

Effect of L-Ascorbic Acid on the Rheological Properties of Wheat Flour-Water Dough

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ABSTRACT

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L-Ascorbic acid (AsA) and its related compounds play an important role as improvers in bread production. Addition of AsA and its related compounds, such as dehydro-L-AsA (DHA) and 2,3-diketo-L-gulonic acid (DKG), affected the rheological properties of flour-water dough during mixing, especially hardness. Addition of 10 or 100 ppm AsA increased the dough hardness of samples as compared with the control dough. Addition of DHA or DKG to dough only slightly increased hardness. Addition of *p*-quinone significantly increased the hardness. Both

glutathione (GSH) and its oxidized form (GSSG) drastically decreased the hardness. Contents of AsA in the treated dough decreased and contents of DHA increased during mixing, suggesting that oxidation occurred. The oxidation rate of AsA was influenced by the concentration of AsA added. The improving effect of AsA on the rheological properties of flour-water dough seemed to be mostly dependent on reactive intermediate oxidation products such as O₂⁻, while the contribution of DHA was rather limited.

Physicochemical interactions among various food components contribute to food quality. For example, L-ascorbic acid (AsA) has been widely used as an antioxidant in food processing and preservation. Also, AsA and its related compounds are suggested to play an important role in the modification of rheological properties of foods and foodstuffs. AsA has long been used as an improver in bread industry (Jorgensen 1939), although the improvement mechanism of AsA is not clearly understood.

It has been suggested that the direct or indirect effect of the substances used as improvers, such as AsA, on the structure and intermolecular interaction of proteins in dough may play an important role in the modification of rheological properties of dough. It is generally accepted that the rheological properties of dough and its three-dimensional network are dependent on the interaction of sulfhydryl (SH) groups and disulfide (SS) bonds of the protein.

It is generally accepted that AsA improves bread dough when it is oxidized to dehydro-L-ascorbic acid (DHA) by oxygen or other oxidants (Elkassabany et al 1980, Elkassabany and Hosney 1980, Nicholas et al 1980). DHA oxidizes SH groups of cysteine residues in protein molecules forming inter- and intramolecular SS bonds (Melville and Shattock 1938, Maltha 1953, Tsen 1965, Lillard et al 1982).

On the other hand, the effect of DHA may be due to the removal of glutathione (GSH) in wheat flour, which would diminish dough strength by SH-SS interchange reaction with gluten molecules (Kuninori and Matsumoto 1963, 1964; Carter and Pace 1965; Bloksma 1972; Boeck and Grosch 1976; Mair and Grosch 1979; Walther and Grosch 1987).

Ascorbic acid is known to be easily oxidized by one- or two-electron oxidation reactions to DHA, the first chemically stable oxidation product. This oxidation of AsA in water at neutral pH is accelerated by the transfer of an electron to dioxygen from a donor, such as transition metals (Khan and Martell 1967a,b and 1968; Ogata et al 1968). Grant and Sood (1980) and Pfeilsticker and Roeung (1980, 1982) showed that a nonenzymic oxidation of AsA, mediated by copper or iron ions, plays an important role in

its improver action. In this oxidative reaction, some free radical species such as superoxide anion radical (O₂⁻) and hydroxyl radical (•OH) were formed from molecular oxygen. Reactive oxygen radicals such as O₂⁻ promote the oxidative scission of proteins (Ogata et al 1968; Samuni et al 1983; Shinar et al 1983; Levine 1984; Marx and Chevion 1985; Uchida and Kawakishi 1986, 1987, and 1989). However, the actual dough system is so complex that there are many questions about the effect of AsA on its rheological properties.

The objective of this study was to clarify the improving effect of AsA and its related compounds on the rheological properties of flour-water dough during the mixing process. In particular, the improver function of AsA as an electron donor that contributes to the structure of protein was studied.

MATERIALS AND METHODS

Reagents

All chemical reagents used were reagent grade. GSH and GSSG were from Sigma Chemical Co (St. Louis, MO). *p*-Quinon and other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Wheat flour was supplied by Nisshin Flour Milling Co., Ltd. (Tokyo, Japan).

