Ascorbic Acid as an Oxidant in Wheat Flour Dough. I. Conversion to Dehydroascorbic Acid[1]

M. ELKASSABANY,[2] R. C. HOSENEY, and P. A. SEIB[3]

ABSTRACT

The fates of L-ascorbic acid (AA) and the stability of dehydro-L-ascorbic acid (DHAA) were studied during dough mixing and subsequent resting. Practically all of the AA was converted to DHAA in optimally mixed yeasted doughs. Yeast accelerated the conversion of AA to DHAA during dough mixing. DHAA was relatively stable in flour-water doughs andextremely stable in yeasted doughs. D-Isoascorbic acid also was effectively converted to dehydro-D-isoascorbic acid during mixing, suggesting that no active AA oxidase system exists in dough.

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The mechanism of ascorbic acid (AA) in bread making has been reported (Cathcart and Edelmann 1944, Dahle and Murthy 1970, Grant 1974, Johnston and Mauseth 1972, Kuninori and Matsumoto 1964, Maltha 1953, Meredith 1966, Tsen 1965, Zentner 1968). Sanstedt and Hites (1945) found that an extract of second clear flour oxidized AA slowly but more rapidly than did a boiled extract. They therefore concluded that the extract contained a thermolabile oxidase. Extracts of an 85% patent flour gave even less oxidation of AA.

Melville and Shattock (1938) also concluded that flour contains the enzyme AA oxidase, which is capable of catalyzing the oxidation of AA to dehydro-L-ascorbic acid (DHAA), and that DHAA was more effective than AA as a flour improver. They also reported that diketogulonic acid showed no improving action. Carter and Pace (1965) also followed the oxidation of AA in a flour slurry. After 6 hr at 20°C, only 18% of the added AA was oxidized. A 12% loss was found for the control slurry containing flour from steamed wheat. They concluded that flour contains a very low level of AA oxidase. Honold and Stahmann (1968) agreed with that conclusion.

The present study was done to examine the conversion of AA to DHAA in flour-water and yeasted doughs and to determine the stability of DHAA in those doughs.

MATERIALS AND METHODS

Reagents

All chemicals used were reagent grade. L-AA was purchased from Sigma Chemical Co. and D-isoascorbic acid (DIAA) from Aldrich Chemical Co. DHAA and dehydro-D-isoascorbic acid (DHIAA) were prepared immediately before use by titration of AA with iodine in aqueous solution. *o*-Phenylenediamine was obtained from Aldrich Chemical Co. and recrystallized from water containing 0.2 g of sodium dithionite (Fieser and Fieser 1967). Norite was obtained from Fisher Scientific Co. and was acid washed.

Flour and Doughs

The flour was a composite of many hard winter wheat varieties grown at many locations. It contained 12.2% protein (N × 5.7) and 0.42% ash. Doughs for the AA and DHAA determinations were prepared from 10.0 g of flour (14% mb) mixed in a 10-g mixograph. Optimum absorption was obtained from mixograph data (Finney and Shogren 1972). The amount of reagents used for treated doughs was expressed in parts per million based on flour weight at 14% mb. Two milliliters of 10% yeast suspension (baker's compressed yeast) was used per 10.0 g of flour. The treatment solutions were added immediately before mixing. Fresh solutions of AA and DHAA were prepared immediately before mixing each series.

Doughs prepared for AA, DHAA, or DIAA determinations were mixed for either 1 or 3½ min, received rest periods of 0, 15, 30, 45, 60, or 180 min at 30°C and 95% rh, then were immediately frozen, lyophilized, ground with a Mourlinex grinder, and refrigerated until analyzed.

Analytical Methods

Total AA was determined by the microflurometric method of

TABLE I
Recovery of Dehydro-L-Ascorbic Acid (DHAA) from Flour Samples

Sample	Total (ppm)	Blank (ppm)	Recovery (ppm)
100 ppm DHAA + flour extract	193.0	98.3	95.0
Flour extract (no norite)	200.0	188.3	11.7
Flour extract (with norite)	106.6	93.9	13.3
100 ppm ascorbic acid (no norite)	216.6	190.0	26.6

