

Fortifying Bread with Each of Three Antioxidants¹

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ABSTRACT

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White bread was fortified individually with fat-coated L-ascorbic acid (AsA), cold-water-dispersible (CWD) β -carotene, and CWD all-*rac*- α -tocopheryl acetate (ToAc) at levels of 64, 5, and 100 mg, respectively, of active ingredient per 100 g of flour (14% mb). The freshly baked pup-loaves retained 76, 67, and 96% of added antioxidant, respectively. To extract ToAc quantitatively from bread or dough, dimethyl sulfoxide (DMSO) gave better results than hexane, which indicated that ToAc was in a partially bound state. "Protein-encased" (PE) β -carotene did not impart a yellow color to bread crumb and had one-fourth higher retention in fresh bread when compared to CWD β -carotene. In loaves stored at

25°C for one to seven days, AsA disappeared rapidly and PE β -carotene disappeared slowly; CWD β -carotene and ToAc were stable. In spite of storage loss, bread fortified with PE β -carotene retained significantly higher levels of β -carotene when compared to CWD β -carotene. One serving size (one slice, 28 g) of three-day-old bread fortified with one of the three antioxidants was calculated to provide 7, 120–150, and 13–16%, respectively, of the adult recommended daily allowances (RDA) for vitamins C, E, and A. When bread was fortified with both fat-coated AsA and CWD β -carotene and stored for five days, no protecting effect on the retention of the antioxidants was found.

Chronic diseases are those that develop over a lifetime, and two such diseases, cardiovascular disease and cancer, account for roughly 75% of all mortalities in the United States (Leaf 1988). Many hypotheses have been proposed to account for chronic diseases at the cellular level. Although no single explanation is sufficient, the free radical hypothesis appears to have some merit (Ames et al 1993, Harman 1993, Sohal and Weindruch 1996). As a result, antioxidants such as vitamin C, vitamin E, and β -carotene have become the focus of studies to determine their protective role because of their ability to scavenge free radicals. Reflecting this view, U.S. Department of Health and Human Service (Anon 1990) recommended Americans increase their intake of fruits and vegetables, which are often rich in antioxidants. Increasing the supply of vitamins E and C, and β -carotene (provitamin A) in staple foods such as bread may provide a safety net for those at the lower end of the economic spectrum. However, recent clinical trials indicated that supplementation of β -carotene in the human diet did not reduce the incidence of malignant neoplasm and cardiovascular disease (Hennekens et al 1996) and raised the possibility of inducing lung cancer among smokers (Omenn et al 1996).

Attempts have been made before to fortify bakery products with antioxidants. Hung et al (1987) compared L-ascorbyl 6-palmitate to fat-coated L-ascorbic acid (AsA) as a source of vitamin C in bread. When either antioxidant was added at a level equivalent to 64 mg AsA per 100 g of flour, the freshly baked bread retained 51% L-ascorbyl 6-palmitate and 58% AsA, but during storage of bread at 25°C, AsA was lost ~3 times faster than L-ascorbyl 6-palmitate. Park et al (1994) fortified bread with three forms of AsA including fat-coated AsA, L-ascorbate 2-polyphosphate, and crystalline AsA, again at the level of 64 mg of AsA eq per 100 g

of flour. At this level, one slice of bread (28 g) would provide 20% of the U.S. adult recommended daily allowance (RDA) (60 mg/day) of vitamin C, if no AsA was lost in breadmaking. Among the three forms of AsA tested, fat-coated AsA gave the highest retention of AsA during breadmaking and storage of bread at 25°C. Three-day-old bread retained 35% from fat-coated AsA, 30% from L-ascorbate 2-polyphosphate, and 10% from crystalline AsA. L-Ascorbyl 6-palmitate and L-ascorbate 2-polyphosphate are not food-approved forms of vitamin C.

When all-*rac*- α -tocopheryl acetate (ToAc) was incorporated into a bread formulation as the source of vitamin E, it showed good stability during processing and storage of bread (Cort et al 1976).

