Stabilities of Several Forms of Vitamin C During Making and Storing of Pup-Loaves of White Pan Bread[1,2]

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ABSTRACT

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Pup-loaves of white pan bread were fortified with three commercial forms of fat-coated L-ascorbic acid (AsA) and with uncoated AsA at a level equivalent to 64 mg of sample per 100 g of flour (14% mb). Sample 1 contained large crystals of AsA coated with 30% fat (mp 58–71°C) and resisted leaching in 6% metaphosphoric acid at 25°C. Sample 3 contained mostly small crystals coated with 10% fat (mp 49–69°C) and lost 70% of its AsA during leaching. Proofed bread dough mixed with Sample 1 retained 80% of fat-coated AsA, whereas proofed bread dough mixed with Sample 3 retained only 7% of fat-coated AsA. Bread (up to three days old) fortified with Sample 1 showed ~20% higher retention of vitamin C than did bread fortified with Sample 3 or with uncoated AsA. Bread fortified with Sample 2 showed intermediate retention of vitamin C. Bread also was fortified with 64-mg equivalents of AsA per 100 g of flour in the forms of L-ascorbate 2-polyphosphate (AsPP) and L-ascorbate 2-monophosphate (AsMP). After mixing, fermenting, and proofing the dough, ~85% of AsPP and AsMP were hydrolyzed to AsA. 2-Phosphorylated AsA remaining in proofed dough largely survived the baking step, whereas the free AsA underwent approximately one-third destruction. After three days of storage, pup-loaves fortified with AsMP or AsPP retained 10–15% higher vitamin C than did those fortified with AsA.

Optimum health and longevity may depend upon adequate levels of antioxidant nutrients in the human diet (Gey et al 1993). One of those nutrients is L-ascorbic acid (AsA) or vitamin C. AsA is unstable in processed foods because it is oxidized and destroyed by air in the combined presence of moisture and certain metal ions (Liao and Seib 1988). To increase intake of vitamin C in the general population, it may be necessary to fortify staple foods.

In the United States, bread has been used as a carrier of added nutrients since the 1940s, (Bauerfeind and DeRitter 1991). However, AsA is unstable in bread (Hung et al 1987). Freshly baked bread fortified with 64 mg and 2,000 mg of AsA per 100 g of flour with no added enrichment retained 58 and 61% AsA, respectively. However, AsA was lost rapidly when the bread at the lower level of fortification was stored in polyethylene bags at 25°C. The objective of this investigation was to fortify bread with vitamin C using uncoated AsA, commercially available fat-coated AsA, and 2-phosphorylated forms of AsA (Fig. 1). The retention of AsA was followed through the breadmaking process and the bagged storage of the loaves.

MATERIALS AND METHODS

Materials

Crystalline AsA, 99.6% pure, was obtained from Fisher Scientific Co. (Pittsburgh, PA). Fat-coated AsA samples were gifts from Balchem Corp. (Slate Hill, NY); Van Den Bergh Foods Co. (Joliet, IL); and Takeda Chemical Industries, Ltd. (Osaka, Japan). Samples 1 and 2 were coated with hydrogenated soybean oil and contained 70% AsA by weight. Sample 3 was coated with hydrogenated vegetable oil and contained 90% AsA. L-Ascorbate 2-monophosphate (AsMP, magnesium salt) was donated by Showa Denko K. K. (Tokyo, Japan). Magnesium salt powder contained 46.8-mg AsA equivalents per 100 mg of flour and 18.4% moisture. L-Ascorbate 2-polyphosphate (AsPP, sodium salt) was prepared by X. Y. Wang in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, using the method of Liao and Seib (1990). Sodium AsPP powder contained 27.2-mg AsA equivalents per 100 mg of flour and 6.3% moisture. The AsPP salt was a mixture of predominantly L-ascorbate 2-triphosphate (AsTP), L-ascorbate 2-diphsophate (AsDP), and AsMP (Fig. 1). Bread flour with a protein level of 11.0% (14% mb) and no additives was obtained from Campbell-Taggart, Inc. (Dallas, TX). Dithiothreitol (DTT 99%) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Compressed yeast was obtained from Universal Foods Co. (Milwaukee, WI). All other chemicals were reagent grade.

General Methods

Protein (N × 5.7) and moisture contents of flour were determined, respectively, on a nitrogen determinator (Leco Corp., St. Joseph, MI) and in a forced-draft oven using method 44-15A (AACC 1983). Moistures of magnesium AsMP and sodium AsPP were determined using a rapid moisture analyzer (IR-100, Denver Instrument Co., Arvada, CO).

