

Stability and Dietary Contribution of Vitamin E Added to Bread

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ABSTRACT

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Daily intake levels of vitamin E in the range of 200–800 IU are now recommended for its antioxidant effect. However, only vitamin E supplements or fortified foods may provide these high intake levels. As a fortified food, breads were prepared containing 200, 400, 800, or 1,600 IU of added vitamin E (dl- α -tocopheryl acetate) per pound loaf. These levels of fortification exerted no adverse effects on bread quality. However, only about two-thirds of the added vitamin E was retained (recovered) in the breads, with retention values showing no further

significant change during the seven-day shelf-life of the product. In fresh breads, vitamin E retention values were nearly the same (range 66.3–68.5%, average 67.2%) at all levels of vitamin E tested; this may hold true for levels not tested. Factoring in an average retention value of 67.2%, and actual potency (81.8%) of the vitamin E source used, a 50-g serving of bread fortified with 1,600 IU of vitamin E per loaf would provide nearly one-fourth of a suggested daily intake of 400 IU.

Vitamin E (tocopherols and tocotrienols) is essential in human nutrition (Bieri 1990, Diplock 1997). Because vitamin E is also a potent antioxidant, it has the ability to prevent oxidative damage to cells through inactivation of free radicals and reactive oxygen species (Diplock 1997). Combined evidence from animal, epidemiological, and clinical studies suggests that prevention of oxidative damage to cells would reduce the risk of human cancer and cardiovascular disease, and improve the immune and other physiological responses (Ames et al 1993, Diplock 1994, Block and Langseth 1994, Meydani and Beharta 1996, Kamal-Eldin and Appelqvist 1996, Eitenmiller 1997, Diplock 1997, Elliot 1999).

As a nutrient, a daily allowance of 12–15 IU of vitamin E is recommended for adults, with proportionally lesser amounts for children (NRC 1989). For nutrition labeling purposes, the “daily value” for vitamin E is set at 30 IU (CFR 1996). These intake amounts are well below the levels of 200–800 IU now recommended for antioxidant effects of vitamin E (Diplock 1997, Elliot 1999).

Vitamin E naturally occurring in foods provides only a fraction of the recommended 200–800 IU intake. For example, several studies of composite diets found daily vitamin E intake in the United States as ranging only between 10–15 IU (Bieri 1990); even these intake levels may become compromised with increasing use of low-fat products. Thus, only vitamin E supplements or fortified foods may provide daily intake in the range of 200–800 IU.

A variety of foods can be fortified with vitamin E. Fortification of a dietary staple such as bread is obviously a better choice. If added to bread, vitamin E may achieve its intended purpose only if the bread quality is not adversely affected, and possible vitamin E losses during breadmaking are quantified and taken into consideration in communicating vitamin E information on finished products. This study was undertaken to address these questions.

MATERIALS AND METHODS

Vitamin E Source

A food-grade source of synthetic vitamin E (dl- α -tocopheryl acetate, in oil base) was obtained from Hoffman La-Roche Inc. (Nutley, NJ) in mid 1998 and stored under refrigeration until used in breadmaking in the early part of 1999. For assessing vitamin E stability, this source was added to breads at levels of 200, 400, or 800 IU (or mg) per pound loaf. An additional level of 1,600 IU was included in the set of breads prepared for evaluating bread quality (Table I).

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Breadmaking and Scoring

White pan breads were made by the standard sponge and dough procedure. The sponge contained 70% flour, 2% compressed yeast, 0.5% yeast food (no oxidants), and 42% water, while the dough contained the remaining 30% flour, 7% sugar, 3% shortening, 2% salt, 0.12% calcium propionate, and the rest (20%) of the water. Vitamin E was added along with the shortening. After baking, breads were cooled on a rack, bagged, and saved. One set of breads was baked for scoring (Table I); two sets used for analysis (vitamin E retention) were baked several weeks apart (Table II).