DHA or DKG Acid Preparations

DHA was prepared by the reaction of AsA in ethanol solution with molecular oxygen in the presence of charcoal (Omori and Takagi 1978). Remaining AsA in DHA was measured by HPLC, and AsA concentration in DHA preparation was ≈0.1%. Potassium salt of DKG was prepared as a hydrolysis product of DHA after the oxidation of AsA in aqueous solution with potassium iodide (Kagawa 1962).

Flour-Water Dough

Dough consisting of 400 g of flour and 260 mL of deionized and distilled water (Kosakaiseiyaku Co., Ltd. Tokyo, Japan) or 10, 100, and 1,000 ppm of aqueous solution of AsA was mixed at room temperature in an air mixer (CS-10, Kanto kongoki kogyo Co., Ltd., Tokyo, Japan) especially designed for bread dough making. Concentration of DHA and DKG were 100 ppm. Concentration of other components such as GSH, GSSG, and *p*-quinone were 0.936 μmol/g of flour (concentration equivalent to 100 ppm of AsA). Mixing was done at medium speed (250 rpm) for 3, 6, 9, and 12 min. After mixing, a dough sample (55 g) was measured for its hardness using a Rheoner (RE-33005, Yamaden

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Co. Ltd., Tokyo, Japan). Dough hardness is the force (dynes/cm²) measured under defined compressive stress conditions. The rheoner had a plunger with a diameter of 8 mm. The sample was placed on the plate, which was set to move at 1 mm/sec in the glass vessel, and pressed with the plunger for a compression distance of 10 mm. Other rheoner conditions were: load cell, 2 kg-force; compressive speed, 1 mm/sec; and sample height, 20 mm. The measurement was completed within 70 sec at room temperature.

Extraction of AsA from Wheat Flour Dough

After mixing, dough sample (10 g) was ground with sea sand in 5% metaphosphate solution using a mortar. The extracts were centrifuged at 3,000 × *g* for 5 min and then filtered with 0.8- and 0.45-μm membrane filters (Toyo Roshi). The clear supernatant was used for the analysis by HPLC. Recovery of AsA added during extraction was ≈100%.

HPLC Analysis

Contents of AsA and DHA in the dough extracts were measured by HPLC with electrochemical detection (HPLC-EC). HPLC-EC was performed on a Shimadzu HPLC system (Shimadzu, Kyoto), with a model LC-9A pump, and equipped with an electrochemical detector (model LC-4B Bas Co. Ltd., Tokyo) with a glassy carbon working electrode, a rheodyne loop injector (20 μL, Rheodyne, Cotati, CA), and a recorder (model C-R6A) attached to the HPLC system. Separation was made using Inertsil ODS-2 column, 4.6 mm i.d. × 15 cm (GL Science Co. Ltd., Tokyo). The mobile phase was 0.05M sodium phosphate buffer (pH 2.30) containing 1 μM ethylenediaminetetraacetic acid (EDTA). The mobile phase buffer was filtered through 0.2-μm membranes (Toyo Roshi) and degassed by ultrasonic generator (model 50a, MRK Inc., Tokyo) under reduced pressure before use. The flow rate was 0.7 mL/min. The column temperature was maintained at room temperature (25–28°C) and the potential of the detector was set at +0.6 V vs. Ag/AgCl reference electrode. AsA concentrations were 0.005–0.01 mg/100 g of sample.