Rogers et al (1993) studied the stability of added β -carotene in selected bakery products. β -Carotene was added to each product at a level of ~1 mg of β -carotene per serving. β -Carotene losses during baking ranged from ~20% in bagels and cakes, to ~30% in cookies. No further loss of β -carotene occurred during storage at room temperature.

The objectives of this study were to fortify bread individually with vitamins C, E, and β -carotene and to demonstrate analytical methods for their determinations in dough and bread. Also, the stabilities of the three nutrients were determined in bread stored at room temperature. In a subsequent study (Park et al 1997), we fortified bread with a combination of the three antioxidants together with dietary fiber.

MATERIALS AND METHODS

Ascorbic acid coated with 30% by weight of hydrogenated soybean oil was a gift from Balchem Corp. (Slate Hill, NY). The following were gifts of Hoffmann-La Roche, Inc. (Nutley, NJ): cold water dispersible (CWD) all-*rac*- α -tocopheryl acetate (ToAc) with 50wt% all-*rac*- α -tocopheryl acetate as an equimolar mixture of eight diastereomers; CWD β -carotene with 10wt% active ingredient; and protein-encased (PE) β -carotene with 10wt% active ingredient. The CWD ToAc (100 mg) was calculated (Machlin 1991) to contain 33.6 mg of RRR- α -tocopherol equivalents (α -TE), and CWD and PE β -carotene (100 mg) to contain 10 mg of all *trans*- β -carotene equivalent to 1.67 mg of retinol equivalents (NAS 1989). Besides the active ingredient, CWD ToAc contained maltodextrin and fish gelatin; CWD β -carotene contained sucrose, fish gelatin, food starch, and peanut oil; and PE β -carotene contained fish gelatin, corn starch, fructose, and glycerol. An endoprotease (Maxatase XL-550,000), from *Bacillus*, was a gift from

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Genencor, Intl. (Rochester, NY). The enzyme was supplied in aqueous propylene glycol with 6.5% protein, and its activity was 550,000 Delft units/gram as defined by the manufacturer. Reference standards for assays were AsA from Fisher Scientific (St. Louis, MO); β -carotene from Sigma Chemical Company (St. Louis, MO); and ToAc from Aldrich Chemical Corp. (Milwaukee, WI). All other chemicals were reagent grade.

Wheat flour was Regional Baking Standard (RBS-93) from the USDA/ARS Hard Winter Wheat Quality Laboratory, Grain Marketing and Production Research Center, Manhattan, KS. The flour contained no additives and had a protein content of 12.0% on a 14% mb. Shortening, instant dry yeast, and malt (50–60 dextrizing units) were from Bunge Foods (Bradley, IL), Fleishmann's Foods (Portland, OR), and Cargill Flour Milling Co. (Wichita, KS), respectively. One dextrizing unit of malt was defined as the grams of soluble starch dextrinized by 1 g of malt in 1 hr at 20°C with an excess of β -amylase added (Standstedt et al 1939). The loss of the blue iodine-staining of starch was the end-point for complete dextrinization.

General Methods

Protein content ($N \times 5.7$) of flour was determined by a Nitrogen Determinator (Leco Corp., St. Joseph, MI) and moisture content by AACC Method 44-15A (1995). Crumb and crust color of bread were measured on one-day-old loaves with a Minolta Chroma Meter (CR-210, Minolta Co., LTD, Ramsey, NJ). The instrument was calibrated with a white tile with $L^* = 97.83$, $a^* = -0.43$, and $b^* = 1.92$, where L^* indicates lightness, $-a^*$ to $+a^*$ indicates green to red, and $-b^*$ to b^* indicates blue to yellow. Total color difference ΔE^* was calculated as: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Crust color was measured on the top of loaves, and crumb color on 0.5 in. (12.7 mm) thick slices produced by a slicer (Thomson Machine Co., Belleville, NJ). All measurements were replicated twice.