Doughs and breads were scored for physical characteristics using a standard procedure typical of the industry (Table I) (Anonymous 1998); for breads, the scoring included sensory characteristics—taste, aroma, and mouthfeel. Evaluations were conducted by two trained bakers on one loaf from each batch of breads.

Extracting Vitamin E

Vitamin E in doughs and breads was extracted using a modification of the procedure by Zaspel and Csallany (1983). Freeze-dried doughs and breads were finely ground in a Waring blender, and stored frozen before analysis. Portions (0.5 g) of the ground samples were weighed into 15-mL glass-stoppered centrifuge tubes, and 5 mL of acetone was added. The slurry formed was vortexed briefly, sonicated for 7 min, revortexed, then centrifuged (500 \times g, 10 min). The supernatant was decanted into a disposable borosilicate test tube, and the pellet was reextracted twice with acetone. Combined supernatants of each sample were placed in a 40°C water bath, and the solvent was evaporated under a stream of nitrogen to <1 mL. Each sample was then filtered into a 4-mL screw-capped amber vial using a glass syringe fitted with a 25-mm PTFE syringe filter with 0.45- μ m pore size. Small portions of acetone were used to carefully rinse the test tube, syringe, and filter, and the combined extract and rinsings were brought up to a total of 3 mL. The filtrates were then stored frozen and refiltered through a 13-mm PTFE syringe filter with 0.45- μ m pore size before analysis.

Determining Vitamin E

Tocopherol standards were purchased from Matreya, Inc. (Pleasant Gap, PA) and stored frozen at –20°C until needed for the analysis. HPLC was performed using a Hewlett-Packard HP 1100 Series (Hewlett-Packard Co., Wilmington, DE) fitted with HP 1100 Quaternary pump, degasser, thermostated autosampler, and two detectors in series: a diode array detector with a standard flow cell (13 μ L, 10 mm path) and a fluorescence detector. Each sample (50 μ L) was loaded onto a C₁₈ reverse phase analytical column (Phenomenex Luna 5 μ C₁₈ (2), 250 \times 4.6 mm). The column was maintained at 25°C, and the chromatography was accomplished using an isocratic mobile phase consisting of methanol and acetonitrile (85:15) at a flow rate of 1 mL/min. The peaks resolved were monitored by diode array detector set at 292 nm (reference 360 nm

TABLE I
Dough and Bread Characteristics of Vitamin E-Fortified Breads

	Dough or Bread				
	A	B	C	D	E
Vitamin E added ^a , IU/loaf					
Based on labeled potency	0	200	400	800	1,600
Based on actual potency	0	164	327	654	1,309
Dough characteristics					
Water absorption, %	62	62	62	62	62
Mixing time, min	6	6	6	6	6
Proof time, min	60	60	60	55	57
Dough and bread scores ^b					
Dough (27)	22.25	22.25	22.25	22.25	22.25
External (18)	15.75	15.50	15.50	15.25	15.00
Internal (55)	44.00	43.75	43.75	43.25	42.25
Total (100)	82.00	81.50	81.50	80.75	79.50
Loaf volume					
Total volume, cm ³	2,700	2,613	2,625	2,625	2,688
Specific volume, cm ³ /g	5.88	5.70	5.70	5.72	5.92

^a As dl- α -tocopheryl acetate (1 mg = 1 IU)

^b Maximum possible points are given in parentheses. Dough scores included sponge out of fermentation, dough out of mixer, and dough at make-up. External bread scores included symmetry, crust character, crust color, and break and shred. Internal scores include grain, texture, crumb body, crumb color, taste and aroma, and mouthfeel.

