Journal of Agricultural and Food Chemistry

MARCH 1992
VOLUME 40, NUMBER 3

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Factors Affecting Stability of Colored Substances in Paprika Powders

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A study was undertaken to investigate the change in carotenoid pigment as well as natural effective antioxidant content of paprika during fruit ripening and storage period of the ground products. By means of modern analytical procedure, paprika fruit was found to contain α-tocopherol in the pericarp and γ-tocopherol in the seeds. Ascorbic acid approached maximum level when the fruit turned red and then declined. Both antioxidants when added to the ground products have substantially reduced color impairment occurring during storage. The color degradation was estimated after 150 days of storage to be 27%, 20%, and 15% at ambient storage conditions and 15%, 13%, and 5% under refrigeration in untreated, δ-tocopherol-treated, and ascorbic acid-treated powders, respectively. Among different paprika cultivars the seeds of F-03 (hot) showed the highest level of tocopherol content, and the powder of such cultivar showed the lowest degradation of carotenoid pigments during storage.

INTRODUCTION

Color impairment during storage of paprika powder (*Capsicum annuum* L.) is a serious problem in many factories and enterprises. This degradation is attributable to many factors such as variety of paprika (Lease and Lease, 1956), moisture content (Malchev et al., 1982), ripeness stage at harvest (Kanner et al., 1977), and healthy state of dry fruits before grinding.

In previous work we studied the development and susceptibility to degradation of different carotenoid pigments of paprika fruit during ripening and postharvest (Biacs et al., 1989). The results showed that red xanthophylls are very susceptible to the oxidative degradation process such as lipoxygenase-catalyzed linoleic acid oxidation, and addition of seeds up to 15% of the total powder caused remarkable deterioration of the major pigments. However, in an attempt to control loss in paprika powder during storage, Okos et al. (1990) added ground seed at different percentages to paprika powder. No difference has been noticed between seed-containing and non-seed-containing samples in color loss for up to 5 months of storage, but at the end of the subsequent 7 months seed-containing paprika lost its color to a lower degree than the paprika without seeds.

It seems that a beneficial application of ground seed to reduce oxidative degradation of paprika pigments depends to a high extent on their antioxidant content.

Among the natural antioxidants tocopherol and ascorbic acid have been found to be the most effective. Positive correlation between these antioxidants and the stability of carotenoid pigments has been indicated at ripeness and postharvest of paprika (Daood et al., 1989).

According to the concluded recommendations of our previously published work, this study was undertaken to investigate the changes in pigment content of different paprika cultivars in connection with the changes in tocopherol and ascorbic acid contents. The effect of added seeds, ascorbic acid, and tocopherol on stability of carotenoid pigments was also included in this work.

EXPERIMENTAL PROCEDURES

Fresh and Dried Paprika Samples. Paprika fruits at various stages of ripening and postharvest from different cultivars (SZ-20, SZ-80, Mihálytelki, SZ-178, and F-03) were obtained from the Paprika Research Development Society, Szeged, Hungary. The dry fruit was ground to pass a sieve of 0.63-mm mesh.

Stock solutions of 10% δ-tocopherols in hexane and 10% ascorbic acid in water (Sigma) were prepared. The ground paprika samples were mixed thoroughly in a mixing machine and sprayed with the aforementioned solutions to give a final concentration of 0.2% for each antioxidant added. Control and treated samples were then packed in nylon packages and stored in a dark cabinet, at ambient and refrigeration temperatures for 150 days.

Extraction Methods. Extraction of pigments from fresh and ground paprika samples was carried out according to a previously described method (Biacs et al., 1989) using a mixture of 2:1:1 chloroform–2-propanol–acetone. The extracted pigments were dissolved in 100 mL of benzene for spectrophotometric deter-

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Table I. Parameters and Conditions Used for the Separation of Ascorbic Acid and Tocopherol of Paprika by HPLC Techniques

parameters	conditions of separation		
	ascorbic acid	tocopherol	red pigments
stationary phase	Lichrosorb C_{18} 5 μm, 250 × 4.6 mm i.d.	Lichrosorb 10 μm, 250 × 4.6 mm i.d.	Chromsil C_{18} 5 μm, 250 × 4.6 mm i.d.
mobile phase	0.01 M KH_2PO_4/MeOH = 97:3 containing 0.75 mM tetrabutylammonium hydroxide	*n*-hexane/2-propanol = 99.5:0.5	acetonitrile/2-propanol/water = 39:57:4
flow rate	1 mL/min	1.5 mL/min	1 mL/min
detection	268 nm	ex = 290 nm em = 320 nm	348 nm
reference	Rizzolo et al. (1984)	Speek et al. (1985)	Biacs et al. (1989)

mination of total carotenoids (Benedek, 1958) or in 5–10 mL of 4:1 eluent–chloroform for HPLC analyses.