Breadmaking

Pup-loaves were made from 100 g of flour (14% mb) and instant dry yeast (1.6 g) using a slight modification of the straight-dough procedure (Finney 1984) and AACC Method 10-10B (1995). 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. The forms and levels of nutrients added per 100 g of flour (14% mb) were fat-coated AsA eq to 64 mg of AsA, CWD or PE β -carotene eq to 5 mg of β -carotene, and CWD ToAc eq to 100 IU of vitamin E. Fat-coated AsA (91.3 mg) and PE β -carotene (53 mg) were dry-blended with the flour, dry yeast, and shortening in the mixing bowl (National Mfg. Co., Lincoln, NE) for 30 sec before adding liquid ingredients. CWD β -carotene (50 mg) and CWD ToAc (200 mg) were added as freshly prepared, aqueous dispersions immediately before mixing.

After proofing, some doughs were quartered; one quarter of a dough was lyophilized and the remaining three quarters were discarded. During lyophilization, bottles containing dough or bread fortified with β -carotene were wrapped with aluminum foil to avoid light-induced degradation. Lyophilized doughs were ground in a Waring blender to pass a U.S. No. 200 wire-mesh screen, and the ground samples were stored at -20°C until assayed. In doughs fortified with PE β -carotene, whole doughs were lyophilized. Bread loaves were tested after baking (0 days) or stored in polyethylene bags (0.038 mm thickness) for 0, 1, 3, 5, and 7 days at room temperature. At the end of each storage period, loaves fortified with AsA, ToAc, and CWD β -carotene were quartered, and one quarter of a loaf was lyophilized. Bread loaves fortified with PE β -carotene were lyophilized without quartering. The lyophilized bread was ground in a Waring blender to pass a U. S. No. 200 wire-mesh screen, and ground samples were stored at -20°C until assay.

Determination of AsA

Ascorbic acid was assayed by reverse-phase, high-performance liquid chromatography (RP-HPLC) with electrochemical detection essentially as described by Park et al (1994). The injection loop of the system had a volume of 20 μ L. A standard curve was constructed as follows. Ascorbic acid (0.5 g) was dissolved in freshly prepared and degassed 6% aqueous metaphosphoric acid (100 mL) containing 0.2% DTT, and an aliquot (1 mL) of the solution made to volume (50 mL) with 6% metaphosphoric acid solution, which formed the stock solution. Immediately before injection, aliquots of the stock solution (25, 50, and 75 μ L) were made to volume (25 mL) with cold degassed 0.05M aqueous perchloric acid. The resulting solutions containing 2.5, 5.0, and 7.5 μ g of AsA in 20 μ L were syringe-filtered (pore size 0.45 μ m and diameter 3 mm) (Gelman Science, Ann Arbor, MI) and injected into the chromatograph.

Fat-coated AsA (0.25 g) was stirred on a magnetic stirrer with a 2:1 (v/v) mixture of chloroform and methanol (50 mL). Aqueous 6% metaphosphoric acid and 0.2% DTT solution (50 mL) was added, the mixture shaken vigorously in a separatory funnel, and an aliquot (1 mL) of the aqueous layer was diluted to 25 mL with 6% metaphosphoric acid. An aliquot (75 μ L) of the resulting solution was made to volume (25 mL) with cold 0.05M perchloric acid, filtered through a syringe filter, and injected into the chromatograph.

Lyophilized and ground dough (1 g) was stirred on a magnetic stirrer for 15 min at 25°C with a 2:1 (v/v) mixture of chloroform and methanol (4 mL). Aqueous 6% metaphosphoric acid and 0.2% DTT solution (40 mL) was added, and the mixture was shaken vigorously in a separatory funnel. The aqueous layer was centrifuged for 20 sec at 8,100 \times g and an aliquot (1 mL) of the clear supernatant was made to volume (25 mL) with cold 0.05M perchloric acid to give a final concentrations of 2.5–7.5 μ g/mL. The resulting solution was syringe-filtered and injected into the chromatograph.

