

# Assay of Dehydroascorbic Acid in Bread and Dough Added as a Crystalline Dimer<sup>1</sup>

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## ABSTRACT

Cereal Chem. 75(2):200–206

The assay of dehydro-L-ascorbic acid (DHAA) in dough and bread was done by reduction of DHAA to L-ascorbic acid (AA) in aqueous dithiothreitol (DTT) at pH 6–7 followed by quantitation of the AA using HPLC with electrochemical detection. At room temperature and pH 6.6, with 4.0 equivalents of DTT, the conversion of DHAA to AA was stoichiometric after 5 min. In mixograms on flour-water doughs, DHAA added in dimeric form at 200 ppm had no effect on absorption but increased mixing time by 9–19% with the same effects occurring in full-formula doughs. AA added to doughs did not affect mixing peak time or absorption. Mixing bread doughs with an initial level of 25–200 ppm of AA based on flour (14% mb) produced DHAA in the freshly mixed doughs at concentrations of 20–51 ppm, or from 80 to 26% of AA added. During ≈120 min of fermentation and proofing, the levels of AA in the doughs increased by 4–10 ppm. Mixing bread doughs with an initial level

of 25–200 ppm of DHAA produced no AA in the freshly mixed doughs, but the proofed doughs and fresh breads contained 4–10 ppm and 7–49 ppm of AA, respectively. Fresh bread made from dough with 200 ppm of AA retained 66% total vitamin C (110 ppm of AA + 21 ppm of DHAA), whereas bread made with 200 ppm of DHAA retained 9.5% total vitamin C (13 ppm of AA + 6 ppm of DHAA). DHAA was 2–4 times more effective in improving loaf volume than an equal weight of AA in no-time dough, and 1.5–2 times more effective in straight-dough. In straight-dough bread made with a commercial bread flour, increasing concentrations of DHAA markedly improved bread up to ≈20 ppm, beyond which overoxidation occurred rapidly. In contrast, increasing concentrations of AA improved bread up to ≈150 ppm with a broad tolerance up to 200 ppm. The improving action of DHAA was independent of the concentration of air in the mixing bowl, and DHAA was much more heat-labile than AA.

Jorgensen (1935) was the first to report that adding L-ascorbic acid (AA) to an elastic wheat dough improved the handling and the volume and grain of bread. The role of AA in breadmaking has been investigated in many laboratories (Melville and Shattock 1938, Sandstedt and Hites 1945, Kuninori and Matsumoto 1963, Carter and Pace 1965, Johnston and Cooke 1971, Elkassabany and Hosenev 1980), and AA is now used worldwide in breadmaking. It is an especially important additive in breadmaking with little or no bulk fermentation of doughs. In recent years, even greater reliance has been placed on AA as an oxidizing improver because of the withdrawal of bromate.

The improving effect of AA is thought to arise from the oxidation of thiol groups in dough by DHAA (Tsen 1965, Mair and Grosch 1979, Kuninori and Nishiyama 1993). Disulfide bonds between protein molecules add elasticity to dough and improve breadmaking quality (Johnston and Mauseth 1972, Ewart 1988, Ewart 1990). Rearrangement plus reformation of disulfide linkages happens during bread dough development when proteins and peptides undergo oxidation-reduction processes mediated by improvers (Bietz and Lookhart 1996). Bread improvement by AA appears to proceed by the vitamin C redox system being coupled to that of glutathione (GSH), and the two systems operate in tandem to remove sulfhydryl groups in dough.

During dough mixing, AA is oxidized at least in part to DHAA by atmospheric oxygen catalyzed by cupric and ferric ions (Martell 1982) or by AA oxidase (Grant 1987). DHAA then oxidizes GSH, which is endogenous in flour, to oxidized GSH (GSSG), the reaction being catalyzed by dehydroascorbate reductase or GSH dehydrogenase (Walther and Grosch 1987, Sarwin et al 1993). Then, the interchange reaction of disulfide and sulfhydryl groups, respectively, on GSSG and on flour protein or low molecular weight peptides promotes the formation and preservation of disulfide cross-links between protein molecules. The reduced GSH generated from the interchange reaction is recycled to GSSG by the action of GSH dehydrogenase plus DHAA. These paired reactions result in

an increased quantity of disulfide bonds between gluten molecules, and increased dough elasticity. Evidence supporting this mechanism includes the enhanced disappearance of GSH when DHAA, but not dehydro-D-isoascorbic acid, was added to dough (Mair and Grosch 1979, Sarwin et al 1992), and the bread-improving action of only the *L-threo*-stereoisomer among the four C<sub>6</sub> dehydroascorbic acids (Lillard et al 1982, Kieffer et al 1990).

AA is not the only substance possessing vitamin C activity. DHAA and the intermediate product of AA oxidation, monodehydro-L-ascorbic acid free radical, have antiscorbutic activity equal to that of AA (Nobile and Woodhill 1981). The vitamin C redox system contains mainly AA, the reduced form, and DHAA, the oxidized form. In aqueous solution, DHAA exists principally in the hydrated hemiketal form (Tolbert and Ward 1982). Numerous assays for vitamin C in food and biological samples have been published in the scientific literature, but many of the old methods suffer from lack of stability, sensitivity, specificity, and substance interference, known as the “four-S syndrome” (Washko et al 1992). Much improved modern methods for AA assay in foods and biological samples rely on HPLC with ultraviolet (UV), electrochemical (EC), coulometric, or fluorometric detection (Sauberlich et al 1982, Howard et al 1987, Behrens and Madere 1992, Graham and Annette 1992, Vander-slice and Higgs 1993, Ito et al 1995, Lykkesfeldt et al 1995).