As for ascorbic acid, aqueous extraction using 4% metaphosphoric acid solution was carried out and followed by centrifugation and filtration of the extract. In the case of fresh paprika, disintegration of the samples with quartz sand in a crucible mortar with pestle before extraction is necessary.

Tocopherols were extracted according to a method similar to that applied for pigments. Extracted fat-soluble residues were saponified by refluxing with 20 mL of 30% methanolic KOH for 40 min at the boiling point of methanol. Saponified tocopherols were then extracted by petroleum ether (30–40 °C) and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum by rotary evaporator.

HPLC Analysis. A Beckman liquid chromatograph was equipped with a Model 114 M pump, a Model 165 variable-wavelength UV–vis detector for ascorbic acid, and a Shimadzu fluorometric detector for tocopherols. The signal was electronically integrated by a Shimadzu Model C-R3A integrator. Conditions for HPLC separations are illustrated in Table I.

For peak identification, the R_f values and maximum absorption spectra of tocopherols and ascorbic acid were compared with those of standard materials. In the case of capsanthin and capsorubin (red pigments of paprika), the identification was based on their behavior in TLC separation and spectral characteristics (Biacs et al., 1989).

RESULTS AND DISCUSSION

The important function of ascorbic acid and tocopherols, as antioxidants, in plant tissues makes the progress in their analysis of special interest. In this respect, HPLC became the outstanding technique and rapidly replaced the routine and classical ones. Figures 1 and 2 show the isocratic separation of organic acids and tocopherol isomers, respectively. To achieve an accurate quantitation of the individual antioxidants, peak purity had to be checked exhaustively. This was carried out by scanning the absorption spectra at different points of each peak automatically. The separated antioxidants were found to be satisfactorily pure so that no further purification was required.

As ascorbic acid is more effective than tocopherol, in particular when water content is relatively high, the rapid decrease in its concentration was expected at the final stages of ripeness (Figure 3). The action of tocopherol could be observed only at the time when paprika fruit lost about 35% of its water content (postripeness stage), indicating that fat-soluble antioxidants act effectively when the whole lipid fraction is directly exposed to oxidizing agents.

At the beginning of storage the ascorbic acid content of ground paprika was 54.8 mg/100 g of dry matter. As a result of 150 days of storage at ambient and refrigeration temperatures decreases of 96% and 77%, respectively, were estimated. At refrigerated storage the value of

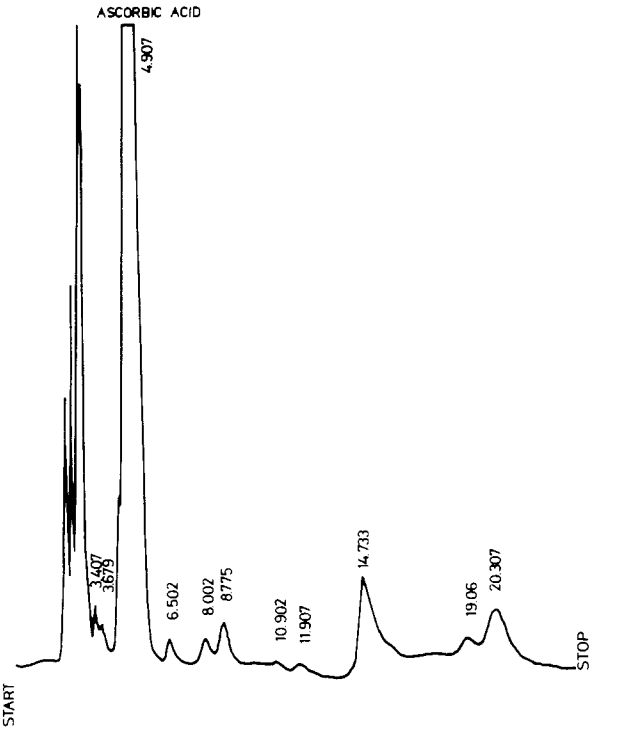

Figure 1. HPLC separation of ascorbic acid extracted from paprika powder. Chromatographic conditions are given in Table I.

oxidative degradation of pigments was reduced by elongation of the interval in which ascorbic acid acts as effective antioxidant (Figure 4).