Lyophilized and ground bread (1–2 g) was vigorously extracted at 25°C with 6% metaphosphoric acid and 0.2% DTT solution (40 mL) on a magnetic stirrer for 15 min. After centrifugation for 20 sec at 8,100 \times g, an aliquot (1–3 mL) of the clear supernatant was made to volume (10–25 mL) with cold 0.05M perchloric acid. The resulting solution was syringe-filtered and injected into the chromatograph.

Determination of CWD β -Carotene

β -Carotene was determined by HPLC on an ODS-Hypersil column (Alltech 260 \times 4.6 mm, particle size 5 μ m, Deerfield, IL) with visible detection using a mixture of methanol, acetonitrile, chloroform, and water (20:25:9/1, v/v) as mobile phase (Speek et al 1986). For a stock solution, β -carotene (20 mg) was dissolved in methylene chloride (10 mL) and made to volume (100 mL) with hexane. An aliquot (1 mL) of this solution was diluted with cyclohexane (200 mL), and the concentration of β -carotene was calculated from absorbance at 457 nm with $E_{1\%} = 2,505$ (Speek et al 1986). A standard curve was prepared daily by dilution of the stock solution with mobile phase to concentrations of 1, 2, and 3 μ g/mL, followed by injection of 40 μ L into the chromatograph. Quantitation of β -carotene was made by comparing peak areas.

CWD β -carotene (50 mg) was placed in warm water (50 mL), and after cooling to room temperature, the mixture was made to volume (100 mL) with water. An aliquot (5 mL) was taken, and 1M hydrochloric acid (2 mL) and sodium sulfate decahydrate (2–3 g) were added. The mixture was shaken mechanically for 10 min against chloroform (20 mL) in a capped flask, centrifuged for 5 min at 2,000 \times g, and the aqueous layer was drawn off with a syringe and discarded. Sodium sulfate anhydrous (2 g) was added to the chloroform phase and the mixture was shaken vigorously for 1 min. An aliquot (2.5 mL) was taken, made to volume (25 mL) with mobile phase, and an aliquot (40 μ L) of the solution was

injected into the chromatograph. Mobile phase (25 mL) was added to lyophilized dough or bread (1 g), and the mixture was stirred on a magnetic stirrer for 20 min in a capped flask wrapped with aluminum foil. The mixture was centrifuged for 8 min at $2,000 \times g$, and an aliquot (40 μL) of the clear supernatant was injected into the chromatograph. Freeze-dried (1 g) bread and dough was spiked with β -carotene (50 μg) and the spiked sample was assayed for β -carotene.

Determination of PE β -Carotene

PE β -carotene (100 mg) was weighed into a 250-mL volumetric flask containing $5.7 \times 10^{-3} M$ ammonium hydroxide (10 mL). The mixture was placed in an ultrasonic bath, and Maxatase (proteolytic enzyme) (~0.5 mL) was added dropwise until disintegration of the sample was complete, which required ~10 min at room temperature. The mixture was diluted to volume (250 mL) with DMSO, and an aliquot of this solution (5 mL) was shaken against cyclohexane (100 mL) for 1 min in a 250-mL capped flask. The cyclohexane phase was centrifuged for 5 min at $2,000 \times g$, and the absorbance of the supernatant measured at 457 nm on a spectrophotometer. The concentration of β -carotene was determined using $E_{1\%} = 2,505$ (Hejro 1964).

Lyophilized and ground dough or bread (5 g) in $5.7 \times 10^{-3} M$ ammonium hydroxide solution (50.0 mL) was digested with gentle stirring at 25°C with Maxatase (1.5 mL) in a glass-stoppered Erlenmeyer flask (500 mL). After 1.5 hr of stirring, DMSO (50 mL) was added, and the mixture was stirred an additional 30 min. Cyclohexane (100 mL) and distilled water (250 mL) were added, and the flask was stoppered. After 15 min of stirring, the mixture was shaken vigorously, and centrifuged for 8 min at $2,000 \times g$. The absorbance of the clear supernatant was read at 457 nm, and the content of β -carotene was calculated using $E_{1\%} = 2,505$. One sample of dried ground bread (5 g) was spiked with 20 mg of PE β -carotene and then assayed. In all assays, duplicate samples were analyzed with two injections per sample.