Scanning electron microscopy (SEM) was done using an ETEC Autoscan SEM (Perkin-Elmer Inc., Hayward, CA) at a magnification of 30×. Samples were mounted on specimen holders with colloidal graphite (Ted Pella Inc., Tustin, CA) and then sputter-coated with gold (Conductavac I, Seevac Inc., Deltona, FL) before viewing.

Differential scanning calorimetry (DSC) was done on a Perkin Elmer DSC-2 (Norwalk, CT) equipped with a flexi-cooler and

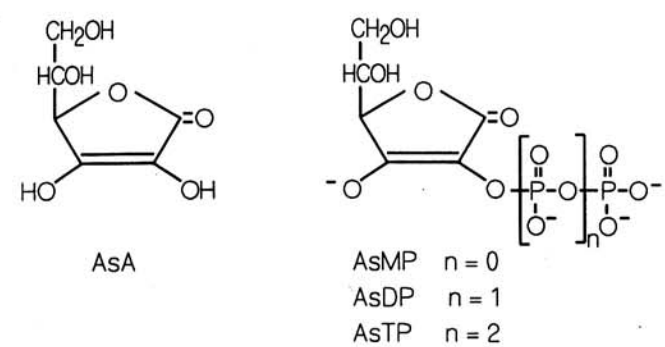

Fig. 1. Structure of L-Ascorbic acid (AsA) and its 2-phosphorylated esters: L-ascorbate 2-monophosphate (AsMP), L-ascorbate 2-diphsophate (AsDP), L-ascorbate 2-triphosphate (AsTP).

[1]Cooperative investigation between the USDA-ARS and the Kansas Agricultural Experiment Station, Manhattan. Mention of a propriety product does not constitute an endorsement by the USDA.

[2]Contribution 94-281-J of the Kansas Agricultural Experiment Station.

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temperature controller (FTS Systems, Inc., Stone Ridge, NY). Samples (5–10 mg) were heated at a rate of 10°C/min from 27 to 220°C at a sensitivity at 0.5 mcal/sec.

Particles of the three samples of fat-coated AsA were separated on U.S. standard sieves with openings of 500, 425, 350, and 250 μm. The sieves were stacked and shaken in a circular motion ~50 times by hand, and the weights of the overs and throughs were recorded.

Breadmaking

Pup-loaves were made from 100 g of flour (14% mb) using a slight modification of the straight-dough procedures of Finney (1984) and AACC approved methods (AACC 1983). The bread formula contained instant dry yeast (1.6 g). Dough was mixed to optimum development and fermented for 90 min at 37°C, proofed for ~40 min at 37°C, and baked for 21 min at 219°C.

Bread was fortified with all forms of vitamin C at a level equivalent to 64 mg of AsA per 100 g of flour. The AsMP and AsPP were added immediately before mixing as freshly prepared aqueous solutions (20 ml of 18 m*M*). In other loaves, uncoated AsA or fat-coated AsA was dry-blended with the flour, dry yeast, and shortening in the mixing bowl for 30 sec before addition of liquid ingredients. Some doughs were assayed without drying, whereas others were lyophilized immediately after mixing or proofing. The dried doughs were ground in a high-speed blender to pass a U.S. No. 200 wire-mesh screen, and the ground material was stored at −20°C until assay. After baking and cooling, the loaves were sampled (day 0) or placed in polyethylene bags (0.038 mm thickness) and stored for one to seven days at room temperature. Loaves were sliced, lyophilized, ground, and stored at −20°C until assay.

AsA Content and Aqueous Leaching of Fat-Coated AsA

AsA content was determined using high-performance liquid chromatography with electrochemical detection (HPLC-EC) (Wang et al 1988). The injection loop of the system had a volume of 20 μl.

Fat-coated AsA (0.25 g) was stirred on a magnetic stirrer with 50 ml of a chloroform and methanol mixture (2:1, v/v) at 25°C. Aqueous 6% metaphosphoric acid containing 0.2% DTT (50 ml) was added with stirring. The mixture was then transferred to a separatory funnel. After vigorous shaking and phase separation, an aliquot (1 ml) of the aqueous layer was made to volume (25 ml) with 6% metaphosphoric acid and 0.2% DTT. An aliquot (75 μl) of the resulting solution was made to volume (25 ml) with cold 0.05*M* perchloric acid. The diluted mixture was filtered through a syringe filter (pore size: 0.45 μm, diameter: 3 mm) (Gelman Science, Ann Arbor, MI) and then injected into the chromatograph.

