Mixograph Studies. IV. The Mechanism by Which Lipoxygenase Increases Mixing Tolerance[1]

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ABSTRACT

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Soy flour lipoxygenase increased mixing tolerance and improved rheological properties of wheat flour dough. Lipoxygenase overcame the effects that potassium iodate had on mixing tolerance. Lipoxygenase had no effect when those compounds were added to defatted flour, showing that free lipids are required for lipoxygenase action. Adding linoleic acid to defatted flour restored the effect of lipoxygenase. Oxygen was not required for lipoxygenase to increase mixing tolerance or to overcome the deletrious effects of fast-acting oxidants or activated double-bond compounds, but it was required for lipoxygenase to improve the rheological properties of dough. The data suggest that lipoxygenase affects mixing tolerance by creating a free radical on certain lipids that competes for activated double-bond compounds indigenous in flour or created by fast-acting oxidants. Lipoxygenase interferes with radioactive fumaric acid's binding with the gluten proteins during dough mixing.

The effect of soy lipoxygenase on wheat flour doughs is clearly multifunctional. Haas and Bohn (1934) patented the use of enzyme-active soy flour as a bleaching agent, and enzyme active soy is used extensively in the commercial production of white bread (Matz 1972, Ponte 1971). The reaction involved in the bleaching action is a coupled oxidation of pigments and unsaturated fatty acids by atmospheric oxygen (Barrett 1975).

Besides its bleaching action, lipoxygenase increases the mixing tolerance of dough (Matz 1972, Barrett 1975). Koch (1956) found that added lipoxygenase did not affect the mixing properties of defatted flour. He also reported that adding back to flour the lipids extracted from lipoxygenase-treated flour reduced mixing tolerance. A loss of flour sulfhydryl (–SH) groups when lipoxygenase is added has been used as evidence that the improvement caused by enzyme-active soy flour is due to oxidation of sulfhydryl groups (Matz 1972, Koch 1956, Tsen and Hlynka 1963).

Frazier et al (1977), studying the improving action of soy lipoxygenase on the rheology of wheat flour dough, showed that dough relaxation times increased, consistent with oxidative improvement of gluten proteins. When lipoxygenase was inactivated, either by heat or by mixing under nitrogen, improving effects were absent. They also reported that the antioxidant nordihydro-guaretic acid greatly inhibited peroxide formation but only marginally impaired rheological effects. Thus, the effect of peroxide on the improving effects is questionable.

The mechanism by which lipoxygenase increases mixing tolerance has not been reported. This study was designed to study that mechanism.

MATERIALS AND METHODS

A composite hard winter wheat flour with a protein content of 12.2% and an ash content of 0.39% (14% moisture basis) and a commercial sample of enzymatically active, defatted soybean flour (200E, Far-Mar-Co., Inc., Hutchinson, KS) were used. Defatted wheat flour was produced by extracting the flour with petroleum ether (38–55°C) in a large Soxhlet apparatus for 24 hr. 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 as a percentage of the flour. The linoleic acid (Matheson, Coleman, and Bell) was technical grade. All other chemicals were reagent grade.

Radioactive fumaric acid (1-^{14}C), 3.03 mCi/mM, was obtained from ICN Pharmaceuticals Inc. and radioactive linoleic acid ^{14}C (universal), 854 Ci/mM, from New England Nuclear. Radioactivity was measured by placing a 1-ml sample in a scintillation vial, mixing with 5 ml of toluene/triton X-100 (2:1) containing 0.4% (w/v) of 2,5-diphenyloxazole, and counting in a Beckman LS-200
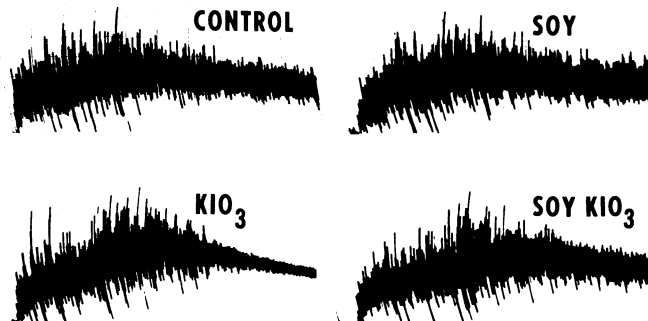

Fig. 1. Mixograms showing the effects of enzyme-active soy flour (soy), potassium iodate, and their combination on mixing properties.