Determination of CWD All-*rac*- α -Tocopheryl Acetate

All-*rac*- α -tocopheryl acetate was assayed by the HPLC on a Lichrosorb Si-60 column (Alltech 250×4.6 mm) with a mobile phase of hexane and diisopropyl ether (93:7, v/v) and fluorescence

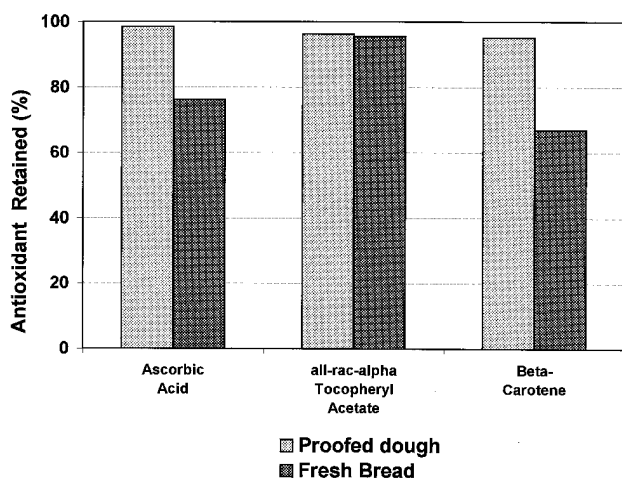


Fig. 1. Stabilities of antioxidants in proofed dough and fresh bread. Doughs were fortified initially with fat-coated L-ascorbic acid (AsA), cold-water-dispersible (CWD) AsA, CWD β -carotene, or CWD tocopherol acetate (ToAc) at levels of 64 mg of AsA eq 5 mg of β -carotene and 100 mg of all-*rac*- α -ToAc, respectively, per 100 g of flour. Doughs were fermented for 90 min, proofed for 40 min at 30°C ; breads were baked for 21 min at 219°C . The samples were lyophilized and ground before analysis. Least significant differences for AsA, β -carotene, and ToAc were 3.8, 3.1, and 4.1, respectively ($\alpha = 0.05$).

detection (Håkansson et al 1987). A stock solution was prepared by dissolving 200 mg of pure ToAc in hexane (100 mL). Working standard solutions of ToAc were prepared from aliquots (0.5, 1.0, and 1.5 mL) of the stock solution made to volume (50 mL) with hexane, which gave 20, 40, and 60 $\mu\text{g}/\text{mL}$ of ToAc, respectively. An aliquot (40 μL) of a standard solution was injected into the chromatograph, and peak areas were used to construct a standard curve for 0.8 to 2.4 μg of ToAc per injection.

In a capped flask, CWD ToAc (100 mg) was stirred gently for 20 min at 25°C with 50 mL of a 9:1 mixture of DMSO and water (v/v) containing 0.05% AsA. Hexane (50.0 mL) was added and the mixture stirred for 30 min on a magnetic plate and centrifuged for 5 min at $2,000 \times g$. An aliquot (1 mL) of the clear supernatant was taken, diluted to volume (10 mL) with hexane, and an aliquot (40 μL) was injected into the chromatograph.

Lyophilized and ground dough or bread (1 g), which had been prepared with CWD ToAc (200 mg per 100 g flour on 14% mb, containing 100 mg of ToAc) was shaken mechanically for 1 hr with 50 mL of a 9:1 (v/v) mixture of DMSO and water containing 0.05% AsA. Hexane (40 mL) was added, the mixture shaken vigorously on a mechanical shaker for 30 min and then centrifuged for 8 min at $2,000 \times g$. An aliquot (80 μL) of the clear supernatant was injected into the chromatograph. Freeze-dried and ground dough (1 g) and bread (1g) were spiked with 20 mg of CWD ToAc and assayed.