Aqueous leaching of fat-coated AsA (0.25 g) was done in 6% metaphosphoric acid and 0.2% DTT (100 ml) at room temperature. At various times, an aliquot (0.1 ml) of the mixture was taken after slight agitation and made to volume (25 ml) with 0.05*M* cold perchloric acid. After filtration through a syringe-filter, the solution was injected into the chromatograph.

Total and Free AsA Levels in Dough and Bread Fortified with Uncoated and Fat-Coated AsA

The levels of free and total AsA were determined in dough, and the level of fat-coated AsA was calculated as the difference. Only free AsA was detected in bread fortified with fat-coated AsA. To assay for total AsA in bread, lyophilized and ground bread (1 g) was extracted vigorously at 25°C with a solution of 6% aqueous metaphosphoric acid and 0.2% DTT (40 ml) on a magnetic stirrer for 15 min. After centrifugation for 20 sec at 8,100 × *g*, an aliquot (1 ml) of the clear supernatant was made to volume (25 ml) with cold 0.05*M* perchloric acid. The resulting solution was syringe-filtered and injected into the chromatograph.

To assay for free AsA in dough containing fat-coated AsA, a portion (2 g) of wet dough was immersed in the 6% metaphosphoric acid and 0.2% DTT solution (100 ml) at 25°C. The remainder of the dough was lyophilized and saved for determination of total AsA. The metaphosphoric acid mixture was stirred very gently by hand for 30 min, using a glass rod to minimize disturbance of the fat coating. After stirring, the extract was analyzed for AsA as before. In all assays, replicate samples were analyzed, and triplicate injections were made into the chromatograph.

To assay for total AsA in dough fortified with fat-coated AsA, the freeze-dried and ground dough (1 g) was added to 50 ml of a mixture of chloroform and methanol (2:1, v/v), and the slurry stirred for 1 hr at 25°C. Then 40 ml of the 6% metaphosphoric and 0.2% DTT solution was added, and the mixture vigorously stirred for 15 min. The aqueous layer was centrifuged; an aliquot (1 ml) of the clear supernatant was made to volume (25 ml) in cold 0.05*M* perchloric acid; and the solution was treated as before.

Total AsA in Dough and Bread Fortified with AsMP and AsPP

The level of AsMP and AsPP remaining in dough or bread was calculated as the difference between total and free AsA (Wang et al 1988). To determine total AsA, lyophilized and ground dough or bread (1 g) was extracted for 15 min at 25°C with 40 ml of a 6% metaphosphoric acid and 0.2% DTT solution. After centrifugation for 20 sec at 8,100 × *g*, an aliquot (1 ml) of the supernatant was made to volume (10 ml) with aqueous 0.13*M* sodium acetate buffer containing 0.2% DTT and 1.1% compressed yeast. Acid phosphatase (9–10 mg) was added to the medium, and the mixture digested for 3 hr under mild agitation on a stir-plate at ~37°C. An aliquot (1 or 2 ml) of the digest was made to volume (10 ml) with cold 0.05*M* perchloric acid. After centrifugation, a small volume of the supernatant solution was syringe-filtered and injected immediately into the chromatograph. The levels of free AsA in doughs and breads fortified with the L-ascorbate 2-phosphates were determined in exactly the same manner as that for total AsA, except the phosphatase addition was omitted. In all assays, replicate samples were analyzed and triplicate injections were made into the chromatograph.

RESULTS AND DISCUSSION

Commercial Samples of Fat-Coated AsA

Sample 1 of fat-coated AsA comprised large and relatively uniform particles of which 67% were >500 μm in size, and none were <250 μm (Table I). SEM showed that the particles in Sample 1 were coated with a continuous and smooth film (Fig. 2). Sample 2 contained a large proportion of particles with sizes 425–500 μm, but also a substantial proportion (30%) of particles <250 μm (Table I). The coating on some particles in Sample 2 was not smooth (Fig. 2), either because of underlying surface irregularities or because of irregular application of the fat coating. Sample 3 contained the smallest particles, with ~75% being <350 μm (Table I). In theory, small particles require more add-on of coating material to cover the increased surface area. Surprisingly, Sample 3 was coated with only 10% fat compared to 30% for Samples 1 and 2. The fat coating in Sample 3 showed signs of peeling from the AsA crystals (Fig. 2).

