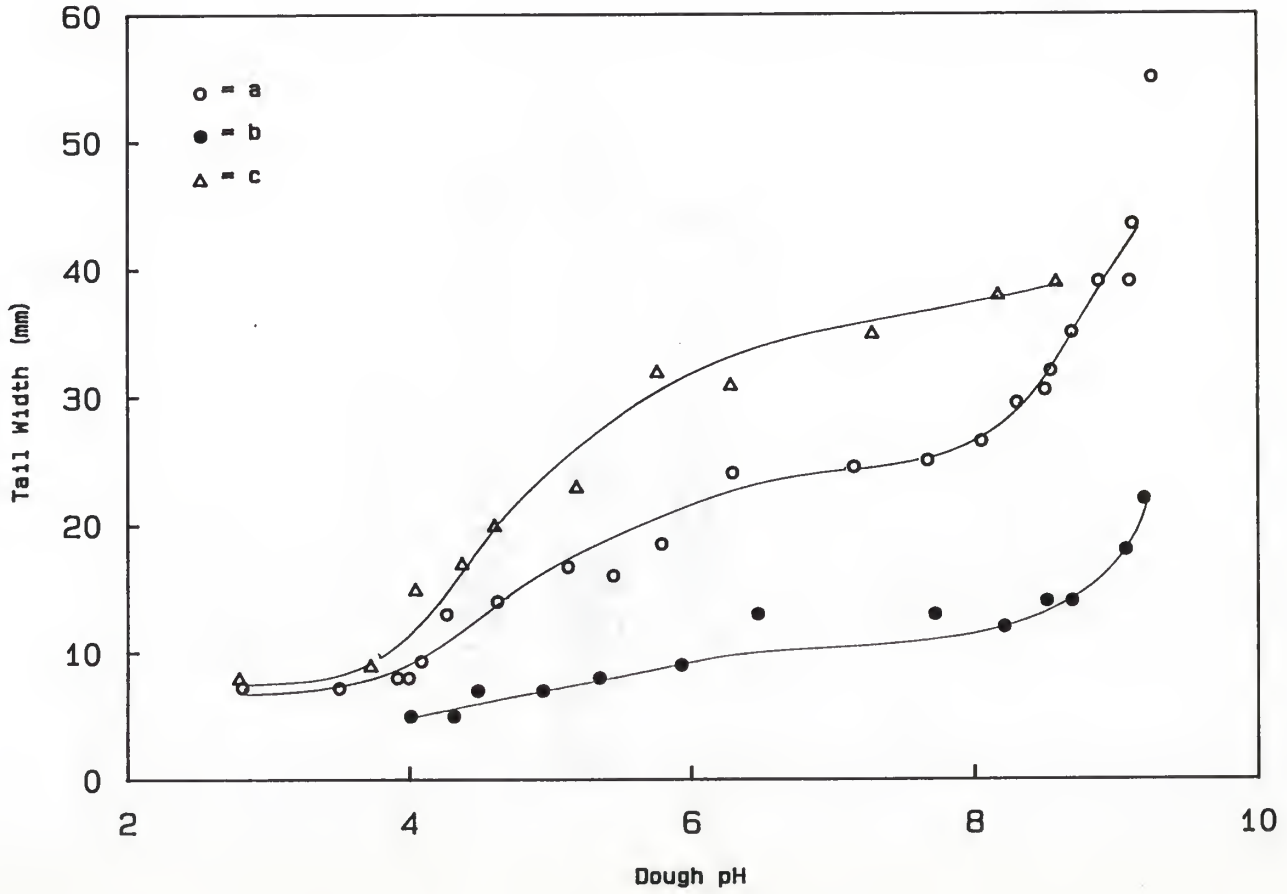
2
4
6
8
10
Tail Width (mm)
Dough pH
o = a
● = b
△ = c

Figure 27. Effect of pH on Flours of Different Strengths.

a = Ross Mills (standard)
b = Siouxland (weak)
c = Eastern High Gluten (strong)


60
50
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20
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0
2
4
6
8
10
o = a
● = b
△ = c
Tail Width (mm)
Dough pH

The different responses of strong and weak flours to changes in pH suggest different protein compositions. Strong flours exhibit greater tolerance to dough breakdown in the presence of positive charge (low pH) than do weak flours. If charge repulsion indeed contributes to dough breakdown, this suggests that proteins of strong flours may have less potential positive charge (in other words, less positively charged amino acids) than do proteins of weak flours.

CONCLUSIONS

This study observed two different aspects of dough behavior. The spread test studied rheological changes in dough after optimal mixing during fermentation, while the mixograph was used to study factors affecting dough overmixing.