Statistical Analysis

All experiments were replicated three times, and analyses for antioxidants were done on duplicate samples with two injections per sample into a chromatograph. Statistical analyses were performed by using the Statistical Analysis System (SAS 1990). Means were compared by the least significant difference (LSD) test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

AsA in Dough and Bread Fortified with Fat-Coated AsA

L-Ascorbic acid is often added at low levels (<100 ppm) to bread dough to modify its rheological properties. But at those levels, practically no AsA remains in freshly baked bread (Thewlis 1971). In this investigation, fat-coated AsA was selected to fortify bread at a level of 64 mg AsA per 100 g of bread flour (14% mb).

The AsA retention in proofed dough, immediately before baking was 99% (Fig. 1). The high survival of AsA in dough was attributed to the yeast, which almost instantly consumes oxygen in

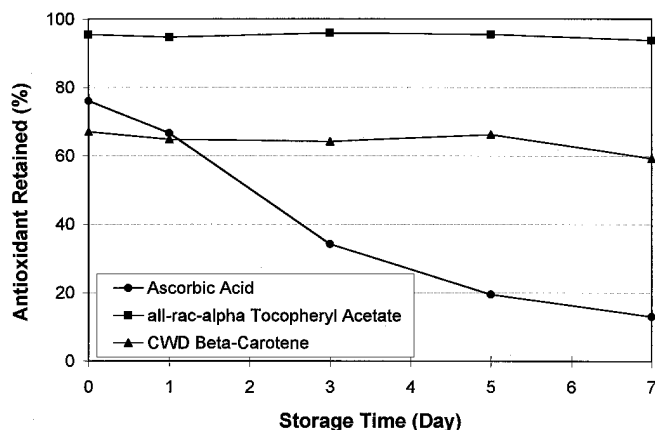


Fig. 2. Stabilities of antioxidants in pup loaves stored at 25°C . Levels of antioxidants and least significant differences are given in Fig. 1. Bread was placed unsliced in polyethylene bags and stored at 25°C .

dough during mixing and provides an anaerobic environment for AsA during fermentation and proofing of dough. The net result is protection of AsA from oxidation (Seib 1985).

During the baking step, ~23% of AsA was destroyed. (Fig. 1), and much more disappeared during seven days of bread storage at room temperature (Fig. 2). After 1, 3, and 5 days of storage, one serving (one slice, 28g) of the bread would provide 13, 7, and 4% of the adult U.S. RDA of AsA, respectively. However, pound-loaves of bread (300 g of flour) with their lower specific surface area would retain more AsA than pup loaves (100 g of flour).

β-Carotene in Dough and Bread Fortified with CWD β-Carotene

Spiking experiments gave 96% recovery of β-carotene added to freeze-dried dough and bread at a level of 50 μg/g. When 5 mg of β-carotene of the CWD form was added to the bread formulation, the retention of β-carotene in proofed dough was 95% (Fig. 1), which agreed closely with Gordon and Bauernfeind (1982), who reported essentially no loss after mixing and proofing of yeast-raised dough. Rogers et al (1993) reported a 5% loss of β-carotene in mixed bagel dough, and no further loss during proofing. The loss of β-carotene during mixing might be attributed to lipoxygenase activity in flour, which is limited by the low level of free linoleic acid in flour (Nicolas and Drapron 1983).

Fresh bread retained 67% of β-carotene from the CWD form, so the baking loss was 28% (Fig. 1). That loss was more than the 10–15% losses reported by Gordon and Bauernfeind (1982) in yeast-raised products, and the 20% loss in bagels reported by Rogers et al (1993). β-Carotene showed good stability in stored bread, which is consistent with results of Rogers et al (1993) and Gordon et al (1985). Bread stored for one to five days retained 67–64% of added β-carotene, and would provide 0.63–0.60 mg of β-carotene per one serving size. The level of β-carotene decreased to 59% in seven-day-old bread (Fig. 2). The addition of CWD β-carotene did not alter water absorption, mixing time, loaf volume, or flavor or texture of bread, but it did give an intense yellow bread crumb.