Quantitative Analysis of AsA and DHA

Quantitative determinations of AsA were made using a calibration curve. The amount of DHA in the sample was estimated by the

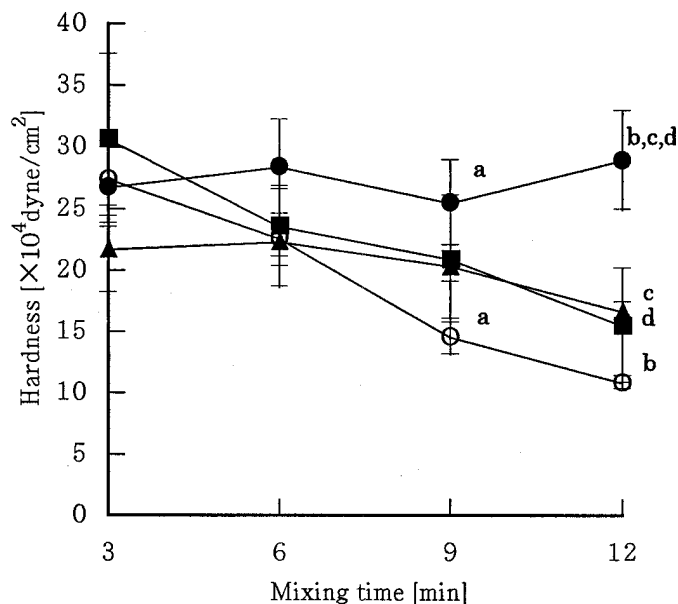


Fig. 1. Effect of additives on hardness of flour-water doughs. Control with no additives (○); 100 ppm of L-ascorbic acid (AsA) (●); 100 ppm of dehydro-L-AsA (DHA) (▲); 100 ppm of 2,3-diketo-L-gulonic acid (DKG) (■). Average ± standard errors of five or more replicates. Values with the same letters are significantly different (*P* < 0.01).

reduction of DHA to AsA with dithiothreitol (DTT). The reduction procedure was as follows. The pH values of the sample solution was adjusted to pH 7.4 with 0.05M phosphate buffer, and 1% DTT solution was added to a final concentration of 0.1% and incubated at 35°C for 30 min. After incubation, the sample was diluted with water suitable for HPLC analysis.

RESULTS AND DISCUSSION

Effect of AsA and Its Related Compounds on Dough Hardness

Dough hardness was used as a measure in the improvement in rheological properties of dough. The effect of AsA and its related compounds on dough hardness is illustrated in Figure 1. Control flour-water dough hardness decreased from ≈28 × 10⁴ dyne/cm² to 11 × 10⁴ dyne/cm² during mixing (3–12 min). Addition of AsA at 100 ppm showed no significant decrease in the hardness of dough. However, at 9 and 12 min, dough hardness was remarkably increased when compared to the control dough. Addition of DHA or DKG showed a tendency to increase the dough hardness after 12 min of mixing, although differences were not statistically significant. Thus, at 12 min of mixing, addition of AsA increased the hardness of water-flour dough. DHA or DKG had a similar but smaller effect on dough hardness.

The effect of a reducing agent such as GSH and an oxidizing agent such as *p*-quinone on the dough hardness is shown in Figure 2. The concentration of GSH, oxidized GSSG, and *p*-quinone was 0.0936 μmol/g of flour (concentration equivalent to 10 ppm of AsA). *p*-Quinone added to dough kept hardness at ≈30 × 10⁴ dyne/cm² during dough mixing. When GSSG was added to the dough during mixing, a similar softening effect was observed. Comparison of the hardness of the control and GSH or GSSG treated dough showed that GSH or GSSG reduced dough hardness rapidly within 6 min of mixing. After that time, a gradual decrease in hardness was observed up to 12 min. Addition of oxidizing agent increased the dough hardness. Addition of reducing agent decreased the dough hardness.