Lipoxygenase was known to affect dough rheology, and these studies found lipoxygenase to exert its maximum rheological effect only if present during the entire mixing period of the dough. The enzyme's effect decreased with decreasing reaction time during mixing. Indeed, when lipoxygenase was present only during the last minute of mixing, the doughs had a spread ratio similar to that of an untreated control. Therefore, lipoxygenase was assumed to react immediately during mixing. Attempts to limit or increase the rheological effects of lipoxygenase in dough through changes in oxygen concentration were unsuccessful. Therefore, whether or not oxygen is a limiting factor in lipoxygenase activity is still undetermined.

Evidence, however, supported the hypothesis that air oxidation is not occurring solely through its role in the lipoxygenase reaction. Subsequent studies combining oxidants and lipoxygenase demonstrated an additive effect in creating more viscous doughs. However, the effect of lipoxygenase was not evident in an ascorbic acid treated dough. It was hypothesized that ascorbic acid is acting as

a free radical scavenger and dispersing the lipid radical created by lipoxygenase.

The spread test showed that yeast exerts an effect on dough rheology over time. It was determined that yeast requires the water soluble fraction of the flour to exert this effect. The spread ratio of a cysteine treated dough was altered during fermentation to a spread ratio similar to that of a control dough. Thus, yeast's action appears to be through its reaction with low molecular weight thiol compounds in doughs. The finding that a free radical scavenger reverses the effect of the yeast indicated a free radical mechanism may be involved in the mechanism of the yeast's action.

Because the water soluble fraction was found to be important for yeast's ability to affect dough rheology, a fractionation experiment in which water solubles were fermented separately from the gluten/starch fraction of the flour was used to clarify the effect of yeast. Results indicated that yeast contains a reducing entity, since doughs containing yeast had higher spread ratios at zero time than their controls. However, during fermentation, yeast appears to exert its effect by reacting with cysteine or related thiol compounds to remove or modify them. The result of this reaction is a dough with reduced viscous flow properties. Therefore, although both yeast and lipoxygenase create a more elastic dough system, they appear to operate

through different mechanisms. Lipoxygenase reacts immediately, while yeast creates a more viscous flow system over time, apparently by removing or modifying low molecular weight thiols present in the water soluble fraction of the flour.

Dough breakdown (over mixing) was also studied with the mixograph. Three seemingly unrelated factors have been reported to affect dough breakdown. However, their mechanism of action is not always clear. A three component model involving all these factors (ADB compounds, charge, and chemical oxidants) was developed to gain a better understanding of the interactions of these factors. Factors that affect dough breakdown were found to shift the response of the dough to pH. The response curve of tail width vs. pH could be shifted either horizontally or vertically, translating into a change in dough breakdown. Changes in dough stability to overmixing (as measured by tail width of the mixogram) were most affected by ADB compounds and/or oxidation in a level region of the curve that corresponded to the region of native flour pH. In addition, the sigmoidal curve of the control could be flattened and/or straightened by ADB compounds and oxidation.

Studies showed that while changes in concentrations of ADB compounds could shift curves both vertically and horizontically, factors affecting oxidation resulted in a vertical shift of the curve. Interestingly, mixing in the

absence of oxygen (N_2 atmosphere) resulted in a linear response of tail width to pH, completely eliminating the sigmoidal shape over the pHs tested.

Regardless of how the dough was treated, charge appears to be the determining factor in controlling dough breakdown. The effect of the bulky ADB compounds and pH suggest that charge repulsion and stearic hindrance are major contributors to dough breakdown.

Further studies of oxidation alone were conducted with the mixograph. Although oxidation is thought to affect the water soluble fraction of the flour, oxidation was found to accelerate breakdown in a gluten/starch dough. The same phenomenon was evident in commercial gluten/starch, eliminating the possibility that oxidation was freeing ADB compounds from the insoluble pentosan fraction. Apparently, either oxidation was directly affecting the gluten/starch fraction, or it was affecting a compound bound in the gluten/starch fraction. Because gluten/starch from defatted flour was susceptible to accelerated breakdown when treated with a fast acting oxidant, the possibility of a compound bound in the lipid fraction of the flour was eliminated. However, the gluten/starch fraction of a flour previously extracted with ethyl acetate showed no accelerated breakdown in response to added oxidation. Although not yet confirmed in flour, hydroquinones, which are a very reactive component of whole wheat flour, are hypothesized to be the compounds

responsible for the effect of fast acting chemical oxidation. Most hydroquinones derivatives are easily oxidized to quinones, a very reactive ADB compound. This was illustrated by the greatly accelerated breakdown that resulted when hydroquinone was added to a flour. By mixing in nitrogen, and therefore preventing oxidation, the activity of the hydroquinone was greatly reduced, indicating that air oxidation is enough to produce the reactive ADB compound.