Because AA and DHAA are equivalent in vitamin C activity, methods have been devised to measure AA plus DHAA. DHAA is insensitive to EC and UV detection, so often it is reduced back to AA by sulfhydryl agents, such as dithiothreitol (DTT), homocysteine, and 1,2-dithioglycerol. DHAA then is determined by difference between the AA levels before and after reduction. The objectives of this investigation were to: 1) devise an analytical scheme for DHAA in bread and dough using HPLC-EC, and 2) compare the properties of AA and dimeric DHAA in breadmaking.

## MATERIALS AND METHODS

### Materials

All chemicals were reagent-grade. L-Ascorbic acid was from Fisher Scientific Co. (Fair Lawn, NJ); DHAA was synthesized by O<sub>2</sub> oxidation of AA in ethanol solution in the presence of activated charcoal (Ohmori and Takagi 1978). The crystalline dimeric form (mp 233–236°C) was produced by dehydration of the monohydrated monomeric DHAA in diethyl carbonate (Lu 1995). The crystalline dimer, on dissolving in water, immediately adds water

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to reform monomeric DHAA (Hvoslef and Pedersen 1979). Thus, a given weight of DHAA dimer forms exactly the same weight of monomeric nonhydrated DHAA in water and dough. Most dough testing and breadmaking were done on a commercial bread flour provided by Cargill Flour Milling Company, Wichita, KS, with 11.5% protein, 13.7% moisture, and 0.47% ash. The flour contained malt (falling number 220) but no other additive, and was obtained in April, 1993. Another flour was a baking standard from North Dakota State University milled from hard red spring (HRS) wheat grown in 1993, and the flour had 13.0% protein, 13.6% moisture, and 0.43% ash. Shortening was a partially hydrogenated mixture of soybean and cottonseed oils and contained mono- and diglycerides (Crisco, Proctor and Gamble, Cincinnati, OH). Instant dry yeast was Fermipan donated by Gist-Brocades (Food Ingredients, Inc., King of Prussia, PA).

### General Methods

The pH levels of buffers and reaction mixtures were determined on a pH meter fitted with a Lab Research Products electrode (catalog no. 5733930-003b, Lincoln, NB). AA was determined by HPLC-EC according to Wang et al (1988).

### Reduction of DHAA to AA with DTT in Aqueous Buffer

Phosphate buffers at  $\approx 0.6M$  with pH levels of 1.2–9.4 were prepared by mixing various proportions of 6.0% aqueous metaphosphoric acid (pH 1.2), 10% potassium diacid phosphate (pH 4.1), and 10% dipotassium acid phosphate (pH 9.4). DHAA (20 mg, 0.11 mmol) was dissolved in a phosphate buffer (100 mL) containing DTT (200 mg, 1.3 mmol), and the mixture was allowed to stand at room temperature for 15 min. An aliquot (100  $\mu$ L) of the reaction mixture was made to volume (25 mL) with cold 0.05M aqueous perchloric acid, and the resulting solution was syringe-filtered and injected into the liquid chromatograph. The extent of reduction of DHAA was measured by an increase in the concentration of AA using HPLC-EC.

Reduction of DHAA with 0.5–12 molar excess of DTT was examined at pH 6.6 and 15 min of reaction time, and reaction time was measured at pH 6.6 and a [DTT]/[DHAA] ratio of 8:1.

### AA and DHAA Dimer in Dough Mixing

The effects of AA and DHAA dimer on dough mixing were examined on the mixograph with a 10-g flour sample (National Mfg. Co., Lincoln, NE). AA or DHAA dimer were added in solid form, whereas L-cysteine hydrochloride was added as a freshly prepared 0.3% aqueous solution. The mixograph was interfaced with a computer, and the characteristics of mixograms were evaluated according to Approved Method 54-40 (AACC 1995).

### AA and DHAA Dimer in Straight-Dough Breadmaking

Pup loaves were made according to Approved Method 10-10B (AACC 1995) from 100 g of flour (14% mb), 4.0 g of nonfat dry milk (NFDm), 3.0 g of shortening, 11.0 mL of a solution of sucrose and sodium chloride containing 6.0 g of sucrose and 1.5 g of sodium chloride, and 2.01 g of instant dry yeast.

Instant dry yeast and solid AA or DHAA dimer were added and blended well with flour and NFDm before adding the solution of sucrose-sodium chloride and water to optimum absorption. In some tests, AA was added as a freshly prepared aqueous solution immediately after adding the liquid ingredients. A dough was mixed to optimum development in a National Special 100 Gram Pin Mixer (TMCO-National Mfg. Co., Lincoln, NE). Optimum mixing time was determined from the appearance of the dough and its film-forming property. The dough piece was fermented in a proof cabinet (TMCO-National Mfg.) at 30°C and 85–90% rh for 90 min with punching and sheeting (3/16-in. gap) of the dough piece after 52 and 77 min of fermentation. Then, the dough was sheeted at a gap of 5/16 in., molded in a drum molder, and the dough cylinder was panned. After proofing at 30°C and 90–95% rh for 33 min, the dough was baked at 218°C for 24 min. Immediately on removal from the electric reel oven (TMCO-National Mfg.), the loaf was weighed and volume measured by rapeseed displacement.