TABLE II
Retention of Vitamin E (dl- α -tocopheryl acetate) Added to Bread

	Added (IU/loaf) ^a	Retained (% of amount added) ^b		
		Experiment I	Experiment II	Both
Set B ^c	200 (164)			
Dough		105.2 \pm 4.5a	103.1 \pm 0.7a	104.5 \pm 3.8a
Bread				
Fresh		66.6 \pm 6.1cd	66.8 \pm 1.4bc	66.7 \pm 5.0b
3 days		78.3 \pm 8.7b	64.0 \pm 3.2bc	73.6 \pm 10.1b
7 days		69.5 \pm 1.9b-d	65.7 \pm 3.3bc	69.5 \pm 1.8b
Set C ^c	400 (327)			
Dough		102.9 \pm 6.8a	100.3 \pm 2.2a	102.0 \pm 5.4a
Bread				
Fresh		65.0 \pm 1.7d	67.6 \pm 3.6bc	66.3 \pm 3.0b
3 days		77.1 \pm 2.8b	61.2 \pm 2.7c	71.8 \pm 8.4b
7 days		67.3 \pm 2.5b-d	61.5 \pm 1.7c	69.1 \pm 6.2b
Set D ^c	800 (654)			
Dough		106.6 \pm 2.5a	106.3 \pm 3.3a	106.5 \pm 2.7a
Bread				
Fresh		67.9 \pm 4.2b-d	69.7 \pm 1.1bc	68.5 \pm 3.5b
3 days		74.8 \pm 2.5bc	71.4 \pm 3.5b	73.6 \pm 3.2b
7 days		72.9 \pm 4.5b-d	71.6 \pm 4.6b	72.0 \pm 4.3b

^a Based on sample label and actual potency (in parentheses) values.

^b Assuming 81.8% actual potency. Within each experiment values not sharing a common letter are significantly different ($P < 0.05$).

^c Sets B–D correspond to breads B–D in Table I.

and slit 4 nm). The tocopherol peaks were identified by comparing their retention time with those of the authentic compound standards (α -, γ -, and δ -tocopherols and α -tocopheryl acetate). The fluorescence scans of the peaks revealed the tocopherol characteristic λ maximum at 296 nm (excitation) and 328 nm (emission). Therefore, these λ settings were used throughout the analysis to confirm the identity of the tocopherol peaks.

Concentrations of the α -tocopheryl acetate in the samples were computed employing a Single Instrument LC 3D Chemstation program (Hewlett-Packard) using a calibration curve generated from the authentic standards.

Statistical Analyses

The data in Table II were analyzed statistically by analysis of variance using the Tukey test for means separation (SigmaStat Statistical Software, Jandel Scientific Software, San Rafael, CA).

RESULTS AND DISCUSSION

Bread Quality

Vitamin E exerted no adverse effect on dough water absorption or dough mixing time, and dough proof times differed only mini-

mally; other dough characteristics did not differ irrespective of the level of vitamin E added (Table I). In breads, vitamin E caused a slight opening of the grain structure, most noticeably in bread E (1,600 IU). However, this did not affect bread quality in any significant way as total bread scores varied within a narrow range of 82–79.5 (Table I). Other than grain structure, no additional bread characteristics were affected, including sensory characteristics (bread A vs. breads B–E). Optimizing bread quality should normalize grain structure but this was not attempted. Loaf volumes and specific loaf volumes also revealed no adverse effect of vitamin E on bread quality, even at the highest (1,600 IU) level of vitamin E addition (Table I).

Vitamin E Analysis

A number of tocopherol isomers in the extracts of dough and bread were well resolved by HPLC (Fig. 1). The added α -tocopheryl acetate was the most prominent peak (by UV detection) in those samples that had been fortified. It was not detected in the control sample. γ -Tocopherol was the most predominant of the tocopherol isomers present in the extracts but trace levels of δ - and α -tocopherols were also observed. Under the conditions of the