Aqueous Leaching Tests and Thermal Properties of Fat-Coated AsA

The quality of the fat-coating on AsA was assessed by leaching tests in water at 25°C. Sample 3 rapidly released 60% of its AsA; an additional 10% was released over the next 30–150 min; after that time, no further leaching was observed up to 420 min (Fig. 3). Sample 1, on the other hand, showed remarkable stability against leaching: no AsA was released within 30 min; and at 420 min, only 2% had been released. Sample 2 was of intermediate quality; it released AsA slowly and linearly up to 420 min, at which time 24% AsA had been leached (Fig. 3). Particle-size analysis of Sample 2 showed the presence of 30% small particles (Table I) that may not have been adequately coated.

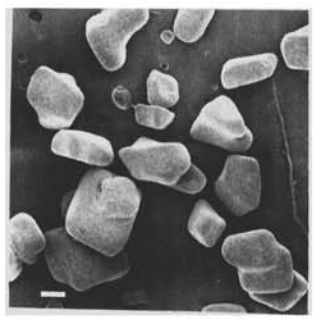
Not only is the the continuity of the fat coating important, but also its melting properties. When examined by DSC, each sample showed two endotherms between 48–71°C and 190–201°C

(Table II). Tristearin and tripalmitin are model compounds for the hydrogenated triglycerides used to coat AsA. Tripalmitin and tristearin melt at 66 and 73°C, respectively; L-ascorbic acid melts at 190–191°C. The fat used in Sample 1 melted at almost 10°C higher than did the fats in Samples 2 and 3. The excellent stability of Sample 1 to aqueous leaching was due to large and uniform crystals with smooth surfaces that were coated uniformly with fat that melted at a higher temperature.

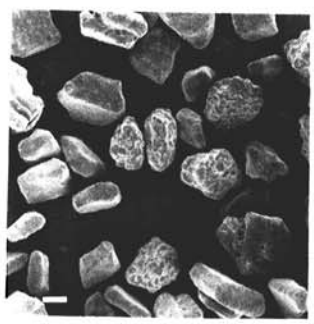
Stability of Vitamin C in Dough Fortified with Uncoated and Fat-Coated AsA

Immediately before baking, the total AsA retained in proofed doughs was 97–100% of that added at the mixer (Table III). The high survival of AsA in dough was due to the presence of yeast. Yeast almost instantly consumes the oxygen in a dough during mixing, which gives an anaerobic environment during fermentation and proofing of dough. The net result is protection of AsA from oxidation in yeasted dough (Elkassabany et al 1980, Seib 1985).

Figure 4 shows the levels of free AsA released from the fat-coated samples into doughs immediately before baking. Samples 1 and 2 released 20 and 50% of total AsA added, respectively; Sample 3 released 92% of AsA added. Most of the coating of Sample 3 was destroyed during mixing, fermenting, and proofing. The order of release of AsA from the coated samples in proofed dough correlated directly with the order found in the leaching tests. However, the extent of release in dough under our conditions was higher than that observed in leaching, probably because of shear during dough mixing and heat during proofing.



Sample 1



Sample 2

Sample 3

Fig. 2. Scanning electron micrographs (30×) of three commercial samples of fat-coated L-ascorbic acid.

TABLE I
Particle-Size Distributions of Commercial Samples of Fat-Coated L-Ascorbic Acids

Sieve Opening (μm)	Weight Over Sieve, %		
	Sample 1	Sample 2	Sample 3
500	62	5	6
425	17	39	16
350	14	13	5
250	7	12	39
250	0	30	34

Stability of Vitamin C During Baking and Storage of Pup-Loaves Fortified with Uncoated and Fat-Coated AsA

The retention of AsA in pup-loaves containing the fat-coated samples (Fig. 5) was related inversely to the level of free AsA in the proofed doughs. Thus, the fat coating on AsA inhibited the destruction of AsA during baking. Proofed dough fortified with Sample 1 retained almost 80% of added AsA in coated form

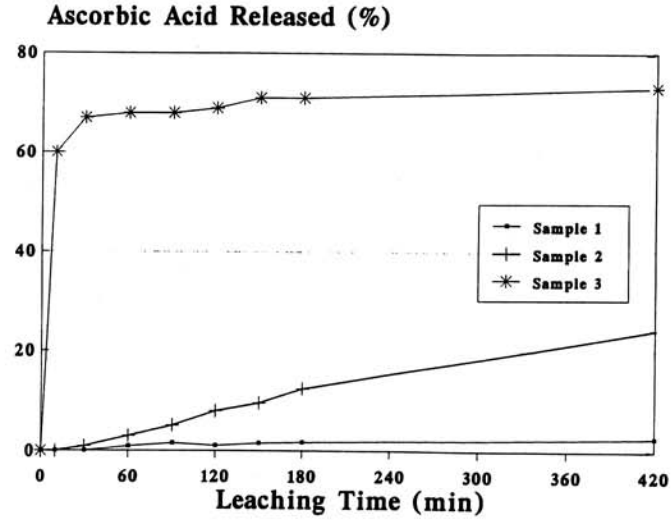


Fig. 3. L-Ascorbic acid (AsA) leached at 25°C from commercial samples of fat-coated AsA. One part of fat-coated sample was stirred gently in 100 parts of 6% aqueous metaphosphoric acid and 0.2% dithiothreitol.