Some doughs were mixed under a nitrogen atmosphere in a plastic bag (catalog no. 47-6-35C, Associated Bag Co., Milwaukee, WI). The air in the dry ingredients was exchanged with nitrogen by subjecting the ingredients to low pressure in a flask and refilling with nitrogen. Air in the sucrose-salt solution and in water was displaced by heating to boiling, and then bubbling nitrogen through the liquids until they reached 25°C.

### AA and DHAA Dimer in No-Time Dough Breadmaking

The ingredients used to produce bread with short-time bulk fermentation were the same as in the straight-dough formula ex-

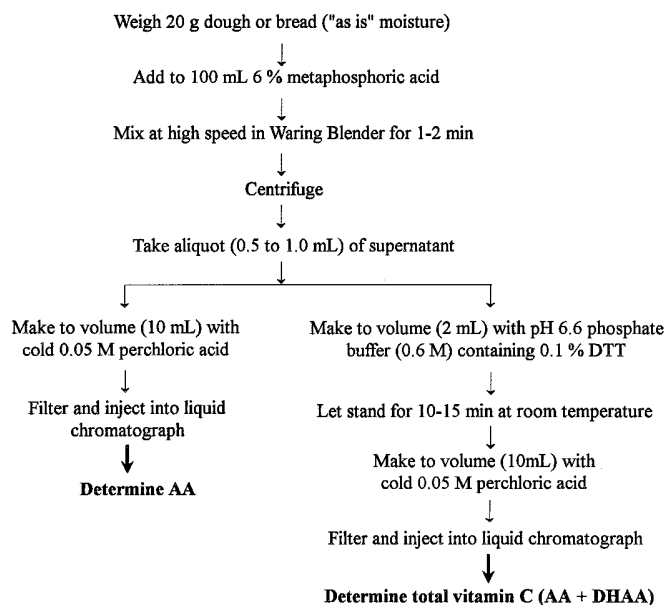


Fig. 1. Analytical schemes to assay L-ascorbic acid (AA) and total vitamin C (AA + dehydro-L-ascorbic acid [DHAA]) in dough and bread by HPLC with electrochemical detection.

TABLE I  
Levels of L-Ascorbic Acid (AA) and Dehydro-L-Ascorbic Acid (DHAA) in Dough and Bread with Various Levels of [AA]<sub>0</sub> (ppm)<sup>a</sup> Added at Mixing

Product	25			50			200		
	AA	Vitamin C <sup>b</sup>	DHAA	AA	Vitamin C	DHAA	AA	Vitamin C	DHAA
Mixed dough	3.8 <sup>c</sup>	24.2 <sup>a</sup>	19.4 <sup>a</sup>	21.6 <sup>b</sup>	47.0 <sup>a</sup>	25.3 <sup>a</sup>	118.0 <sup>b</sup>	169.0 <sup>a</sup>	51.1 <sup>a</sup>
Proofed dough	10.8 <sup>a</sup>	22.7 <sup>a</sup>	11.9 <sup>b</sup>	25.5 <sup>a</sup>	42.9 <sup>b</sup>	17.4 <sup>b</sup>	123.8 <sup>a</sup>	168.3 <sup>a</sup>	44.5 <sup>b</sup>
Bread	9.4 <sup>b</sup>	12.3 <sup>b</sup>	3.4 <sup>c</sup>	15.1 <sup>c</sup>	20.5 <sup>c</sup>	5.4 <sup>c</sup>	110.1 <sup>c</sup>	138.1 <sup>b</sup>	28.0 <sup>c</sup>
LSD <sup>d</sup>	0.9	1.5	2.5	0.9	1.6	1.0	2.0	2.9	1.8

<sup>a</sup> [AA]<sub>0</sub> was the initial level of L-ascorbic acid added at the mixing stage. Concentration was based on the weight of flour at 14% moisture basis.

<sup>b</sup> AA + DHAA.

<sup>c</sup> Values followed by the same letter are not significantly different ( $P < 0.05$ ). Values are the means of three replicates.

<sup>d</sup> Least significant difference ( $P < 0.05$ ).

cept for L-cysteine hydrochloride, which was added at 30 ppm (1.0 mL of 0.3% aqueous solution) before dough mixing. The breadmaking procedure was followed as in the straight-dough procedure, except bulk fermentation time was reduced to 20 min, with punching and sheeting (3/16-in. gap) after 10 min of fermentation. Molding was done after 20 min of fermentation at a roll setting of 5/16 in., and proofing of all doughs was to a height of 7.5 cm ( $\approx$ 50 min) before baking.

### Assay of AA and Total Vitamin C in Dough and Bread

Mixed dough, proofed dough, or bread (center slice of loaf) were assayed immediately on removal from the mixer, fermentation cabinet, or oven, respectively. The sample (20.0 g, wb) was blended with 100 mL of 6% metaphosphoric acid solution in a blender (model 31BL91, Waring Products Division, Dynamics Corporation of America, New Hartford, CT) for  $\approx$ 2 min. A portion

( $\approx$ 1.5 mL) of the mixture was centrifuged at  $4,500 \times g$  in an Eppendorf microcentrifuge (Brinkmann Instruments Co., Westbury, NY) for 1.5 min to obtain a clear supernatant. To assay AA, an aliquot (1.0 mL) of the supernatant was diluted immediately with 0.05M cold perchloric acid to a certain volume, shaken, syringe-filtered, and injected into the liquid chromatograph. The volume of perchloric acid solution was chosen to obtain a level of DHAA plus AA in the solution of 0.05–to 0.20  $\mu$ g/mL. To assay total vitamin C, an aliquot (1.0 mL) of supernatant was mixed with 1 mL of phosphate buffer (pH 7.2) containing 0.1% DTT, and the resultant mixture was allowed to stand at room temperature for 10–15 min. After dilution with 0.05M cold perchloric acid, the level of AA, which equaled total vitamin C, was determined after injection into the chromatograph (Fig. 1). The DHAA content in dough or bread was calculated by subtracting AA content ([AA]) from total vitamin C content ([AA+DHAA]).