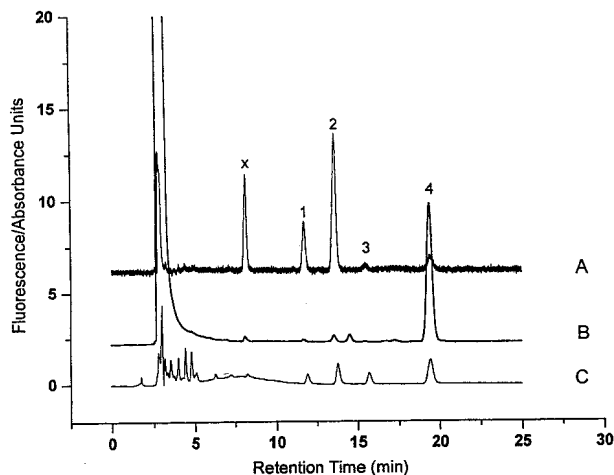


Fig. 1. Chromatograms showing peaks of standard tocopherols used in the identification of tocopherols found in dough and bread extracts. A, fluorescence profile of the sample tocopherols; B, tocopherol peaks from bread extract detected by UV diode array; and C, standards detected by UV diode array; 1, δ -tocopherol; 2, γ -tocopherol, 3, α -tocopherol; and 4, α -tocopheryl acetate. X is unknown peak with the characteristics of tocopherols.

analysis, we also detected one unknown that eluted early and had typical fluorescence characteristics for tocopherols (maximum excitation at 296 and emission at 328). It is possible that this peak was a breakdown product of tocopherols or it may have been another component structurally related to tocopherols.

Vitamin E Potency

A survey of foods and supplements marketed locally indicated that dl- α -tocopheryl acetate is the predominant form of vitamin E used in these products. As such, this was the form used in this study.

Although the vitamin E sample used was labeled dl- α -tocopheryl acetate, trace amounts of other forms of vitamin E may also have been present (Eitenmiller 1997). For this reason, and the possibility that some loss of vitamin E probably occurred during shipment and storage, the activity of dl- α -tocopheryl acetate measured only 81.8% of the label value. Consequently, the actual additions of dl- α -tocopheryl acetate to breads B–E (Table I) were somewhat lower than the label-based values of 200, 400, 800, or 1,600 IU. Table I lists both sets of values.

Vitamin E Stability

Vitamin E source material and all doughs and breads were analyzed for vitamin E (Table II). Breads were analyzed as fresh breads and as three-day and seven-day old breads stored at room temperature; seven days represent the typical shelf life of white pan bread marketed in North America.

As a percent of potency-corrected amounts added, vitamin E retention in doughs ranged between 100.3 and 106.6% (Table II). Retention values slightly exceeded 100%; however, these differences are not significant ($P > 0.05$). Results clearly suggest that no loss of vitamin E occurred in the doughs.

Vitamin E losses in breads were substantial ($P < 0.05$) (Table II, Fig. 2) and were observed in both sets of breads made (experiments I and II). During baking, about one-third of the added vitamin E was lost. This contrasts with the results of Park et al (1997) that showed only 4% loss in freshly baked pup loaves made with flour containing 100 mg of all-*rac*- α -tocopheryl acetate as well as vitamin C and β -carotene. This discrepancy is unclear, but may be due to differences in the vitamin E sources and baking techniques used or the possible protective effects of vitamin C and β -carotene, known antioxidants, on vitamin E in the pup loaves. In agreement with the results of Park et al (1997), no additional

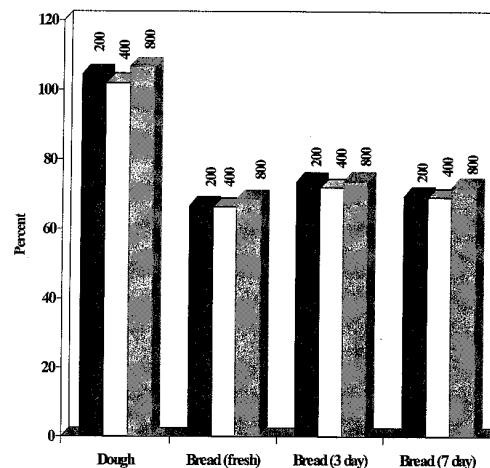


Fig. 2. Retention (%) of added vitamin E in breads. Levels added (IU/loaf) are indicated.