The addition of both GSH and GSSG at a level of 0.936 μmol/g of flour (concentration equivalent to 100 ppm of AsA) drastically reduced the dough hardness, making it almost impossible to measure. GSH is a tripeptide with two negatively charged amino

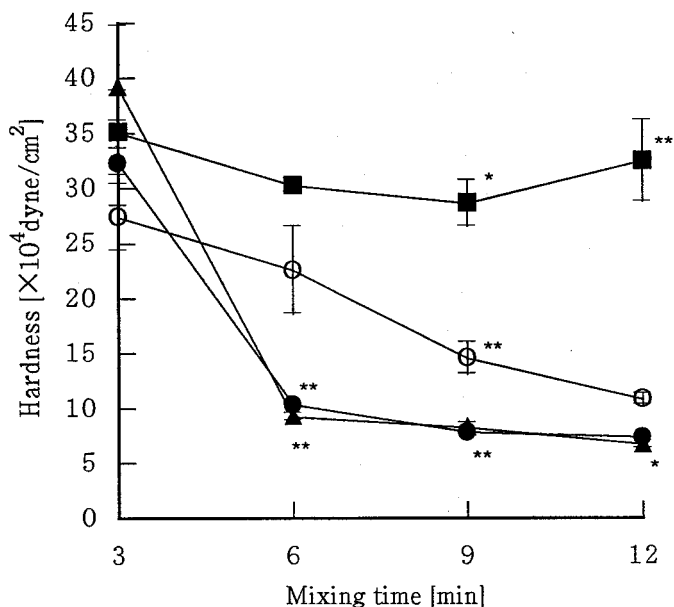


Fig. 2. Effect of glutathione or *p*-quinone additives (0.0936 μmol/g of flour) on hardness of flour-water doughs. Control with no additives (○); reduced form of glutathione (●); oxidized form of glutathione (▲); *p*-quinone (■). Average ± standard errors of four or more replicates. *,** = Significantly different at *P* < 0.05 and < 0.01, respectively.

acid residues. The most reactive functional group in GSH is the SH group of the cysteine side chain. As a reducing agent, this SH group of GSH might cleave a portion of the cross-linking SS bonds in protein and create free SH groups, leading to the decrease in the hardness of dough. In contrast, an oxidizing agent might create interprotein molecular SS bonds, thus increasing dough hardness.

It was suggested that AsA was rapidly oxidized to DHA in dough (Elkassabany et al 1980a,b; Nicholas et al 1980) and then DHA oxidized GSH to form GSSG in flour (Kuninori and Matsumoto 1963, 1964; Carter and Pace 1965; Bloksma 1972; Boeck and Grosch 1976; Mair and Grosch 1979; Walther and Grosch 1987). Thus, the oxidation of AsA to DHA is considered to be an improving factor of AsA in bread dough making. The experimental results of this study indicated that the reduction of hardness as a consequence of the addition of GSSG might be partly due to the increase in SH-SS interchange reaction among protein molecules in the dough.

From these data, it was suggested that improving function of AsA was due not only to its function as a reductant but as a potential oxidant. However, DHA, the major oxidant produced during the oxidation process of AsA, showed a small effect on the dough hardness (Fig. 1) and did not increase the dough hardness as much as AsA did. Thus the function of AsA as dough improver may be due to the development process of its electron-donating function or to reactive intermediates formed during oxidation process of AsA.

Effect of AsA Concentration on Dough Hardness

Further experiments were made to ascertain the effect of various amounts of AsA on dough hardness (Fig. 3). Dough with 10 ppm of added AsA kept its hardness at $\approx 25 \times 10^4$ dyne/cm². The hardness of dough seemed to increase with the increase in AsA concentration (10 and 100 ppm). Dough with 1,000 ppm of added AsA showed little change in hardness when compared with the control. This may be due to the slow oxidation rate of AsA at higher concentration. Consequently, it was considered that the oxidation process of AsA might play an important role in the hardness of wheat dough.

Determination of AsA in Dough

Parallel with the measurements of dough hardness as a rheological parameter, determinations of AsA and its oxidation products were also undertaken to correlate the degraded AsA amount to the corresponding change in rheological properties. Degradation