TABLE II
Endotherms in Differential Scanning Calorimetry of Commercial Samples of Fat-Coated L-Ascorbic Acids (AsA)[a]

Sample	Fat-Coating			AsA		
	T_o (°C)	T_p (°C)	ΔH (cal/g)	T_o (°C)	T_p (°C)	ΔH (cal/g)
1	58	71	35	190	199	67
2	48	68	31	190	199	63
3	49	69	59	191	201	66

[a] Enthalpy (ΔH) values were calculated based on the level of coating reported by the manufacturer. T_o and T_p are initial and peak temperatures, respectively.

TABLE III
Vitamin C Stability in Dough and Bread Fortified with Fat-Coated and Uncoated L-Ascorbic Acids (AsA)[a]

Sample	Age (days)	Uncoated	Fat-Coated		
			Sample 1	Sample 2	Sample 3
Dough[b]	...	63.5 ± 0.4 a	64.0 ± 2.9 a	62.1 ± 2.9 a	63.4 ± 1.7 a
Bread	0	38.0 ± 0.2 a	51.2 ± 0.3 b	47.2 ± 1.0 c	38.0 ± 0.8 a
Bread	1	20.3 ± 1.3 a	37.6 ± 1.0 b	30.3 ± 2.1 c	20.4 ± 1.0 a
Bread	3	6.6 ± 0.3 a	22.1 ± 1.9 b	15.0 ± 1.3 c	6.6 ± 0.4 a
Bread	5	1.5 ± 0.8 a	8.8 ± 0.4 b	5.1 ± 0.3 c	2.2 ± 0.3 a
Bread	7	1.0 ± 0.6 a	6.7 ± 0.8 b	3.7 ± 0.4 c	1.0 ± 0.2

[a] 64-mg AsA equivalents per 100 g of flour, 14% mb.
[b] Proofed dough immediately before baking. All doughs were fortified with 64-mg equivalents of AsA per 100 g of flour. Breads were held unsliced in polyethylene bags at 25°C. Values of AsA retained in breads and doughs are reported in milligrams of AsA equivalents per pup loaf. Means in the same row not sharing a common letter are significantly different ($P < 0.05$) by t-test.

and 20% in the free form (Fig. 4). The freshly baked loaves with Sample 1 retained 80% of AsA (Fig. 5). Because the fat coatings melted below 95°C, the AsA in bread was all in free form. Proofed dough containing Sample 3 retained 7% of added AsA in coated form and 92% in free form (Fig. 4). The baked bread containing Sample 3 retained only 59% of AsA (Fig. 5).

The rate of loss of AsA was the same in all stored pup-loaves (Fig. 5). However, stored bread that had been baked with Samples 1 or 2 contained a higher concentration of AsA than did that baked with Sample 3 or with uncoated AsA (Fig. 5 and Table III). This is because the coatings on Samples 1 and 2 partially protected AsA during baking. In this work, fat-coated AsA Sample 1 gave the highest retention of vitamin C in bread among all forms tested. The rate of loss of AsA previously observed by Hung et al (1987) was slower than that found in this study. Many variables affect the rate of destruction of AsA in bread. The most likely differences are different gauge of polyethylene film or the concentrations of copper and iron ions in the bread.

White pan bread sold in supermarkets is retrieved by wholesale bakers when it is three days old. One serving size (1 slice, 28 g) of three-day-old bread fortified with 64-mg AsA equivalents from Samples 1, 2, or 3 would provide 7, 5, or 2%, respectively, of the adult recommended dietary allowance of vitamin C. Sample 1, with its almost perfect fat coating, gave a two- to threefold improvement in vitamin C levels in bread for up to three days of storage compared to the vitamin C levels in bread with uncoated AsA (Table III). We would expect that pound-loaves (300 g of flour) would retain more AsA after baking than would pup-loaves (100 g of flour). The pup-loaves have an increased crust-to-crumb ratio, and loss of AsA may be high in the crust.