Protecting Effect Between AsA and CWD β-Carotene in Proofed Dough and Bread

In certain foods, antioxidants protect one another. Walsh et al (1970) found that addition of ascorbic acid to semolina decreased losses of carotenoids during spaghetti making. In our work, when AsA was added to bread dough in combination with β-carotene, the retention of β-carotene increased significantly from 95 to

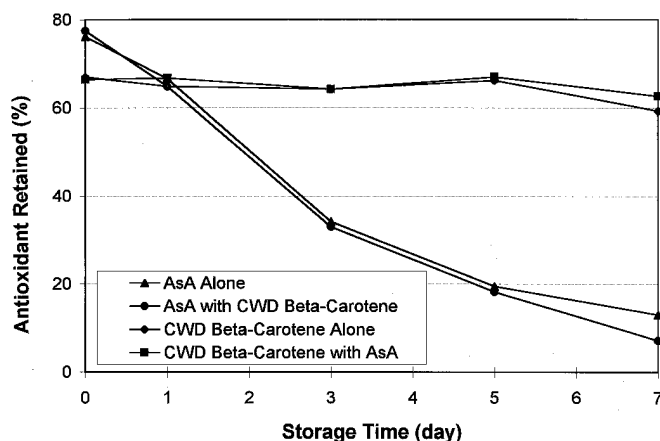


Fig. 3. Survival of ascorbic acid (▲ and ●) and β-carotene (◆ and ■) in bread stored at 25°C when both antioxidants were present in bread. Levels of antioxidants are given in Fig. 1. Bread was placed unsliced in polyethylene bags and stored at 25°C. Least significant differences for assays of ascorbic acid and β-carotene were 3.8 and 3.1, respectively ($\alpha = 0.05$).

101% in proofed dough. Park et al (1994) previously found that when bread dough was fortified with fat-coated AsA, ~20% of the coated AsA was released as free AsA during dough mixing. This AsA released from fat-coated AsA appeared to protect β-carotene from oxidation in dough. On the other hand, AsA failed to protect β-carotene in the stored bread and vice versa (Fig. 3).

PE β-Carotene in Breadmaking

To circumvent the problem of yellow-colored bread crumb, we tested PE β-carotene, which is a beaded product in which the β-carotene appears to be entrained in a network of fish gelatin. That alleged structure is based on the release of β-carotene when PE β-carotene is digested with a protease. Bread prepared with CWD β-carotene showed an intense yellow crumb color as indicated by a high b^* value compared to that of the blank bread (Table I). On the other hand, bread containing the same level of added β-carotene in the PE form did not have a yellow-colored crumb, although on close inspection, some of the tiny beads were visible. Because of the dark color of the crust, no difference in the crust color was discernible to the eye.

The freeze-dried dough or bread spiked with PE β-carotene (4 mg/g) showed 101% recovery of β-carotene. Proofed dough retained 98% of added β-carotene when fortified with 5 mg eq of PE

TABLE I
Color of Bread Crust and Crumb Fortified with Cold-Water-Dispersible (CWD) and Protein-Encased (PE) β-Carotene^a

Bread	Color Value ^b			
	L^*	a^*	b^*	ΔE^*
Crust				
Blank ^c	42.0	11.6	17.0	...
CWD	40.6	12.4	17.2	1.7
PE	44.8	12.0	20.0	4.1
LSD ^d	3.0	0.6	2.5	...
Crumb				
Blank	76.9	19.0	13.4	...
CWD	70.9	4.7	48.9	36.7
PE	75.9	-1.9	15.1	2.0
LSD	2.3	0.3	2.3	...

^a Bread doughs were fortified initially with CWD or PE β-carotene at a level of 5 mg of β-carotene eq per 100 g of flour.

^b Minolta chromameter values: L^* indicates lightness, $-a^*$ to $+a^*$ indicates green to red, and $-b^*$ to b^* indicates blue to yellow. Total color difference ΔE^* was calculated as: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

^c Blank contained no added β-carotene.

^d Least significant difference ($\alpha = 0.05$).