### Stability of Vitamin C During Storage of Bread

Loaves of bread were made using the no-time dough procedure with 200 ppm of AA or DHAA dimer added at mixing. The pup loaves were cooled  $\approx$ 1 hr, held unsliced at 25°C in sealed polyethylene bags, and the levels of AA, total vitamin C, and DHAA in freshly baked and stored loaves of bread were determined as described above.

### Statistical Analysis

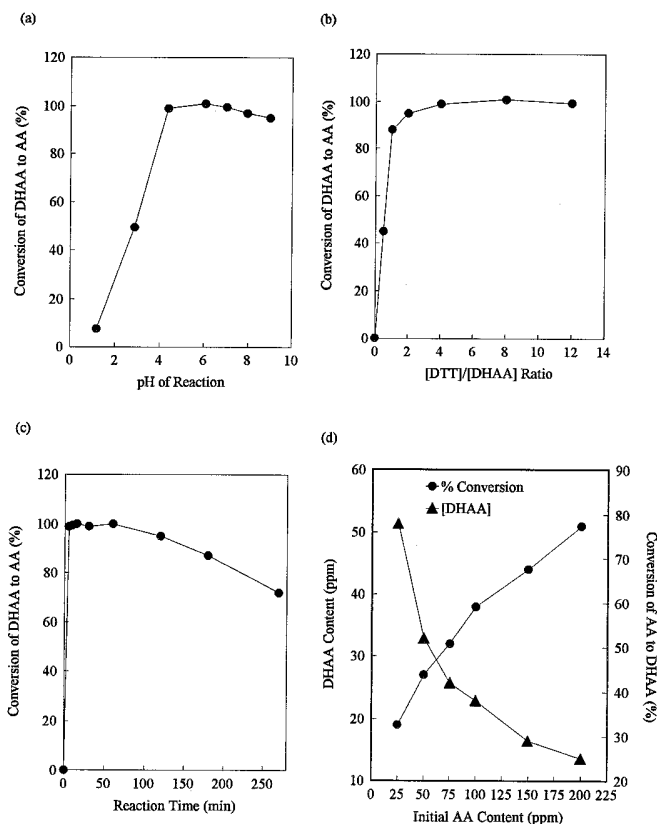
Data were evaluated statistically using the one-way analysis of variance procedure with the least significant difference test (SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

### Reduction of DHAA to AA with DTT

The reduction of DHAA to AA with DTT at room temperature was examined at different pH levels, molar excesses of DTT, and reaction times. Figure 2a shows that the reduction was quantitative in 15 min at pH 4–7 when the reaction mixture contained a 11.8 molar excess of DTT. It appears the rate of reduction was slow below pH 4, and that DHAA probably began to degrade above pH 7. The reduction at pH 6.6 with a reaction time of 15 min was stoichiometric when the molar excess of DTT exceeded 4.0 (Fig. 2b). However, 1 hr after reduction, the AA in the phosphate buffer began to degrade (Fig. 2c). Thus, quantification of the AA produced in the reduction reaction should be completed before that time.

The conditions selected for routine reduction of DHAA at concentrations <200  $\mu$ g/mL were pH 6.6 buffer containing 10 mM DTT (DTT:DHAA > 8) and a time of 10–15 min. In analyses of bread and dough, the concentration of DHAA in a reduction reaction was usually <20  $\mu$ g/mL. Before being injected into the chromatograph, a reaction mixture was diluted with cold perchloric acid to bring the AA (reduced DHAA) concentrations within the range of 0.1–0.4  $\mu$ g/mL. The peak heights of AA (reduced DHAA) solutions at those concentrations showed a linear response with a regression coefficient of 0.999 when assayed by HPLC-EC.



**Fig. 2.** Reduction of dehydro-L-ascorbic acid (DHAA) to L-ascorbic acid (AA) by a 11.8 molar excess of dithiothreitol (DTT) for 15 min at 25°C (a). Reduction of DHAA to AA by DTT in 15 min at 25°C in pH 6.6 phosphate buffer (b). Reduction of 1.2 mM DHAA to AA at 25°C with 8 equivalents of DTT in pH 6.6 phosphate buffer (c). Conversion of AA to DHAA after 4 min of dough mixing with the commercial bread flour at 67% absorption (d).