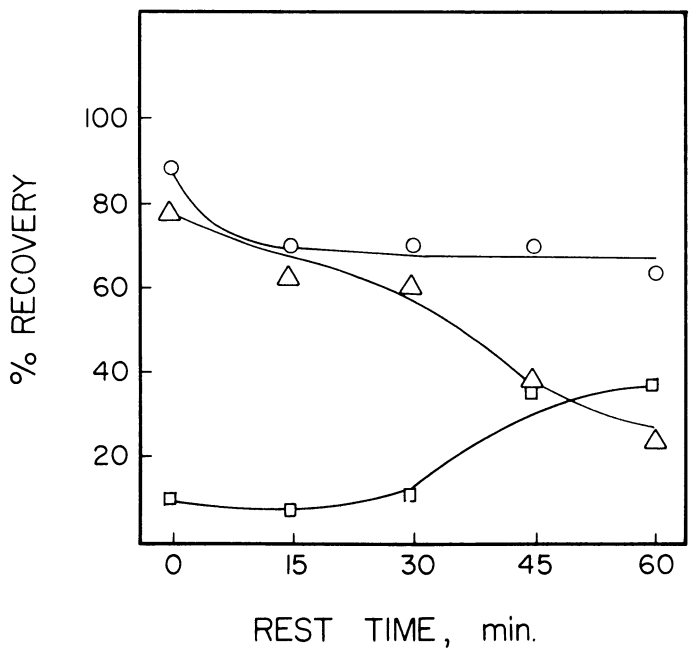

Fig. 1. Effects of mixing (1 min) and rest time on conversion of ascorbic acid (AA) to dehydro-L-ascorbic acid (DHAA): o = total, □ = DHAA, Δ = AA.

[1]Contribution 79-266j, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan 66506.
[2]Present address: Adolph Coors Co., Golden, CO 80401.
[3]Graduate research assistant, professor, and professor, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan 66506.

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Deutch and Weeks (1965). AA, boric acid, and *o*-phenylenediamine solutions were prepared immediately before being used. Metaphosphoric acid, trichloroacetic acid, 0.1 *N* sulfuric acid, and sodium acetate solutions were prepared weekly.

To determine the amount of AA converted to DHAA during treatments, the analytical procedure was modified by omitting the Norite oxidation. Thus, presumably only AA oxidized by the treatment was measured.

RESULTS AND DISCUSSION

Determination of Ascorbic Acid in Doughs

Justification of Analytical Method. The following preliminary experiments were performed to ascertain the suitability of the microflurometric method (Deutsch and Weeks 1965) for analyzing AA and DHAA in flour dough systems.

Two levels of AA (100 and 200 ppm based on flour wt) were added to flour (14% mb), and AA was determined as DHAA by the microfluorometric method. Flour gave a significant background fluorescence. Different flour samples gave significantly different levels of fluorescence. However, good recovery was obtained after deducting the flour fluorescence from the sample fluorescence. Recoveries of 95.3 and 201.7 ppm were obtained from flour samples containing 100 and 200 ppm of added AA. Standard deviation was 2.58 ppm. Thus, the microfluorometric method (Deutsch and Weeks 1965) gave reliable recovery of AA from flour.

Recovery of DHAA. To determine the amount of AA converted to DHAA during certain treatments, the analytical procedure was

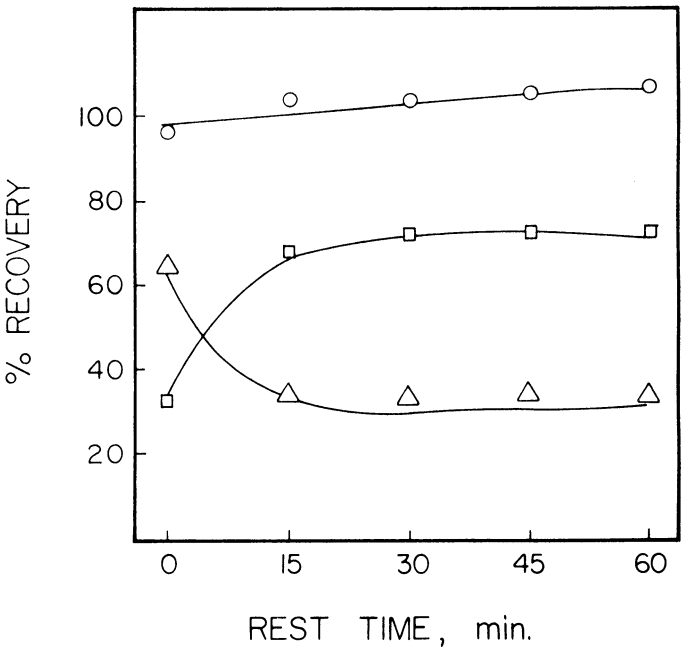

Fig. 2. Effects of mixing (1 min) and rest time on ascorbic acid (AA) oxidation in yeasted doughs: o = total, □ = dehydro-L-ascorbic acid, Δ = AA.