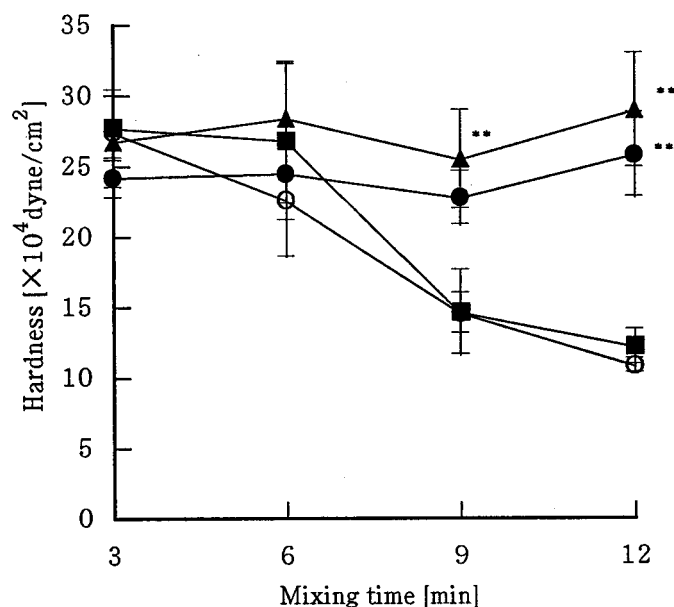


Fig. 3. Effect of added L-ascorbic acid (AsA) on hardness of flour-water doughs. Control with no additives (○); 10 ppm of AsA (●); 100 ppm of AsA (▲); 1,000 ppm of AsA (■). Average \pm standard errors of five or more replicates. ** = Significantly different at $P < 0.01$.

tion of AsA on flour-water dough kneaded for 12 min is shown in Figure 4. AsA added to the dough at the 10 ppm level was immediately oxidized to DHA in the first 3 min, ≈ 3 ppm of the AsA remained. After 3 min, the AsA concentration decreased gradually, and after 12 min, only 1.5 ppm of the added AsA remained. At 12 min, ≈ 7.3 ppm of total AsA (AsA+DHA) remained (Fig. 4C). AsA added to the dough at the 100 ppm level was partly oxidized to DHA after 12 min, ≈ 65 ppm of AsA remained. During 12 min of mixing, total AsA was kept at the original level of 100 ppm