B scintillation spectrophotometer (Turner 1968). Mixed doughs were lyophilized and fractionated as reported previously (Sidhu et al 1980).

The 10-g mixograph was used to mix dough by the procedure of Finney and Shogren (1972). To produce a nitrogen atmosphere, we placed the mixograph in a glove bag. The bag was flushed with a large excess of nitrogen. The flour was evacuated to remove air. Dough rheology was measured by the spread test (Hoseney et al 1979).

RESULTS AND DISCUSSION

Adding small quantities of soy flour lipoxygenase to wheat flour greatly improved its mixing stability, as shown in the mixograph, (Fig. 1). Earlier work (Schroeder and Hoseney 1978) had shown that lipoxygenase overcomes the deleterious effects on dough stability of such activated double-bond compounds as fumaric acid. Fast-acting oxidants, like potassium iodate, also decrease mixing stability (Weak et al 1977). Adding lipoxygenase to wheat flour containing KIO_3 overcame the oxidant's deleterious effect on mixing stability (Fig. 1).

When lipoxygenase and KIO_3 were both added to defatted flour (Fig.

2), lipoxygenase did not overcome the deleterious effect of KIO_3. This supported previous work (Schroeder and Hoseney 1978) showing that free lipids (extractable from wheat flour with petroleum ether) were necessary for lipoxygenase to be effective. Adding linoleic acid and lipoxygenase to defatted flour increased mixing stability (Fig. 2) —evidence that lipoxygenase, and not other enzymes in the enzyme-active soy flour, increased mixing stability.

Frazier et al (1977) showed that oxygen was required for lipoxygenase to be effective, and Baker and Mize (1937) reported that mixing doughs in a nitrogen atmosphere increased mixing stability. Therefore, we determined mixograms in a nitrogen atmosphere. The curves for flour and flour plus lipoxygenase (Fig. 3) are similar, and one could easily conclude that lipoxygenase has no effect in a nitrogen atmosphere. However, adding KIO_3 or fumaric acid to flour and mixing in nitrogen still gave a mixing curve with decreased mixing stability. Surprisingly, lipoxygenase overcame the effect of both agents even when the dough was mixed in a nitrogen atmosphere (Fig. 3). Thus, oxygen is not required for lipoxygenase to increase dough stability.

Careful examination of the data of Frazier et al (1977) shows the major effect reported is on the rheology of the dough, not on mixing stability. Dough rheology, measured by the spread test (Hoseney et al 1979), was determined for dough with and without added lipoxygenase, mixed in air and in nitrogen (Table I). Doughs mixed in air gave a slightly lower spread ratio than those mixed in nitrogen. Adding lipoxygenase decreased the spread ratio of

TABLE I
Effect of Soy on the Rheology of Doughs Mixed in Air and in Nitrogen

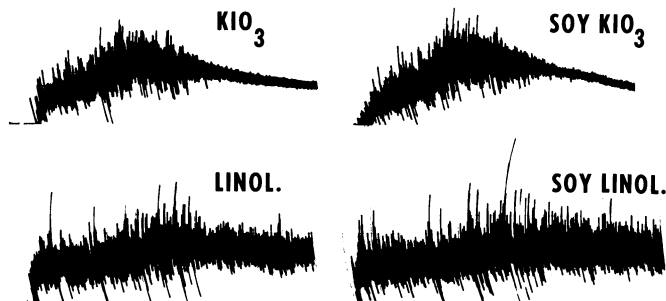| Treatment	Spread Ratio[a]
Flour mixed in air	2.8
Flour mixed in nitrogen	3.2
Flour + 1% soy mixed in air	1.7
Flour + 1% soy mixed in nitrogen	3.0

[a]Width/height, zero fermentation time.