**TABLE II**

Levels of L-Ascorbic Acid (AA) and Dehydro-L-Ascorbic Acid (DHAA) in Dough and Bread with Various Levels of [DHAA]<sub>0</sub> (ppm)<sup>a</sup> Added at Mixing

Product	25			50			200		
	AA	Vitamin C <sup>b</sup>	DHAA	AA	Vitamin C <sup>b</sup>	DHAA	AA	Vitamin C <sup>b</sup>	DHAA
Mixed dough	0.0b <sup>c</sup>	22.1a	22.1a	0.0c	43.4a	43.4a	0.0c	189.0a	189.0a
Proofed dough	5.7a	18.5b	12.8b	4.1b	34.6b	30.5b	10.1b	184.0b	173.9b
Bread	6.5a	11.1c	4.6c	7.7a	19.4c	11.7c	48.5a	69.5c	22.0c
LSD <sup>d</sup>	0.6	1.9	1.8	1.4	2.4	2.7	1.9	2.6	2.9

<sup>a</sup> [DHAA]<sub>0</sub> was the initial level of dehydro-L-ascorbic acid added at the mixing stage. Concentration was based on the weight of flour at 14% moisture basis.

<sup>b</sup> AA + DHAA.

<sup>c</sup> Values followed by the same letter are not significantly different ( $P < 0.05$ ). Values are the means of three replicates.

<sup>d</sup> Least significant difference ( $P < 0.05$ ).

The recovery of DHAA in aqueous solution was 99.0–101.2% at DHAA levels >10 ppm, while the recovery of DHAA was 96.4–99.8% if it was spiked into dough pieces at levels of 25–200 ppm. Precision of the determination was >95% at levels of DHAA >25 ppm. The limit of quantitation was  $\approx 1 \mu\text{g}$  of DHAA/g of sample.

### Conversion of AA and DHAA in Dough and Bread

In the HPLC-EC assay methods for AA and total vitamin C (AA+DHAA) in bread and dough (Fig. 1), the key step is the stoichiometric reduction of DHAA by DTT at pH 6.6 in phosphate buffer. Levels of DHAA in bread and dough are calculated by difference between total and reduced vitamin C.

It is well known that AA is oxidized to DHAA during dough mixing. The amount of DHAA formed during mixing a full-formula dough in this work depended on the initial level of AA. When 25 ppm of AA was added to flour, 20 ppm (80%) of AA was oxidized to DHAA after mixing, but when 200 ppm of AA was added to flour, only 51 ppm (26%) of AA was converted to DHAA after mixing. At levels between 25 and 200 ppm of AA added to the mixer, the absolute amount of DHAA produced during dough mixing increased, but the percentage converted decreased (Fig. 2d).

The factors controlling the extent of oxidation of AA in dough mixing are not known with certainty. The rate of air oxidation of AA depends on the concentrations of the monoanion of AA ( $\text{pK}_1 = 4.2$ ), oxygen, and cupric and ferric ions, whereas the rate of enzymic conversion depends on enzyme concentration and oxygen levels. The concentration of oxygen in dough mixing may be rate-limiting, because yeast rapidly absorbs oxygen in dough, although the rate of uptake of oxygen by the active-dry yeast used in this work may be somewhat slower than that by compressed yeast.

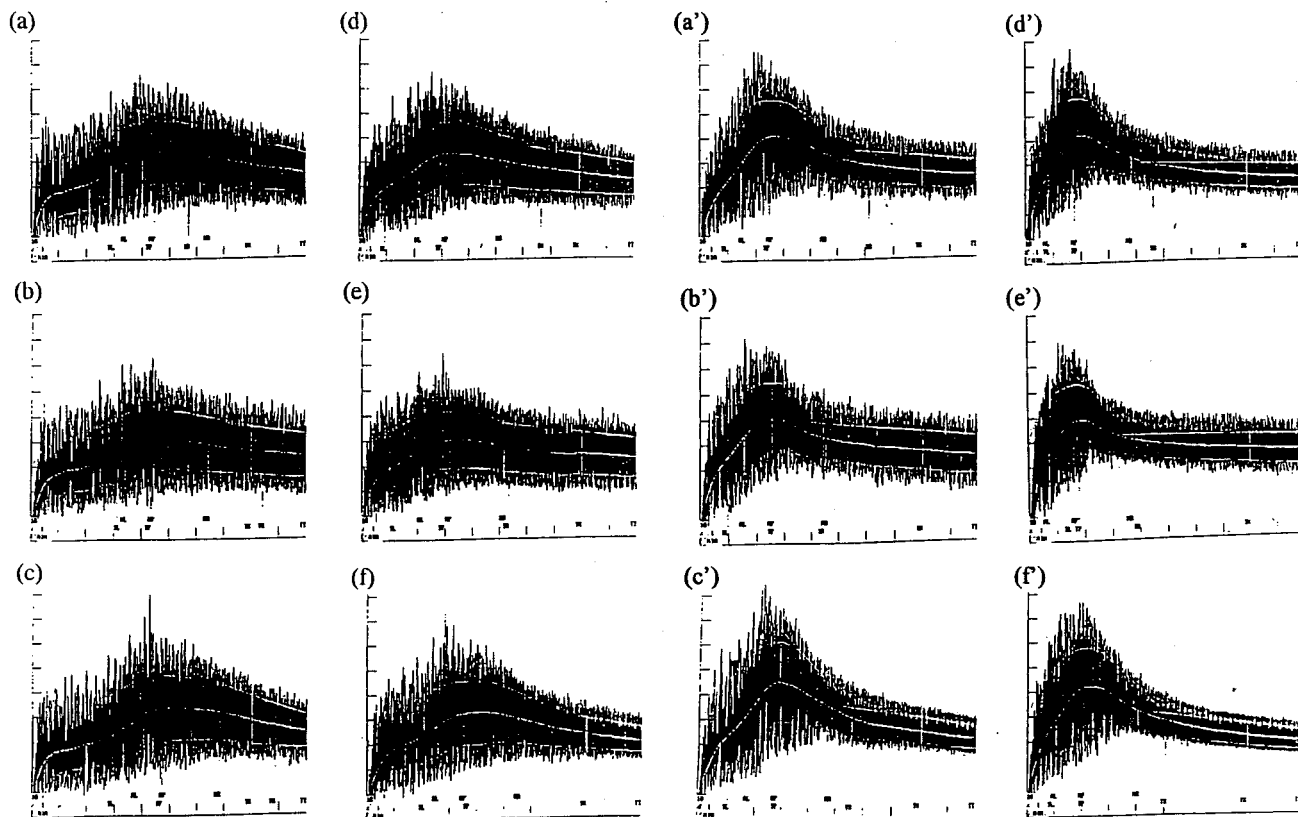
Fully proofed dough contained 3–7 ppm higher levels of AA and 7–10 ppm lower levels of DHAA than did freshly mixed dough when AA was added at the mixer (Table I). Those changes