After a 2-month storage at ambient and refrigeration temperatures, paprika powders lost about 90% of their original tocopherol content (Figure 5). The complete destruction of the major tocopherol isomer was observed after 3 months of storage. The results indicated that, unlike ascorbic acid, tocopherols are temperature-independent antioxidants and can effectively contribute to color protection under various storage conditions. While both antioxidants exhibited great destruction, carotenoid content was slightly decreasing during a 5-month storage (Figure 6), revealing the high potency of the original natural antioxidants.

Paprika powders produced from different cultivars showed different color stability when stored under the same conditions (Figure 7). The stability of pigments of the powders from hot cultivars was better than that of pigments of sweet ones, most likely due to their higher antioxidant content (Table II). Positive correlation with r values of 0.973 and a 0.98 was obtained when the percent of retained pigment was plotted vs ascorbic acid and to-

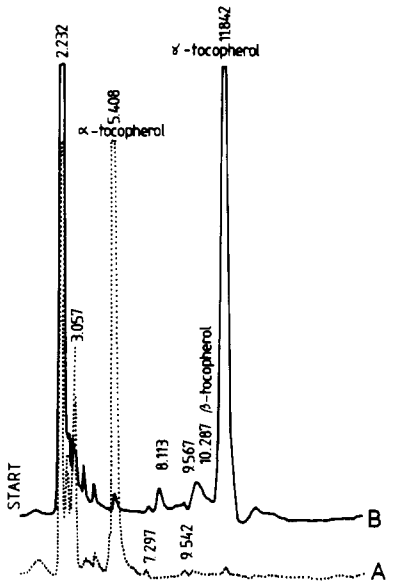

Figure 2. Typical HPLC elution profiles of tocopherols from paprika pulp (A) and seed (B).

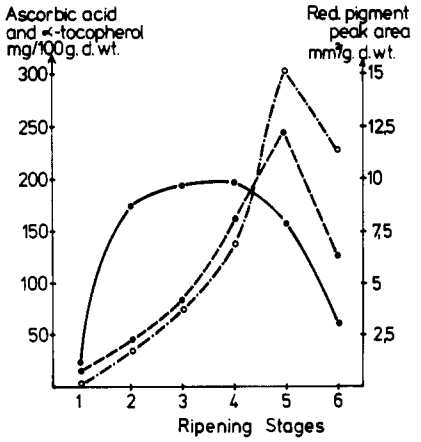

Figure 3. Changes in antioxidant and red pigment content of paprika fruit during ripening: (—) ascorbic acid; (- - -) α-tocopherol; (— • —) red pigment. 1, Green; 2, chlorophyll discoloration 1; 3, chlorophyll discoloration 2; 4, faint red; 5, red; 6, 6 weeks after harvesting.

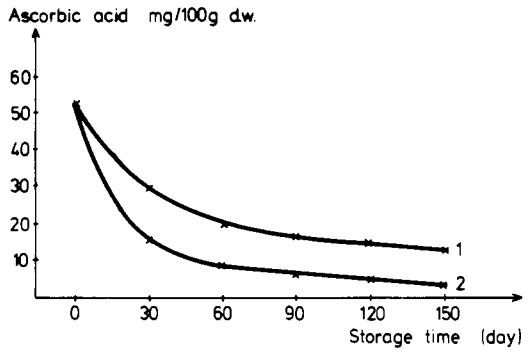

Figure 4. Effect of storage temperature on the ascorbic acid content of paprika powder: (1) refrigerated; (2) ambient.

copherol content of powder at prestorage, respectively. Unfortunately, the high capsaicin content of hot paprika products extremely limits their large-scale use as food colorants, particularly in European countries where hot spices are not commonly used in daily meals.