Fig. 2. Mixograms showing the effects of potassium iodate, enzyme-active soy flour plus potassium iodate (soy KIO_3), linoleic acid (linol.), and of soy plus linoleic acid on the mixing stability of defatted flour.
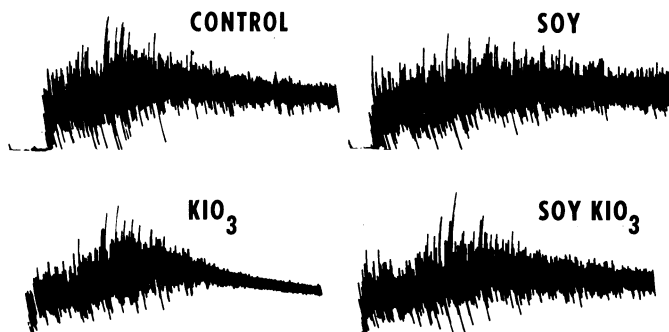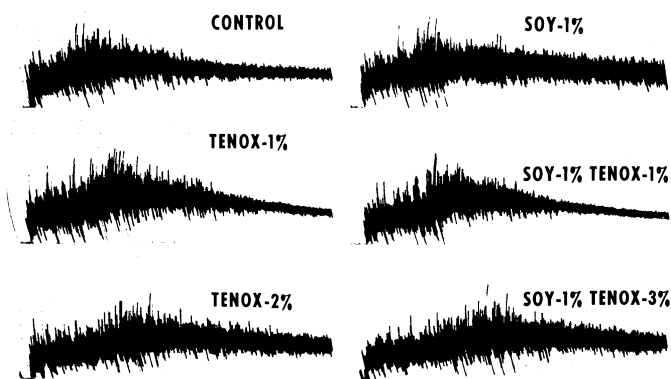

Fig. 3. Mixograms showing the effects of enzyme-active soy flour (soy), potassium iodate, and their combination on the mixing properties of flour mixed under nitrogen.

Fig. 4. Mixograms showing the effects of enzyme-active soy flour (soy), Tenox (20% butylated hydroxyanisole, 20% butylated hydroxytoluene, 60% corn oil), and their combination on mixing properties.
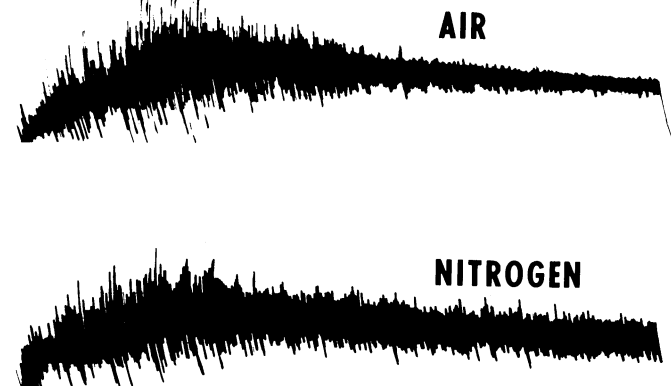

Fig. 5. Mixogram showing the effects of Tenox (20% butylated hydroxyanisole, 20% butylated hydroxytoluene, 60% corn oil) on mixing properties of flour mixed in air and in nitrogen.
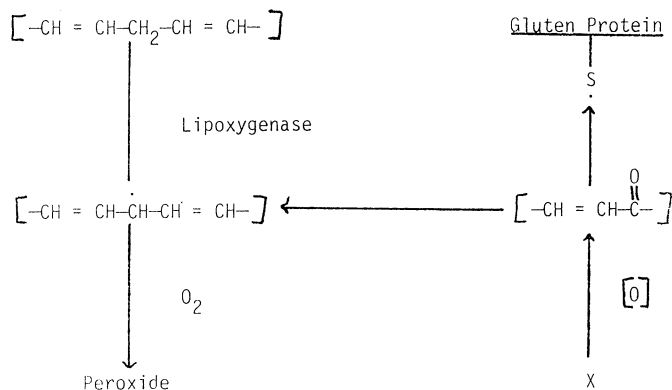
Fig. 6. Diagrams of envisioned reactions of activated double-bond compounds and lipoxygenase products during dough mixing.

doughs mixed in nitrogen only slightly but greatly decreased the spread ratio of dough mixed in air. Thus, oxygen is required for lipoxygenase to have a major rheologic effect. These findings agree with those of Frazier et al (1977).