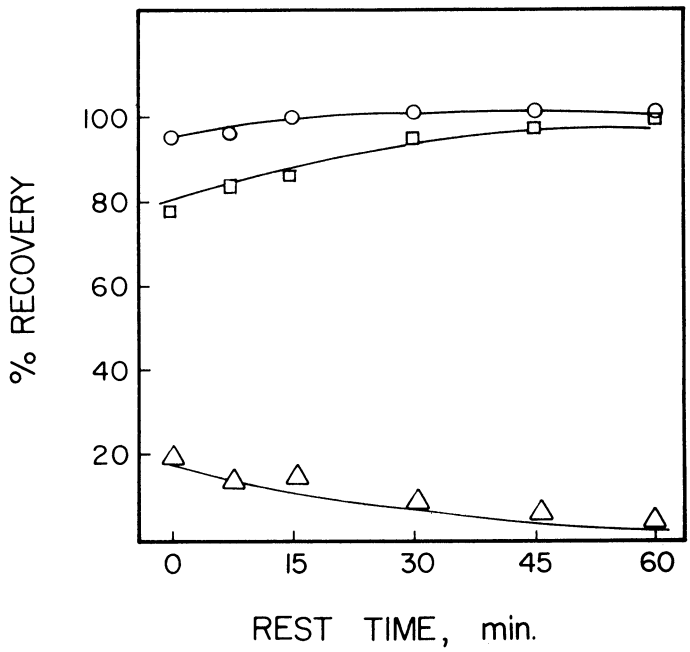

Fig. 4. Effects of mixing (3½ min) and rest time on ascorbic acid (AA) oxidation in yeasted doughs: o = total, □ = dehydro-L-ascorbic acid, Δ = AA.

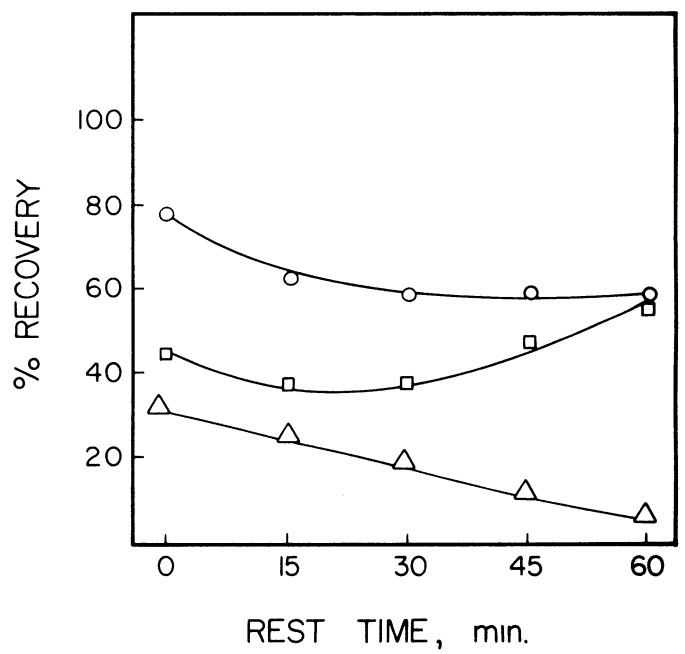

Fig. 3. Effects of mixing (3½ min) and rest time on ascorbic acid (AA) oxidation in flour-water doughs: o = total, □ = dehydro-L-ascorbic acid, Δ = AA.

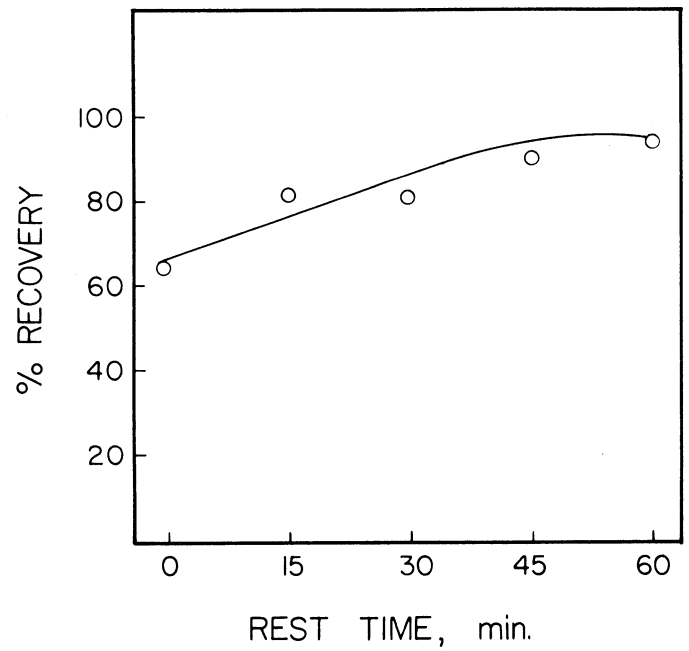

Fig. 5. Stability of D-isoascorbic acid in flour-water yeasted doughs mixed 3½ min.