LITERATURE CITED

BLOKSMA, A. H. 1963. Oxidation by Molecular Oxygen of Thiol Groups in Unleavened Doughs From Normal and Defatted Wheat Flours. J. Sci. Fd Agric. 14:529.

BLOKSMA, A.H. 1975. Thiol and Disulfide Groups in Dough Rheology. Cereal Chem. 52:170r.

BUNGENBERG DE JONG, H. L., KLAAR, W. J., VLIEGENTHART, J. A. 1953. A Precursor of Methoxy-para-benzoquinone in Wheat Germ. Nature 172:402.

COSGROVE, D. J., DANIELS, D. G. H., WHITEHEAD, J. K., and GOULDEN, J. D. S. 1952. Fermentation Products of Wheat Germ. (a) Identification of Methoxy- and 2:6-Dimethoxy-p-benzoquinone. (b) Infra-red Absorption of Some Quinones and 1:2-Dicarbonyl Compounds. J. Chem. Soc. p. 4821.

DANNO, G. and HOSENEY, R. C. 1982. Effects of Dough Mixing and Rheologically Active Compounds on Relative Viscosity of Wheat Proteins. Cereal Chem. 59:196.

DANNO, G. and HOSENEY, R. C. 1982. Effect of Sodium Chloride and Sodium Dodecyl Sulfate on Mixograph Properties. Cereal Chem. 59:202.

DANNO, G. and HOSENEY, R. C. 1982. Changes in Flour Proteins During Dough Mixing. Cereal Chem. 59:249.

DRONZEK, B. and BUSHUK, W. 1968. A Note on the Formation of Free Radicals in Dough during Mixing. Cereal Chem. 45:286.

FAUBION, J. M. and HOSENEY, R. C. 1981. Lipoxygenase: Its Biochemistry and Role in Breadmaking. Cereal Chem. 58:175.

FAUSCH, H., KUNDIG, W., and NEUKOM, H. 1963. Ferulic Acid as a Component of a Glycoprotein from Wheat Flour. Nature 199:287.

FINNEY, K. F. 1984. An Optimized, Straight-Dough, Bread-Making Method After 44 Years. Cereal Chem. 61:20.

GALLIARD, T. 1986. Oxygen Consumption of Aqueous Suspensions of Wheat Wholemeal, Bran and Germ: Involvement of Lipase and Lipoxygenase. J. Cereal Sci. 4:33.

GRAVELAND, A., BOSVELD, P., LICHTENDONK, W. J., and MOONEN, J. H. E. 1984. Isolation and Characterisation of (3-Methoxy-4-hydroxyphenyl)-B-Cellotrioside from Wheat Flour; a Substance Involved in the Reduction of Disulphide-Linked Glutenin Aggregates. J. Cereal Sci. 2:65.

HOSENEY, R. C. and BROWN, R. A. 1983. Mixograph Studies. V. Effect of pH. Cereal Chem. 60:124.

HOSENEY, R. C., HSU, K. H., and JUNGE, R. C. 1979. A Simple Spread Test to Measure the Rheological Properties of Fermenting Dough. Cereal Chem. 56:141.

HOSENEY, R. C., RAO, H., FAUBION, J. and SIDHU, J. S. 1980. Mixograph Studies. IV. The Mechanism by Which Lipoxygenase Increases Mixing Tolerance. Cereal Chem. 57:163.

JACKSON, G. M., and HOSENEY, R. C. 1986. Effect of Endogenous Phenolic Acids on the Mixing Properties of Wheat Flour Doughs. J. Cereal Sci. 4:79.

JACKSON, G. M., and HOSENEY, R. C. 1986. Fate of Ferulic Acid in Overmixed Wheat Flour Doughs: Partial Characterization of a Cysteine-Ferulic Acid Adduct. J. Cereal Sci. 4:87.

KOCH, R. B. 1956. Mechanisms of Fat Oxidation. Bakers Dig. 30:48.

KUNINORI, T., NISHIYAMA, J., and MATSUMOTO, H. 1976. Effect of Mushroom Extract on the Physical Properties of Dough. Cereal Chem. 53:420.

MACRITCHIE, F. 1975. Mechanical Degradation of Gluten Proteins during High Speed Mixing of Doughs. J. Polymer Sci. Polymer Sci. Symp. 49:85.

MAURITZEN, C. M. 1967. The Incorporation of Cysteine-^{35}S, Cystine-^{35}S, and N-Ethylmaleimide-^{14}C into Doughs Made from Wheat Flour. Cereal Chem. 44:170.