support the dehydroascorbate reductase mechanism that has been proposed to explain the improving action of AA on dough (Walther and Grosch 1987). Dehydroascorbate reductase should convert some DHAA to AA when it catalyzes the oxidation of GSH to GSSG. The increase in the level of AA during bulk fermentation and proofing was approximately one-half the loss of DHAA. Reduced AA is stable in fermenting dough because of a lack of oxygen, but DHAA is probably hydrolyzed slowly to 2,3-diketogulonic acid at pH 5–6 in dough, or it may react with some functional groups on proteins, such as amino or sulfhydryl.

When 25–200 ppm of DHAA dimer was added at mixing, no AA was found in freshly mixed dough (Table II). However, the proofed dough contained 4–10 ppm of AA, which agrees with the findings of Pfeilsticker and Marx (1986) and again supports the dehydroascorbic acid reductase mechanism. Even more AA was produced from DHAA during baking, such that three-fourths of the vitamin C in the bread was present as AA. Overall losses of vitamin C were 60–66% when DHAA was added at the mixer, compared to the 30–50% losses with added AA.

### Effects of AA and DHAA Dimer on Mixing of Elastic Doughs

The mixograms of two flours, one a commercial bread flour, and the other a baking standard flour milled from hard red spring (HRS) wheat (Fig. 3), showed AA and DHAA dimer had no effect on optimum absorption (data not shown). AA at 200 ppm had little or no effect on peak time, but slightly widened the curve after development, or in other words, AA slightly increased tolerance to overmixing. On the other hand, DHAA dimer at 200 ppm (curves in Fig. 3c and c') increased mixing time by 9% over the 4.3-min mixing time of the bread flour and 16–19% over the 2.9-min mixing time of the HRS baking standard flour. Moreover, DHAA dimer caused a moderate narrowing of the width of the mixing curve after optimum development, indicating a decreased

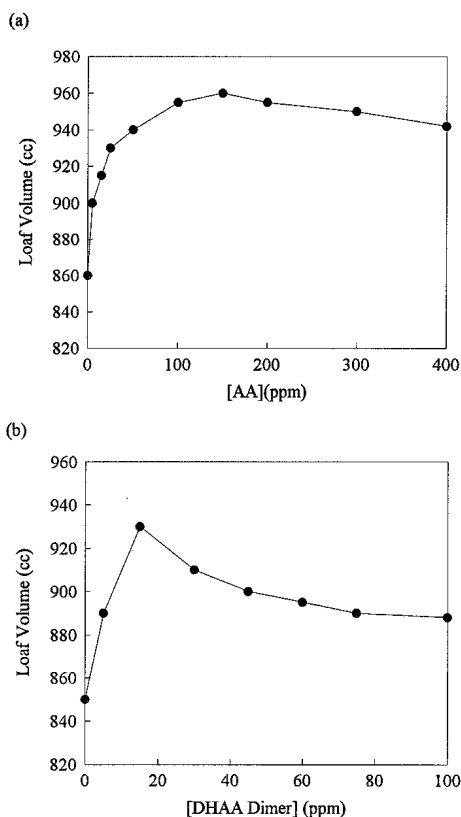


**Fig. 3.** Mixograms of commercial bread flour at 63% absorption. Control (a); 200 ppm of L-ascorbic acid (AA) (b); 200 ppm of dehydro-L-ascorbic acid (DHAA) dimer (c); 30 ppm of cysteine (d); 30 ppm of cysteine and 200 ppm of AA (e); 30 ppm of cysteine and 200 ppm of DHAA dimer (f). Mixograms of baking standard bread flour at 66% absorption. Control (a'); 200 ppm of AA (b'); 200 ppm of DHAA dimer (c'); 30 ppm of cysteine (d'); 30 ppm of cysteine and 200 ppm of AA (e'); 30 ppm of cysteine and 200 ppm of DHAA dimer (f').