modified by omitting the Norite oxidation. Thus, only AA oxidized by the treatment should be measured. The data (Table I) showed good recovery of added DHAA. Omitting the Norite oxidation gave a much higher blank. Subtracting the blank both with and without Norite gave low values for the flour extracts containing no added AA. When 100 ppm AA was added to flour, however, about 25 ppm was recovered as DHAA even though the Norite oxidation was omitted. Thus, part of the AA was converted to DHAA during the analytical procedure. Therefore, the modified procedure probably would not be useful in detecting small amounts of AA after various treatments.

Conversion of AA to DHAA During Mixing and Rest

When a flour-water dough was mixed for 1 min, 78% of the added AA was still in the reduced form after mixing (Fig. 1). The 78% is essentially equal to the recovery of AA with the modified procedure. Thus, essentially no AA was converted to DHAA. During the first 30 min of the rest period, no conversion of AA to DHAA was found. After 30 min the conversion rate slowly increased, and after 60 min only 23% of the added AA remained in the reduced form. The recovery of AA (AA + DHAA) declined from 89% at mixing to 60% after a 60-min rest period.

When yeast was included in the flour-water dough, more AA was converted to DHAA during the 1-min mix than was converted without yeast (Fig. 2). With 15 min of rest time, only 33% of the added AA remained in the reduced form. Further rest time did not change the ratio of AA to DHAA with the yeasted dough; the recovery of added AA (AA + DHAA) was essentially 100% for all rest times.

In a flour-water dough mixed to optimum (3½ min), almost half of the added AA was converted to DHAA during mixing (Fig. 3). As with the flour-water system mixed for 1 min, AA was slowly converted to DHAA during a 30-min rest. The recovery of AA (AA + DHAA) declined from 78% at mixing to 58% after a 60-min rest (Fig. 3). When yeast was included in the optimum-mixed dough, essentially all the AA was converted to DHAA during mixing (Fig. 4). Once again the recovery of AA (AA + DHAA) in the yeasted system was essentially 100%.

The much higher recovery of AA obtained with yeasted dough systems than with unyeasted doughs is assumed to stem from CO_2 production of the yeasted system. AA is more stable in the low O_2 atmosphere and at lower pH than at higher ones.

The minimumly mixed doughs showed less AA converted to DHAA than did similar doughs mixed to optimum. This is assumed to be because more oxygen is incorporated into the optimum-mixed dough. Oxygen and yeast both appear to be important in converting AA to DHAA during mixing.

The slow conversion of AA to DHAA in unyeasted doughs after a 30-min rest may result from AA oxidase. The reports of Kuninori and Matsumoto (1964), Carter and Pace (1965), Meredith (1966), and Honold and Stahmann (1968) that flour contains no AA oxidase suggest, however, that the oxidation may result from other enzymatic systems.

Conversion of DIAA to DHIAA

Sanstedt and Hites (1945), Cathcart and Edelmann (1944), and Maltha (1953) have showed that DIAA does not have the oxidizing improver effect on flour. Kuninori and Matsumoto (1964), comparing DHAA and DHIAA as substrates for the reductase system in flour extracts, found that DHAA was a suitable substrate but DHIAA was not.

Their report did not make clear, however, whether DIAA was converted to DHIAA during bread making. If AA is converted to DHAA by a specific AA oxidase, then DIAA may not be converted to DHIAA. On the other hand, if DIAA is converted to DHIAA during mixing, that is additional evidence that a specific AA oxidase is not active in the system.

Therefore, yeast and DIAA were added to flour, and a dough was mixed to optimum (3½ min). The DIAA was oxidized to DHIAA, and the DHIAA was stable in the dough (Fig. 5). The oxidation of DIAA or AA during mixing, therefore, appears not to be the result of a specific enzyme. The increasing recovery of DHIAA with increased rest time is difficult to explain. It may be because of decomposition of the DIAA in the test solution before mixing, since the 60-min rest sample was prepared first and the 0-min rest sample last.

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