MAURITZEN, C. M., and STEWART, P. R. 1966. The Disulphide and Sulphydryl Content of Fractions Prepared by the Ultracentrifugation of Doughs Made from Wheat Flour. Aust. J. Biol. Sci. 19:1111.

MEREDITH, P. and BUSHUK, W. 1962. The Effects of Iodate, N-Ethylmaleimide, and Oxygen on the Mixing Tolerance of Doughs. Cereal Chem. 39:411.

SCHROEDER, L. F., and HOSENEY, R. C. 1978. Mixograph Studies. II. Effect of Activated Double-Bond Compounds on Dough-Mixing Properties. Cereal Chem. 55:348.

SIDHU, J. S., HOSENEY, R. C., FAUBION, J., and NORDIN, P. 1980. Reaction of ^{14}C-Cysteine with Wheat Flour Water Solubles Under Ultraviolet Light. Cereal Chem. 57:380.

SIDHU, J. S., NORDIN, P., and HOSENEY, R. C. 1980. Mixograph Studies. III. Reaction of Fumaric Acid with Gluten Proteins During Dough Mixing. Cereal Chem. 57:159.

SOSULSKI, F., KRYGIER, K., and HOGGE, L. 1982. Free, Esterified, and Insoluble-Bound Phenolic Acids. 3. Composition of Phenolic Acids in Cereal and Potato Flours. J. Agric. Food Chem. 30:337.

TSEN, C. C. 1969. Effect of Oxidizing and Reducing Agents on Changes of Flour Proteins during Dough Mixing. Cereal Chem. 46:435.

TSEN, C. C. and ANDERSON, J. A. 1963. Determination of Sulfhydryl and Disulfide Groups in Flour and Their Relation to Wheat Quality. Cereal Chem. 40:314.

VUATAZ, L. 1950. Isolation and Study of a Substance Appearing during Wheat Germ Fermentation, and Capable of Being Condensed with Glutathion. Helv. Chim. Acta 33:433.

WALLACE, J. M., and WHEELER, E. L. 1975. Lipoxygenase from Wheat. An Examination of its Reaction Characteristics. J. Agric. food Chem. 23:146.

YEH, Y. F., HOSENEY, R. C., and LINEBACK, D. R. 1980. Changes in Wheat Flour Pentosans as a Result of Dough Mixing and Oxidation. Cereal Chem. 57:144.

STUDIES OF DOUGH
DURING FERMENTATION
AND OVERMIXING

by

Christine L. Kivett

B.S., Kansas State University, 1985

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1987

The chemical bases for two important facets of dough behavior were investigated. The spread test was used to study the rheology of fermenting dough and the Mixograph was used to study the phenomenon of overmixing.

Using the spread test, the enzyme lipoxygenase was found to exert its rheological effects immediately during mixing. It remained unclear whether or not oxygen is a limiting factor in lipoxygenase activity. It was clear that air oxidation does not occur solely through the lipoxygenase mechanism.

Oxidants (KIO_3 and $KBrO_3$) and lipoxygenase had an additive effect in making a dough more elastic. However, lipoxygenase had no effect in an ascorbic acid treated dough. It was hypothesized that ascorbic acid acted as a free radical scavenger and dispersed the lipid radical created by lipoxygenase.

The spread test demonstrated that yeast has its rheological effect over time. To exert this rheological effect, yeast requires the water soluble portion of the flour. Yeast initially appeared to act as a reducing entity. However, its rheological activity appears to be based on its ability to modify low molecular weight thiol compounds during fermentation. The fact that a free radical scavenger reverses the rheological effect of yeast in a dough indicates that a free radical mechanism may be involved.

The interaction of three factors (activated double bond

compounds, oxidation, and charge) known to affect dough breakdown was studied using the mixograph. The width of the mixogram tail 3 min. after peak indicated dough tolerance to overmixing. pH was found to be the overriding factor in promoting or retarding dough breakdown. When oxidants and ADB compounds were studied over a range of pHs, the sigmoidally shaped response curve could be shifted either horizontally or vertically, as well as flattened or straightened. Both of these factors were most effective in a level region of the curve that occurred in the area of the native flour pH. The combined effects of pH and bulkly ADB compounds suggested that charge and stearic repulsion were primary contributing factors to dough breakdown.

Further studies of the effects of oxidation alone revealed that oxidation affects not only the water soluble portion of the flour, but also the gluten/starch fraction. While gluten/starch from defatted flour still demonstrated accelerated breakdown in response to fast acting oxidants (eliminating the lipid fraction), the gluten/starch fraction of ethyl acetate extracted flour does not show accelerated breakdown in response to a fast acting oxidant. Hydroquinone derivatives are removed by ethyl actate and are suspected as compounds responsible for the effect of fast acting oxidants.