A Note Comparing Effects on Dough Mixing of Thioctic Acid (Lipoic Acid) and Yeast-Fermented Flour Extracts

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A recent publication from this laboratory reported the dough-weakening action of exogenous free thioctic acid in a yeast-fermented system, which was attributed to reduction of thioctic acid by yeast enzymes (1). Endogenous thioctic acid recovered from flour has always required vigorous hydrolysis prior to separation (2,3). This suggests a strong covalent linkage of thioctic acid to another moiety. It is probably similar to the amide linkage of thioctic acid to the protein which occurs in *Escherichia coli* (4). It is not likely that such an intact protein could pass through a yeast cell wall and thus become accessible to the yeast enzymes.

Several flours were examined for the presence of endogenous free thioctic acid. In addition, aqueous extracts of these flours were incubated in yeast-sugar suspensions and then mixed with intact patent flour on the farinograph. For comparison, thioctic acid was added to an extract prior to incubation. The evidence indicates that endogenous thioctic acid is present in a form not accessible to the yeast enzymes. Thus, it is not able to exert important dough-weakening effects.

The enzymes in yeast capable of reducing thioctic acid also are capable of nonspecific reduction of many disulfides such as oxidized glutathione, cystine, and insulin (5). The addition of oxidized glutathione to a flour extract prior to incubation gave a dough-weakening effect on the farinograph similar to reduced glutathione. Kuninori *et al.* (6,7) found glutathione in flour, but in amounts too small to be observable by the farinograph procedure used here. The absence of dough-weakening effects from yeast-fermented flour extracts in farinograph mixing tests suggests that little of the various disulfides present in aqueous flour extracts are in a form accessible to the yeast enzymes.

For our experiments, untreated patent, first clear, and second clear flours, from both a hard red spring and a winter wheat, were used. A sensitive test for thioctic acid, described below, was employed.

Thioctic acid was reduced and then spotted on a Gelman ITLC Type SC glass microfiber sheet; this was followed by chromatography in petroleum ether-ethyl ether (90:10). The reduced thioctic acid then was detected with a spray solution (100 mg. per 50 ml.) of 5,5′dithiobis-(2-nitrobenzoic acid) in phosphate buffer, pH 8.0. This gave a yellow spot. Thioctic acid was conveniently reduced in 80% acetic acid by shaking in the presence of zinc for 1 hr. Decantation, followed by addition of 5% of concentrated hydrochloric acid, gave a solution suitable for chromatography.

As a test of flour samples for free thioctic acid, they were extracted

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with chloroform and the extracts treated with 5% sodium bicarbonate to remove acids; then the aqueous extracts were acidified and re-extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate, stripped to dryness *in vacuo,* and then subjected to the above test for thioctic acid. No free thioctic acid was detected.

For the farinograph tests, an extract of flour was obtained by suspending 100 g. in 150 ml. of 0.5*M* sodium chloride, allowing the suspension to stand for 30 min., and then centrifuging it at 2,000 r.p.m. The supernatant extract was decanted; 30 ml. of extract was combined with 2 g. of yeast and 0.5 g. of sugar and incubated at 30°C. for 1 hr. The incubated mixture then was combined with 50 g. of patent flour in the farinograph and mixed for 15 min. Curves were run above the 500 line to give more mixing stress. In some cases, mixing was continued 20 min. For comparison, thioctic acid was added to the extract prior to incubation.

The data shown in Fig. 1 are typical of the results obtained with all

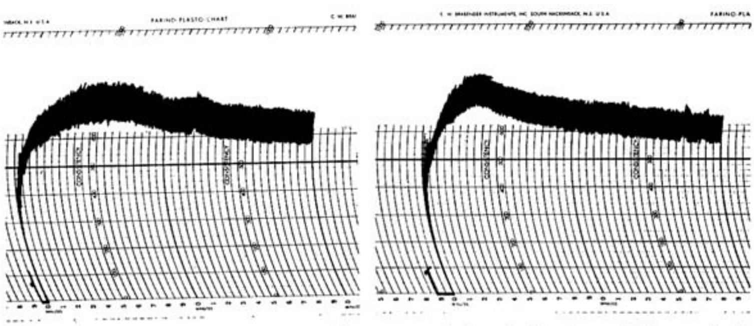

Fig. 1. Yeasted farinogram. Effect on mixing behavior of fermented aqueous second clear flour extracts (winter wheat). The one on the right shows the effect of adding free thioctic acid (0.25 μmol./g. flour) to the extract prior to fermentation.

the samples. There was no perceptible weakening of the mixing curve of the patent flour in the presence of fermented aqueous extracts of the patent, first clear, or second clear flours. This was true of both spring and winter wheat flours. The amount of flour extract used corresponded to the saline solubles obtained from 20 g. of flour. The addition of this amount to 50 g. of flour in the farinograph should have shown an effect if reducible disulfides were present in significant amounts. Such reducible disulfides might be expected to occur in greater quantity in first and second clear flours than in patent flour. Perceptible weakening of the farinograph curve was always obtained when thioctic acid was added to the extract prior to incubation.

It can be concluded that very few of the disulfides present in aqueous flour extracts are available to the enzymes within the yeast cell for enzymatic reduction.

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[Received June 21, 1967. Accepted December 9, 1967]