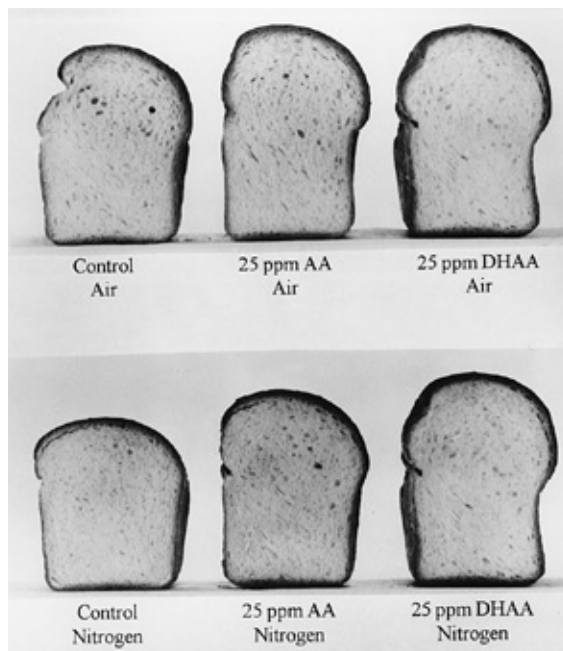
tolerance to overmixing. Cysteine at 30 ppm in flour reduced mixing time and tolerance as expected (Weak et al 1977, Lang et al 1992), and the effects of AA and DHAA dimer in dough with cysteine were addable to either of those alone.

In straight-dough breadmaking with 90 min of fermentation time, adding AA at 5–400 ppm to the commercial flour increased

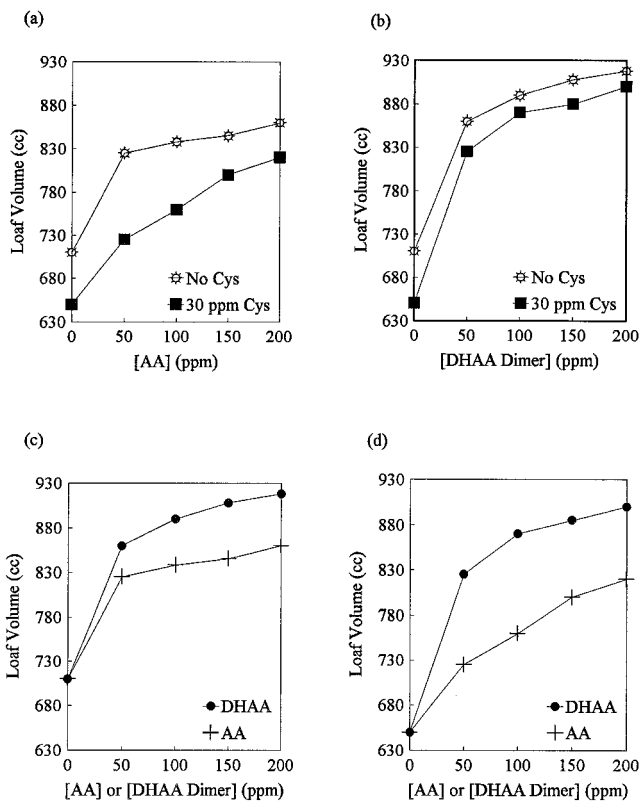
loaf volume and gave optimum volume (950 cm<sup>3</sup>) at a level of 100–200 ppm of AA (Fig. 4a). At levels of AA >200 ppm, bread crumb grain became open; loaf volume decreased ≈5% at 400 ppm.



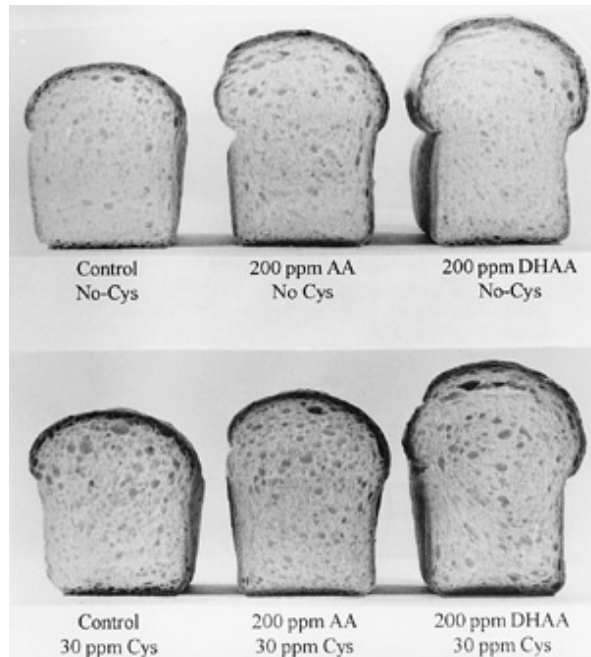
**Fig. 4.** Loaf volumes of pup loaves made with commercial bread flour: L-ascorbic acid (AA) (a); dehydro-L-ascorbic acid (DHAA) dimer (b). Least significant difference ( $P < 0.05$ ) was 20 cm<sup>3</sup>.



**Fig. 5.** Breads made from doughs with or without 25 ppm of L-ascorbic acid (AA) or dehydro-L-ascorbic acid (DHAA) dimer in the commercial bread flour and mixed under air or nitrogen atmosphere.



**Fig. 6.** Effect of L-ascorbic acid (AA) and dehydro-L-ascorbic acid (DHAA) dimer levels on loaf volume in no-time doughs made from commercial bread flour with and without 30 ppm of L-cysteine in the doughs. AA with or without L-cysteine (a); DHAA dimer with or without L-cysteine (b); comparison of AA and DHAA dimer on loaf volume with 30 ppm of L-cysteine addition (c); comparison of AA and DHAA dimer on loaf volume without L-cysteine addition (d).



**Fig. 7.** Breads made by no-time breadmaking procedure with or without 30 ppm of L-cysteine and L-ascorbic acid (AA) and dehydro-L-ascorbic acid (DHAA) dimer at 200 ppm in commercial bread flour.