Effect of Added Antioxidant. To reduce the oxidative degradation of color substances, ground paprika samples were sprayed with ascorbic acid (aqueous solution) or with δ-tocopherol (hexane solution) up to 0.2% of the total

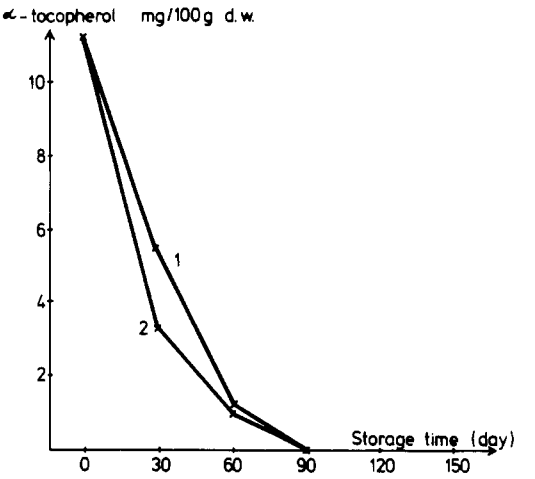

Figure 5. Effect of storage temperature on the α-tocopherol content of paprika powder: (1) refrigerated; (2) ambient.

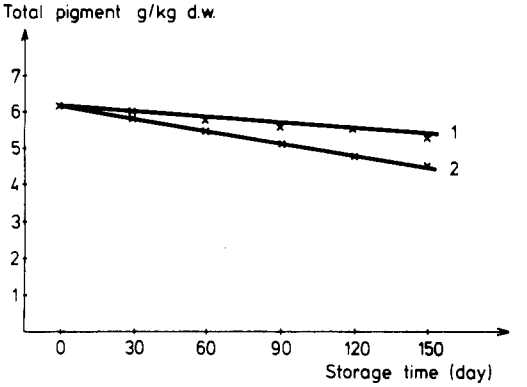

Figure 6. Color loss in paprika powder during storage at different temperatures: (1) refrigerated, $y = -0.0056x + 6.22$, $r = 0.98$; (2) ambient, $y = -0.0117x + 6.25$, $r = 0.99$.

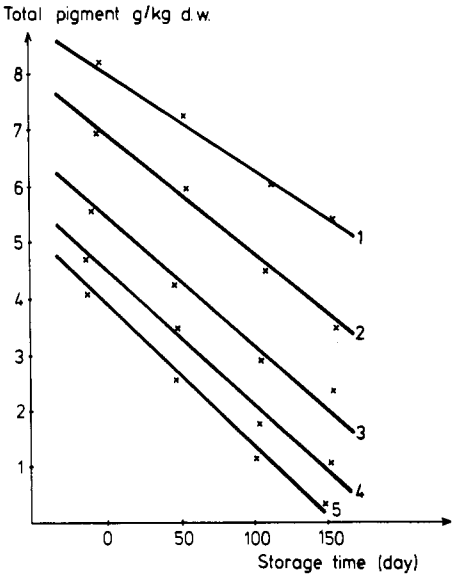

Figure 7. Change in pigment content in the ground pod of five cultivars during storage: (1) FO3; (2) SZ-178; (3) SZ-20; (4) SZ-80; (5) Mihálytelki.

weight. In both treated and untreated samples lower loss of color substances was estimated at refrigeration conditions. Linear curves were indicated with correlation coefficients (r) values between 0.97 and 0.99 when storage time was plotted vs carotenoid content of ground paprika (Figures 8 and 9). In the case of ascorbic acid-treated samples the loss was only 5% under refrigeration and 15%

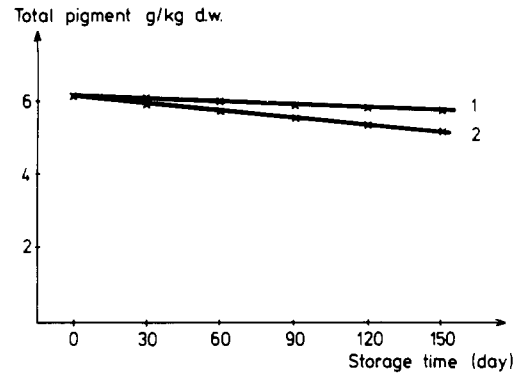

Figure 8. Effect of ascorbic acid treatment on the pigment stability of paprika powder: (1) refrigerated, $y = -0.0027x + 6.25$, $r = 0.99$; (2) ambient, $y = -0.0065x + 6.22$, $r = 0.99$.