We should mention that AsA losses observed in this work were measured on bread with unenriched flour. Using enriched flour would not increase AsA losses if reduced iron was used in the enrichment, but losses would dramatically increase if ferric or ferrous salts were added (K. S. Ra and X. Y. Wang, *personal communication).*

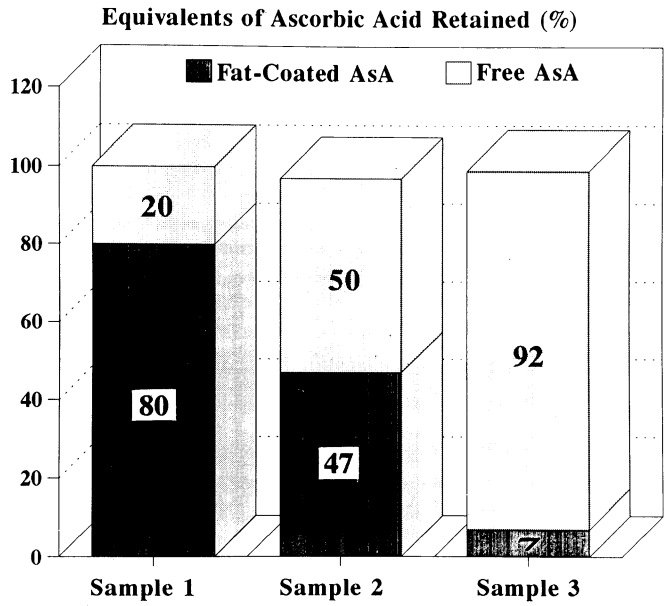


Fig. 4. Proportion of free and fat-coated L-ascorbic acid (AsA) in proofed bread dough immediately before baking. All doughs were fortified with 64-mg AsA equivalents per 100 g of flour (14% mb).

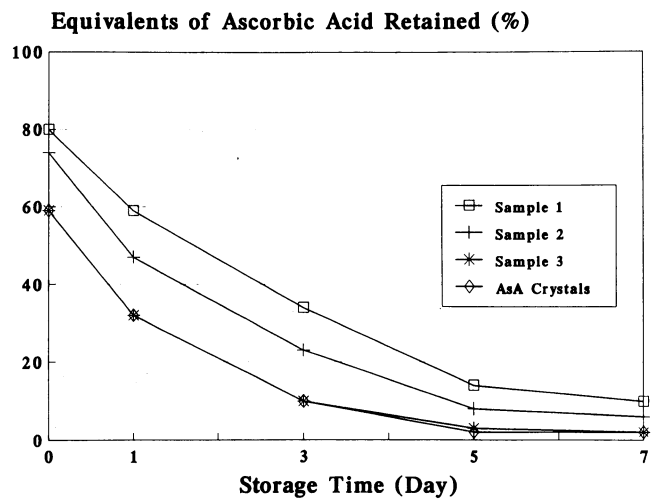


Fig. 5. Stability of L-ascorbic acid (AsA) in stored bread fortified with fat-coated AsA and with free AsA. All breads were fortified with 64-mg AsA equivalents per 100 g of flour (14% mb). Loaves were stored unsliced in polyethylene bags at 25°C.

Hydrolysis of AsPP and AsMP to AsA in Bread Dough

Because AsA losses were extensive in stored bread, we examined the use of 2-phosphorylated forms of L-ascorbate. AsMP and AsPP are equivalent to AsA in vitamin C potency (Machlin et al 1979, Liao and Seib 1990). However, wheat contains acid