TABLE III
Dietary Contribution of Vitamin E in Fortified Breads

Bread ^a	IU in a 50-g		
	Serving of Bread ^b	Daily Value (%) ^c	Antioxidant Value (%) ^c
B	12	40	3
C	24	80	6
D	48	160	12
E	97	323	24

^a Breads B–E correspond to breads B–E in Table I.

^b Based on average vitamin E retention value of 67.2%.

^c Daily value 30 IU, antioxidant value 400 IU.

vitamin E losses occurred during the typical shelf life of the bread. This is true when the two experiments are considered individually or combined (Table II, Fig. 2). Vitamin E retention values tended to be a little higher in stored breads than fresh breads, but statistical analysis of the data revealed no significant differences ($P > 0.05$) in vitamin E retention values between fresh breads and stored breads (Table II).

Dietary Contribution

Because of losses during baking, only about two-thirds of the added vitamin E was retained in the fresh breads. Since the percent retention was nearly identical at all three—200, 400 or 800 IU—levels of fortification tested, it is likely that retention values at levels not tested may be similar.

Quantitatively, the overall retention of vitamin E in fresh breads averaged 67.2% (combined data). Information in Table III used this factor to calculate the dietary contribution of vitamin E in breads that were analyzed (breads B–D) or not analyzed (bread E) for vitamin E content. A serving of these breads (50 g or about two slices) would provide a substantial amount of vitamin E as a nutrient (Table III), but an insignificant amount as an antioxidant unless breads are fortified at levels well above 400 IU. At a fortification level of 1,600 IU, a 50-g serving of bread would provide nearly one-fourth of the widely recommended intake level of 400 IU (Table III).

CONCLUSIONS

Vitamin E can be added to bread at relatively high levels without adversely affecting sensory or other bread characteristics. Irrespective of the level added, about one-third of the added vitamin E is lost during baking, with no additional loss occurring during the typical shelf life of bread. Baking losses need to be factored in, or overages added to cover these losses, when communicating nutrition information on vitamin E-fortified breads.

LITERATURE CITED

- Ames, B. N., Shigenara, M. K., and Hagen, T. M. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci.* 90:7915-7922.
- Anonymous. 1998. *Milling and Baking News/Baking and Snack*, 1998-1999. Reference Source, page 99. Sosland Publishing: Kansas City, MO.
- Bieri, J. G. 1990. Vitamin E. In: *Present Knowledge in Nutrition*, 6th ed. International Life Sciences Institute, Nutrition Foundation: Washington, DC.
- Block, G., and Langseth, L. 1994. Antioxidant vitamins and disease prevention. *Food Technol.* 48:80-84.
- CFR. 1996. Nutrition labeling of foods. 21 Code of Federal Regulations (101.9). U.S. Government Printing Office: Washington, DC.
- Diplock, A. T. 1994. Antioxidants and disease prevention. *Mol. Aspects Med.* 15:293-376.
- Diplock, A. T. 1997. Will the 'Good Fairies' please prove to us that vitamin E lessens human degenerative disease. *Free Rad. Res.* 26:565-583.
- Eitenmiller, R. R. 1997. Vitamin E content of fats and oils—Nutritional implications. *Food Technol.* 51:78-81.
- Elliot, J. G. 1999. Application of antioxidant vitamins in foods and beverages. *Food Technol.* 53:46-48.
- Kamal-Eldin, A., and Appelqvist, L. A. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* 31:671-701.
- Meydani, S. B., and Beharta, A. A. 1996. Recent developments in vitamin E and immune response. *Nutr. Rev.* 56:S49-S58.
- NAS/NRC. 1989. *Recommended Dietary Allowances*, 10th ed. National Academy of Sciences/National Research Council: Washington, DC.
- Park, H., Seib, P. A., Chung, O. K., and Seitz, L. M. 1997. Fortifying bread with each of three antioxidants. *Cereal Chem.* 74:202-206.
- Zaspel, B. J., and Csallany, A. S. 1983. Determination of alpha-tocopherol in tissues and plasma by high-performance liquid chromatography. *Anal. Biochem.* 130:146-150.

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