Table II. Antioxidant Content of Dry Paprika Pulp and Seeds[a]

	concn, mg/100 g of dm		
cultivar	α-tocopherol of pulp	γ-tocopherol of seed	ascorbic acid of pulp
SZ-20	47.2	7.0	128.6
Mihálytelki	32.8	4.5	111.1
SZ-80	37.6	5.2	124.2
F-03	61.3	11.3	156.5
SZ-178	56.3	8.3	142.4

[a] The values represent the mean of three determinations.

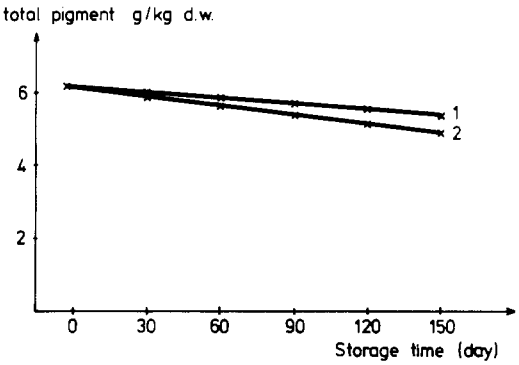

Figure 9. Effect of δ-tocopherol treatment on the pigment stability of paprika powder: (1) refrigerated, $y = -0.0051x + 6.27$, $r = 0.98$; (b) ambient, $y = -0.0080x + 6.14$, $r = 0.97$.

at room temperature. When compared to 15% and 27% determined in control samples, color loss with added ascorbic acid is significantly lower. Such results are of economical importance for producers and consumers as well. In the presence of ascorbic acid lipid hydroperoxides are reduced to alcohols, and thus propagation of free radical during lipid oxidation is aborted to a high extent. Prevention of color degradation by addition of ascorbic acid reinforced the belief that color loss during storage of paprika products is a terminal action of lipid oxidation. Further protection of pigments could be achieved at refrigeration temperature since formation of free radicals is also reduced.

As for tocopherol, although the most effective isomer (δ) was applied, it was not so capable as ascorbic acid to inhibit color degradation (Figure 9). Tocopherol-fortified samples lost 13–20% of their color substances. This range is considerably higher than the 5–15% found in the sample treated with ascorbic acid. In fact, neither of the applied antioxidants exhibited the actual antioxidation potency, probably due to the difficulty in redissolvation and distribution of ascorbic acid and tocopherol in the hydrophilic and lipophilic phases of ground paprika, respectively. The method and conditions of antioxidant application should be modified to improve their solubility and even distribution in the whole powder sample.

Table III. Effect of Added Seed Level on the Total Pigment Content during Storage[a]

	total pigment content, g/kg of dw		
sample	initial	final (150 days)	retention of color, %
SZ-20			
pulp	5.5	2.9	53
+5% seed	5.2	2.6	50
+15% seed	5.0	2.4	48
Mihálytelki			
pulp	3.8	1.4	37
+5% seed	3.6	1.2	33
+15% seed	3.4	1.0	30
SZ-80			
pulp	4.3	1.9	44
+5% seed	4.1	1.7	41
+15% seed	3.9	1.4	36
F-03			
pulp	8.1	5.4	67
+5% seed	7.7	5.1	66
+15% seed	7.5	4.8	64
SZ-178			
pulp	7.1	4.2	59
+5% seed	6.7	3.9	58
+15% seed	6.4	3.6	56

[a] The values represent the mean of three determinations.

Addition of Seeds to Ground Paprika. As an attempt to control color degradation of paprika powders during storange, Hungarian and other world enterprises add ground seeds up to 15% of the total product. The mechanism by which ground seed can improve color stability of stored paprika is still unclear. The effect has preliminarily been attributed to the tocopherol content of the seeds since they contain a considerable amount of the γ isomer that is more effective than the α isomer existing in the pulp (Table II). In contrast, addition of seeds was found to be detrimental to the major carotenoid pigments of the stored paprika powder (Biacs et al., 1989). From the available comprehensive data touching upon these topics, the following points can be raised:

1. Paprika seeds contain an effective antioxidant, e.g., γ-tocopherol, which inhibits oxidation of the red carotenoids especially during the first stage of storage.
2. After grinding, oil from the seeds diffuses and surrounds most of the particulates of paprika powder, so it can protect the pigments from the surrounding oxidizing agents and improve the visual appearance of the products.
3. Excessive addition of seeds is an advantage in the oxidative degradation of red pigments which is facilitated through an auto- and enzyme-catalyzed coupled oxidation of polyunsaturated fatty acids originating mainly from the seeds. The presence and activity of lipoxygenase and lipid hydroperoxidase, the enzymes intimately involved in the oxidation of polyunsaturated fatty acids, have been evident in the seeds of paprika fruits (Daood and Biacs, 1986).