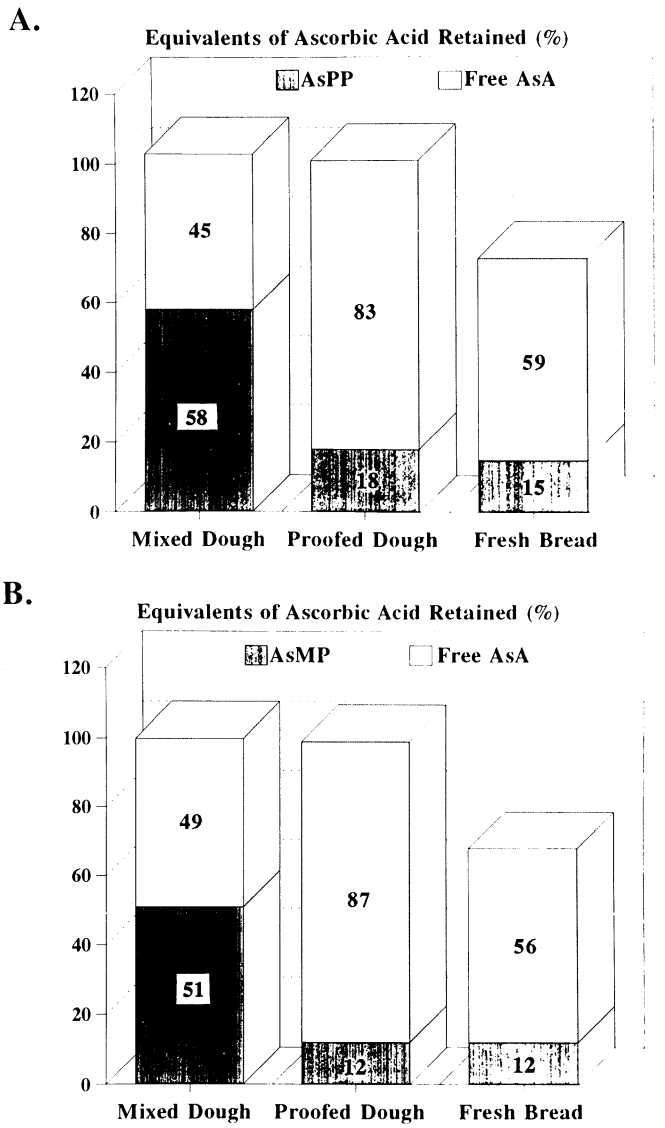


Fig. 6. Hydrolysis of **A,** L-ascorbate 2-polyphosphate (AsPP). **B,** L-ascorbate 2-monophosphate (AsMP) at three stages of the breadmaking process. Doughs were fortified with 64-mg AsA equivalents per 100 g of flour (14% mb) and mixed for 5 min. Doughs were fermented and proofed at 37°C for 90 and 40 min, respectively. Bread was baked at 219°C for 21 min.

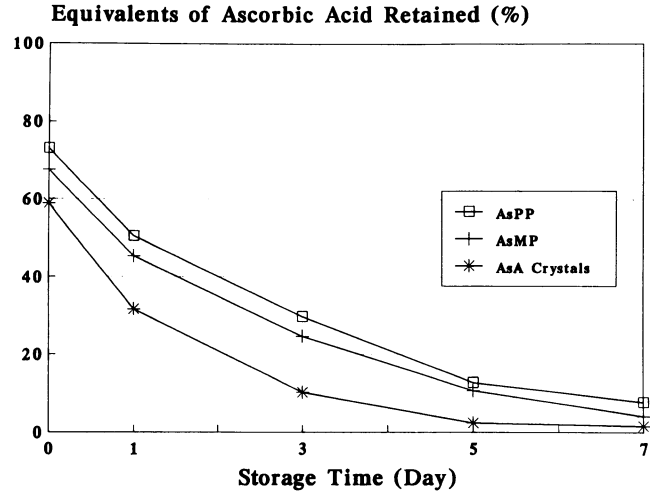


Fig. 7. Retention of L-ascorbic acid (AsA) equivalents in stored bread fortified with L-ascorbate 2-monophosphate (AsMP), L-ascorbate 2-polyphosphate (AsPP), and free AsA at levels equivalent to 64 mg of AsA per 100 g of flour (14% mb). Loaves were stored unsliced in polyethylene bags at 25°C.

phosphatase (Stauffer 1987), and wheat flour contains a phosphatase with activity towards a variety of organophosphate esters (Booth 1944).

We followed the hydrolysis of AsPP and AsMP to AsA during dough mixing, proofing, and baking. After being mixed with added AsPP for 5 min, dough retained all added equivalents of AsA; 45% was present as free AsA and 58% remained as AsPP (Fig. 6A). During the next 130 min, when the dough was fermented and proofed, an additional 38% of AsPP was converted to AsA, resulting in 83% conversion in the proofed dough. Finally, the fresh bread baked from the dough contained 74% of the added equivalents of AsA; 59% was present as free AsA and 15% was in AsPP form (Fig. 6A).

The results of adding AsMP to bread dough were similar to those of adding AsPP, except that the quantities of AsMP retained after mixing, proofing, and baking were 7, 6, and 3% less, respectively (Fig. 6B). The extra phosphate groups released during enzymolysis of AsPP appeared to increase the competitive inhibition of phosphatase, which explained the somewhat increased level of retention for AsPP over that for AsMP in bread.