Free radical scavengers overcome the deleterious effects that activated double-bond compounds have on mixing stability (Schroeder and Hoseney 1978). The effect on dough stability of 2% Tenox, a commercial preparation of 20% BHA and 20% BHT in corn oil, is strikingly similar to that of lipoxygenase (Fig. 4). At lower levels (1% or less) Tenox gives greater mixing stability for a short period of time, but with extended mixing, the mixogram breaks down to give a narrow curve. That data suggested that BHA or BHT was being oxidized by oxygen during the extended mixing and was forming a quinone (an activated double-bond compound) that decreased mixing stability. Mixing 0.5% Tenox with flour under a nitrogen atmosphere gave good mixing stability even under extended mixing (Fig. 5). Thus, BHA and BHT in their reduced forms act as free radical scavengers and give mixing stability. However, if they are oxidized, they decrease mixing stability. Although both lipoxygenase and Tenox increase mixing stability, certain combinations of the two give doughs lacking mixing stability (Fig. 4). An excess of either increases mixing stability.

An explanation of this phenomenon is that lipoxygenase mixed in air provides peroxides that oxidize BHA and BHT. When an excess of BHA or BHT is added, part of it remains in the reduced form and acts as a free radical scavenger, thus increasing mixing stability.

Recent work (Sidhu et al 1980) has shown that activated double-bond compounds, such as fumaric acid, add to a free radical created by the rupture of certain disulfide bonds during mixing. The grafting of the charged species to the protein is responsible for the rapid decrease in dough stability. Indigenous compounds in flour apparently also add to that radical and thus are responsible for the dough breakdown normally found with overmixing. Both Tenox and lipoxygenase either stop the reaction or compete for the reactive site and thus increase mixing stability.

The reactions envisioned are diagrammed in Fig. 6. In flour with no additives the indigenous activated double-bond compound adds to the thiyl radical and alters the surface of the protein, which decreases mixing stability (reaction 1). Adding fumaric acid or similar compounds gives more reactants and thus less mixing stability. Adding Tenox will allow BHA or BHT to disperse the free radical and thus maintain the natural protein surface, which gives good mixing stability. Adding lipoxygenase creates a free radical on certain of the lipids (reaction 2). That radical then can react with activated double-bond compounds and thus preserve mixing stability (reaction 3).

If the above is correct, then adding lipoxygenase should reduce the addition of activated double-bond compounds to the thiyl radical. That idea was investigated by mixing dough with added ^{14}C-fumaric acid both with and without added enzyme-active soy flour. The results (Table II) show that about half as much fumaric acid was incorporated into the dialyzed gluten protein isolated

from dough mixed with soy as was incorporated into the control dough containing no soy. The dialyzed gluten protein was extracted with chloroform/methanol (2:1) to remove lipids bound during dough mixing. A major part of the radioactivity was removed with the lipids (Table II). About 66% of the acitivity in the dialyzed gluten from the control and 75% of the acitivity in the dialyzed gluten from the soy-containing dough were associated with the lipids. However, about three times more activity was bound to the gluten protein from the control dough than to the gluten protein from the soy-containing dough. Thus, the enzyme-active soy flour interferes with the addition of activated double-bond compounds to the thiyl radical.

The fact that a large part (66%) of the radioactivity was found with the lipid fraction raises a question about the role of the lipids in rapid dough breakdown. However, both KIO_3 (Fig. 2) and fumaric acid (Schroeder and Hoseney 1978) caused a rapid breakdown with defatted flour, which indicates that the lipid role is minor.

The possibility that the radical produced on unsaturated lipids by lipoxygenase could add to the thiyl radical was also considered. ^{14}C-linoleic acid was mixed (both by hand and with the mixograph) with defatted flour both with and without soy flour (Table III). After the lipids bound during wetting and mixing were removed with chloroform/methanol (2:1), only insignificant radioactivity was bound to the gluten proteins. Thus, the unsaturated lipid radical does not add to the thiyl radical.

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