When DHAA dimer was added to the commercial flour at 5–100 ppm, the optimum loaf volume was 935 cm<sup>3</sup> at a level of 15 ppm (Fig. 4b). Unlike the broad optimum level of 100–200 ppm of AA, the optimum level of DHAA dimer appeared more sharply defined. High levels of DHAA dimer (>60 ppm) caused overoxidation of bread as evidenced by reduced loaf volume, open crumb, and rounded edges on the loaves.

The improving action of DHAA dimer, but not AA, as expected from the work of Collins et al (1991), was shown to be independent of the atmosphere in a mixer. Dough mixed with 25 ppm of AA under nitrogen had pup loaf volume of 730 cm<sup>3</sup>, much lower than the 900 cm<sup>3</sup> volume when mixed in air. The dough mixed with 25 ppm of DHAA dimer under either nitrogen or air both gave loaves with 900 cm<sup>3</sup> volume (Fig. 5).

### Improving Bread Quality with AA and DHAA Dimer in No-Time Dough

L-Cysteine is often added along with AA in formulas for breads made with a short fermentation period (no-time doughs) and conventional mixing. L-Cysteine reduces mixing time, whereas AA improves loaf volume and grain. Figure 6a shows L-cysteine lowered the improving action of AA on loaf volume, but its detrimental effect was minor when DHAA replaced AA (Fig. 6b). Substituting DHAA dimer for the same level of AA in dough at 50–200 ppm in the presence of 30 ppm of L-cysteine increased pup loaf volume by 80–100 cm<sup>3</sup> (Fig. 6d). In the absence of L-cysteine (Fig. 6c), substituting DHAA for AA gave only one-half the loaf volume improvement.

It appears DHAA is especially effective in counteracting the reducing activity of L-cysteine in dough after mixing is complete. Even so, the DHAA still allowed L-cysteine to reduce mixing time, although L-cysteine was only 50% as effective in reducing mixing time in the presence of DHAA dimer as in the presence of AA. Crumb grain and loaf volume (Figs. 6 and 7) show that

DHAA dimer in no-time doughs gave better bread at optimum oxidant levels than did equal levels of AA, either with or without the use of 30 ppm of L-cysteine to reduce mixing time. The same results were obtained when the baking standard flour was used.

### Stability of Vitamin C During Storage of Bread

Fresh bread made with 200 ppm of AA added at mixing of the no-time dough contained 131 ppm of total vitamin C (based on flour weight with 14% moisture content), whereas fresh bread made with 200 ppm of DHAA dimer contained only 19 ppm of total vitamin C (Fig. 8). The retention of vitamin C in the fresh bread made with 200 ppm of DHAA dimer added in the no-time dough was much lower than that in bread made by the straight-dough procedure (Table II), which may be attributed to the lower conversion of DHAA to AA in the shorter fermentation time of the no-time dough. In both cases, DHAA was much more labile than AA during bread baking. Even so, AA disappeared rapidly in stored bread, so that after five days, breads made with either AA or DHAA contained little vitamin C (Fig. 8).

## CONCLUSIONS

The key step for the reduction of DHAA to AA by DTT is adjusting the pH level of the reaction media to 6.0–7.0. The conversion of DHAA to AA with 4 equivalents of DTT at room temperature is stoichiometric after 5 min at pH 6–7. In breadmaking, the oxidation of AA to DHAA is mainly at the mixing stage. The formation of AA in dough and bread supports the dehydroascorbate reductase mechanism of the improving action of AA in breadmaking. DHAA dimer produces higher bread volume and better crumb grain than an equal level of AA in no-time doughs, especially those containing L-cysteine. DHAA dimer also is an effective bread improver for doughs mixed in limited oxygen mixers, but DHAA is much more heat-labile than AA.

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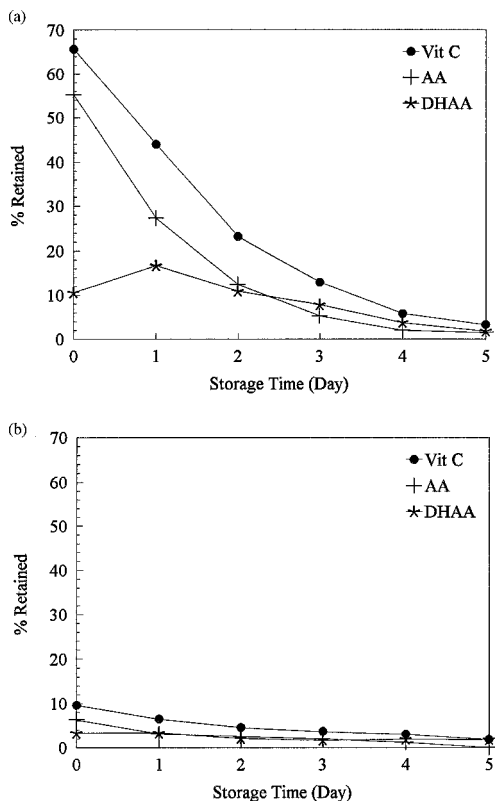


Fig. 8. Retention of total vitamin C, L-ascorbic acid (AA), and dehydro-L-ascorbic acid (DHAA) in stored bread made by the no-time dough procedure with 200 ppm AA (a) or DHAA dimer (b) added to the commercial bread flour at the mixer.

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[Received July 17, 1997. Accepted November 12, 1997.]