In this work we added ground seeds of the different cultivars at 5% and 15% of their own ground pulps. It is noted that a great unusual color loss rate was observed for all of the cultivars harvested during the growing season of 1989. This rapid decay might be related to the virus disease that was widespread in the area of paprika production during the same season. The decay could not be moderated by seed addition (Table III). None of the applied seed types brought about a significant effect in color retention during storage even when δ-tocopherol-rich seeds (of F-03 cultivar) were used for this purpose.

This phenomenon was also indicated with the excessive addition of seeds (15%). Moreover, in some cases seed addition resulted in more color loss. The later findings support our previous conception that when storage is nearing its end, enzymes of the lipid oxidation pathway, such as lipoxygenase and lipid hydroperoxidase, released from the seeds contribute to such a color loss (Biacs et al., 1989). Furthermore, at this stage the antioxidation potency of the whole powder is too weak to inhibit the activity of such enzymes.

Finally, the type and concentration of antioxidants in the seeds of different paprika cultivars have to be clarified. Further work is also required to highlight the factors relevant to oxidation processes in the pulp as well as in the seeds of paprika fruit.

LITERATURE CITED

Benedek, L. *Z. Lebensm. Unters. Forsch.* **1958**, *107*, 228–232.

Biacs, P.; Bodnár, J.; Hoschke, Á. Separation and identification of carotenoid-esters in red pepper during ripening. In *The Metabolism, Structure and Function of Plant Lipids*; Stumpf, P. K., Mudd, J. B., Nes, W. D., Eds.; Plenum Press: New York, 1987; pp 135–139.

Biacs, P.; Daood, H.; Pavisa, A.; Hajdu, F. Studies on the carotenoid pigments of paprika. *J. Agric. Food Chem.* **1989**, *37*, 350–353.

Daood, H.; Biacs, P. Evidence for the presence of lipoxygenase and hydroperoxide-decomposing enzyme in red pepper seeds. *Acta Aliment.* **1986**, *15*, 307–318.

Daood, H.; Kiss-Kutz, N.; Czinkotai, B. Lipid and antioxidant content of red pepper. In *Biological role of plant lipids*; Biacs, P., Gruiz, K., Kremmer, T., Eds.; Akadémia Kaidó: Budapest, 1989; pp 491–494.

Kanner, J.; Mendel, H.; Budowski, P. Carotene oxidizing factors in red pepper fruits. *J. Food Sci.* **1979**, *43*, 709–712.

Lease, J. G.; Lease, E. J. Effect of fat-soluble antioxidants on the stability of the red colour or peppers. *Food Technol.* **1956**, *10*, 403–405.

Malchev, E.; Ioncheva, N.; Tanchev, S.; Kalpakchieva, H. Quantitative changes in carotenoids during storage of dried pepper. *Nahrung* **1982**, *26*, 415–420.

Okos, M.; Csorba, T.; Szabad, F. The effect of paprika seed on the stability of the red colour of ground paprika. *Acta Aliment.* **1990**, *19*, 79–93.

Rizzolo, A.; Forni, E.; Polessello, A. HPLC assay of ascorbic acid in fresh and processed fruits and vegetables. *Food Chem.* **1984**, *14*, 189–199.

Speek, A. J.; Schrijver, F.; Shreurs, H. P. Vitamin E composition of some oils as determined by HPLC with fluorimetric detection. *J. Food Sci.* **1985**, *50*, 121–124.

Received for review April 19, 1991. Revised manuscript received October 3, 1991. Accepted October 12, 1991.

Registry No. Ascorbic acid, 50-81-7; α-tocopherol, 59-02-9; γ-tocopherol, 7616-22-0; δ-tocopherol, 119-13-1.