We should emphasize that little or no loss of 2-phosphorylated L-ascorbate occurred during the mixing, fermenting, and proofing steps (Fig. 6). Instead, the AsA equivalents lost during baking were mainly from the free form in proofed dough. Because AsPP and AsMP in the proofed dough survived the baking step, these forms of vitamin C would be ideal for fortifying bread if hydrolysis of the phosphate ester could be inhibited during mixing, fermenting, and proofing.

Adding Inorganic Phosphate to Bread Dough to Inhibit Phosphatase Action on AsMP

Brouillard and Ouellet (1965) showed that orthophosphate (0.166 m*M*) gave 50% inhibition of the three isozymes of acid phosphatase purified from wheat germ. We added inorganic phosphate in the form of sodium monobasic phosphate (1 g/100 g of flour) to bread dough to inhibit phosphatase activity. The hydrolysis of AsMP to AsA in mixed dough decreased by one third compared to that in dough without added inorganic phosphate (16 vs. 49%). However, after proofing, the conversion of AsMP to AsA was almost identical in the doughs with (86%) and without (87%) added inorganic phosphate.

The addition of sodium monobasic phosphate to dough increased mixing, fermenting, and proofing times by 29, 10, and 22%, respectively. We concluded that adding inorganic phosphate to dough was not feasible because of its detrimental effects on dough processing. Furthermore, the bread had a bitter aftertaste.

TABLE IV
Vitamin C Stability in Dough and Bread Fortified with L-Ascorbate 2-Monophosphate (AsMP), L-Ascorbate 2-Polyphosphate (AsPP), and L-Ascorbic Acid (AsA)[a]

Sample	Age (days)	AsA	AsPP	AsMP
Dough[b]	...	63.5 ± 0.4 a	64.0 ± 1.2 a	64.0 ± 1.1 a
Bread	0	38.0 ± 0.2 a	46.7 ± 0.6 b	43.3 ± 0.5 c
Bread	1	20.3 ± 1.3 a	32.3 ± 0.1 b	29.0 ± 0.6 c
Bread	3	6.6 ± 0.3 a	19.1 ± 1.2 b	15.8 ± 0.3 c
Bread	5	1.5 ± 0.8 a	8.1 ± 0.5 b	2.6 ± 0.3 c
Bread	7	1.0 ± 0.6 a	4.9 ± 0.4 b	2.6 ± 0.3 c

[a]64-mg AsA equivalents per 100 g of flour (14% mb).
[b]Proofed dough immediately before baking. All doughs were fortified with 64-mg equivalents of AsA per 100 g of flour. Breads were held unsliced in polyethylene bags at 25°C. Values of AsA retained in breads and doughs are reported in milligrams of AsA equivalents per pup loaf. Means in the same row not sharing a common letter are significantly different ($P < 0.05$) by *t*-test.

Stability of Vitamin C in Bread Fortified with AsA, AsMP, and AsPP

Figure 7 and Table IV show the retention of total AsA equivalents during room-temperature storage of bagged pup-loaves fortified with vitamin C in the forms of AsMP and AsPP. The fresh breads fortified with AsMP and AsPP retained 12 and 15% of total AsA in the original forms (Fig. 6), which explains the increased levels of total AsA retained during storage of those loaves (Fig. 7). AsPP gave consistently higher retention (~5%) of total AsA in bread than did AsMP, which supports the concept of a secondary mechanism by which 2-phosphorylated L-ascorbate protects vitamin C in bread. Inorganic phosphate released by phosphatase action would surround the released AsA in dough and chelate metal ions, thereby hindering oxidation of AsA in bread.

CONCLUSIONS

L-Ascorbic acid uniformly coated with 30% fat (mp 58–71°C) gives two to three times better retention of vitamin C than does uncoated AsA in one- to three-day-old pup-loaves fortified with 64 mg of AsA/100 g of flour (14% mb). One slice of three-day-old bread fortified with the fat-coated AsA would provide 7% of the adult recommended dietary allowance of vitamin C. The phosphatase in wheat dough hydrolyzes the phosphate esters on 2-phosphorylated derivatives of AsA. After mixing (5 min), fermenting (90 min), and proofing (40 min), ~85% of AsMP and AsPP are hydrolyzed to AsA. The AsMP and AsPP that remains in the proofed dough largely survives the baking step. Pup-loaves fortified with AsMP and AsPP at a level of 64-mg equivalents AsA per 100 g of flour show 10–15% higher retention of AsA for up to five days of storage at 25°C compared to loaves baked with AsA.

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