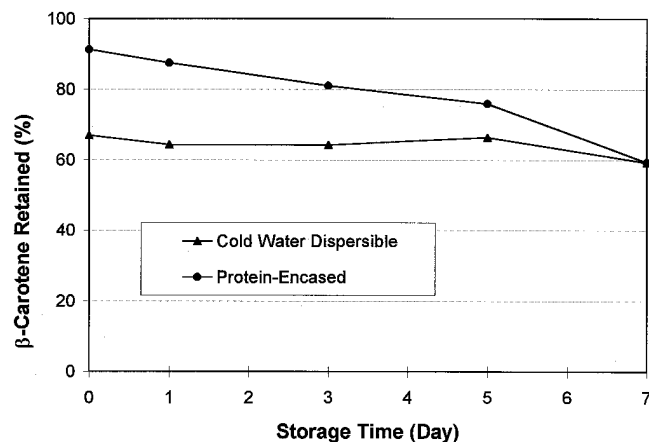


Fig. 4. Stability of β-carotene in bread stored at 25°C when fortified with 5 mg of β-carotene eq of cold-water-dispersible (CWD) and protein-encased (PE) forms of β-carotene. Bread was placed unsliced in polyethylene bags and stored at 25°C. Least significant difference was 4.3 ($\alpha = 0.05$).

β -carotene. The baking loss with PE β -carotene was 7%, which was 21% lower than the loss of CWD β -carotene. The added stability of the PE β -carotene was attributed to the protein coating, which may have inhibited the approach of prooxidant metal ions towards the β -carotene molecule. Over a seven-day storage period, however, the PE β -carotene in bread slowly deteriorated, giving a 59% retention in seven-day-old bread (Fig. 4). That storage loss was unexpected, because there was no storage loss of CWD β -carotene in bread after five days. The reason for those results is unknown. In spite of its storage loss, bread fortified with PE β -carotene still provided significantly higher amounts of β -carotene per serving of bread up to five days old. For example, three-day-old bread fortified with PE β -carotene would provide 0.76 mg of β -carotene per serving size compared to 0.60 mg for CWD β -carotene. Since 6 mg of all *trans*- β -carotene equals 1 mg of retinol equivalents (RE) (NAS 1989), one serving of three-day-old bread would provide 13–16% of the daily RDA (0.8–1.0 mg RE) of vitamin A.

All-*rac*- α -ToAc in Dough and Bread Fortified with CWD All-*rac*- α -ToAc

With hexane as extractant, the recovery of ToAc from freeze-dried dough or bread was ~50% (data not shown), but was ~96% when extracted with 9:1 (v/v) DMSO and water. French and Zobel (1967) reported that DMSO complexes with helical amylose, and if an inclusion complex is formed between amylose and ToAc, a large excess of DMSO in the extraction medium dissociates the complex.

When 200 mg of CWD ToAc, which equals 67 mg of RRR- α -tocopherol equivalents (α -TE), was added to the formula of a pup-loaf, proofed dough retained 96% of added ToAc (Fig. 1). No further loss of ToAc occurred during baking and seven days of storage (Fig. 2), in agreement with the prior findings of Harris (1968) and Cort et al (1976). Stabilization of ToAc is attributed to acetylation of the phenolic hydroxyl group on α -tocopherol. One serving size (28 g, one slice) of the bread fortified with 67 mg α -TE/100 g of flour would provide ~120–150% of the adult daily RDA of vitamin E (8–10 mg α -TE) (NAS 1989). The addition of ToAc did not alter water absorption and mixing time of bread dough, or loaf volume and the flavor and texture of bread.

CONCLUSIONS

Fresh bread retained three-fourths of AsA from a fat-coated form, but AsA disappeared rapidly in bread during storage. Fresh bread fortified with protein-encased β -carotene retained over 90% of that added antioxidant, and its rate of storage loss was slow. Bread prepared with tocopherol acetate lost none of that nutrient during baking or storage. When bread was fortified with both AsA and β -carotene, AsA failed to protect β -carotene in stored bread.

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