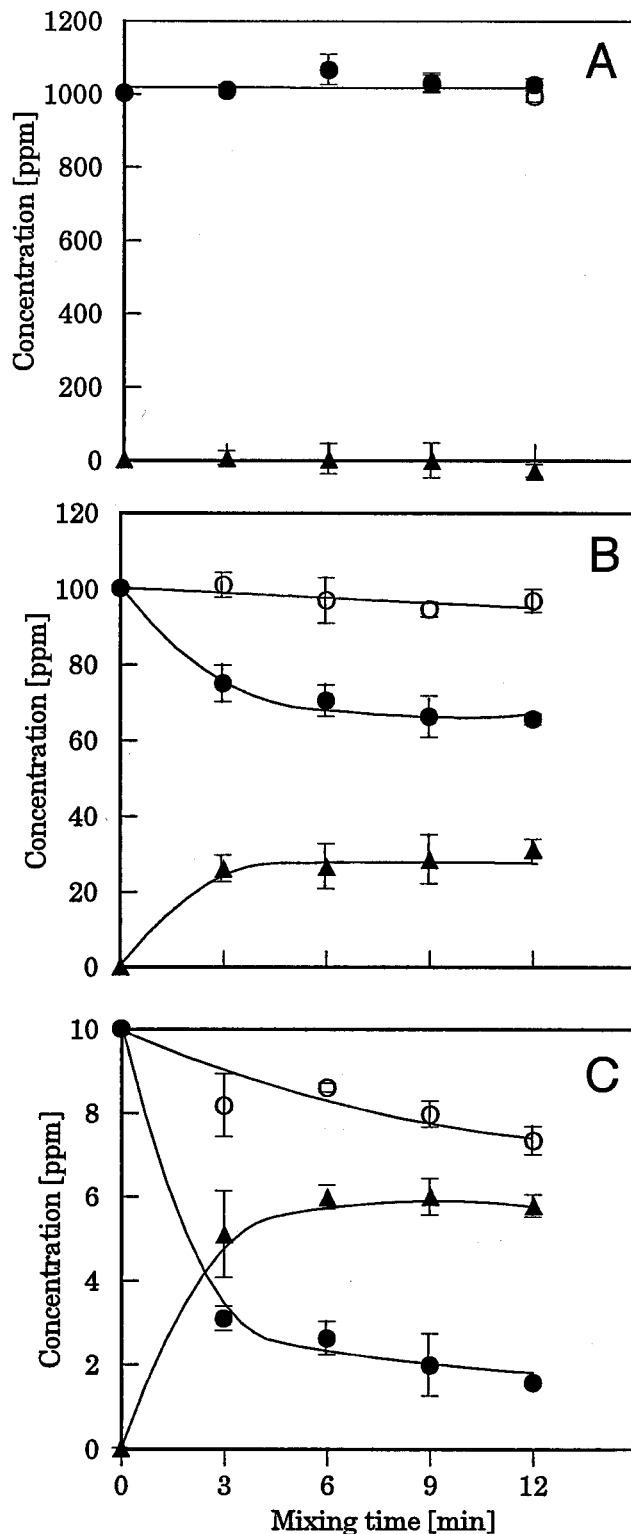


Fig. 4. Degradation of L-ascorbic acid (AsA) during dough mixing. A, 1,000 ppm; B, 100 ppm; C, 10 ppm. Total (○); AsA (●); dehydro-L-AsA (DHA) (▲). Average \pm standard errors of three or more replicates.

TABLE I
AsA Concentration (ppm) in Flour-Water Dough Containing Dehydro-L-AsA (DHA)^{a,b} at Various Mixing Times

0 min	3 min	6 min	9 min	12 min
0.09 ± 0.034 ^c	0.21 ± 0.022	0.20 ± 0.030	0.24 ± 0.080	0.26 ± 0.036

^a Added concentration of DHA was 100 ppm.

^b Remaining AsA in DHA measured by HPLC was ≈0.1%.

^c Values ± standard errors of three or more replicates.

(Fig. 4B). Thus, the hydrolysis reaction of DHA to DKG was not apparent in the oxidative degradation of AsA at 100 ppm. The results shown in Fig. 4B and C for different concentrations of AsA in the dough during mixing (10 and 100 ppm, respectively) were similar to the results reported by Elkassabany et al (1980). DHA was not detected in dough with AsA added at 1,000 ppm (Fig. 4A). The rate of AsA oxidation in dough depended on the concentration of AsA added. The same was true for AsA oxidation in aqueous solution (Lin and Agalloco 1979). Thus, the hardness of the dough containing AsA was confirmed to be related to the rate of AsA oxidation.

AsA concentration in flour-water dough with 100 ppm of DHA is given in Table I. DHA was prepared from AsA according to the method of Ohmori and Takagi (1978). Measured by HPLC, the remaining AsA in the DHA sample was ≈0.1%. However, throughout the 12 min of mixing, ≈0.1 ppm of AsA was formed in the dough. This small increase of AsA might be related to the tendency to increase hardness observed in the dough containing DHA, although it was not statistically significant.

From these data, it seems that the hardness of dough containing AsA was related to the oxidation of AsA, which possibly affected the inter- or intramolecular SH-SS interchange reaction that would contribute to the structure of protein. In the oxidation of AsA, both the oxidation of SH groups of protein and the SH-SS interchange reaction might have taken place, and the formation of intermolecular protein SS bonds might have occurred consequently. The oxidation of AsA in water at neutral pH is accelerated by superoxide (O₂⁻) generated by the transfer of an electron to dioxygen from a donor such as transition metals (Khan and Martell 1967a,b, and 1968; Ogata et al 1968). Oxygen radicals such as O₂⁻ promote the oxidative scission of proteins (Levine 1983; Shinar et al 1983; Uchida and Kawakishi 1987, 1989). Hence, the improvement mechanism of AsA on the rheological properties of flour-water dough was mostly due to the development of its electron-donating capacity and reactive products such as O₂⁻.

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