Content and Stability of α-Tocopherol in Fresh and Dehydrated Pepper Fruits (*Capsicum annuum* L.)

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The content of α-tocopherol in pepper fruits (*Capsicum annuum* L.) and its stability during dehydration and storage were determined. Our data show that the three pepper varieties used in this study, regardless of stage of maturity, contained from 9000 to 10 000 μg of α-tocopherol/g of oil (oleoresin). The α-tocopherol content in the fresh pepper and its dry matter was found to depend on the content of lipids which in turn depends on ripening stage and genetic variety factors. During dehydration the loss of α-tocopherol in red pepper fruits was less than 5%. The α-tocopherol was found to be unstable in powdered pepper stored at low water activity, a_w, but very stable at high a_w. The large amount of α-tocopherol found in the fresh ripe fruits, ca. 3–10 mg/100 g, indicates that this vegetable could become an important source of vitamin E in the human diet.

Pepper fruits (*Capsicum annuum* L.) are used for preparing various dehydrated, canned, and frozen products besides their consumption as fresh vegetable. The lipid fraction from the fruit, the oleoresin, is generally used as a natural food colorant.

In our previous studies, fractions from the aqueous pepper extracts were purified, characterized, and tested for carotene-bleaching activity in an aqueous carotene–linoleate model system (Kanner et al., 1976, 1977). The activities of these fractions were also tested in a solid model consisting of cellulose powder impregnated with oleoresin (Kanner et al., 1978).

One of the notable findings of our previous study was that the oleoresin of paprika is especially rich in α-tocopherol (10 mg/g of oil).

Tocopherols are widely distributed in nature. The main sources of the tocopherols are plants, which, in their growing phase, usually contain α-tocopherol (Booth and Bradford, 1963). The distributional pattern of α-tocopherol in plant foods is modified by species variety, stage of maturity, season, processing procedures, and storage time. Green (1970), Draper (1970), and Bauernfeind (1977) reviewed the tocopherol contents of several important sources, as obtained by analytical methods that can be considered reliable. Nuts, seeds, and vegetable oils are superior sources. It was found that among the commercial seed oils, cottonseed and safflower oils are the richest sources of vitamin E (800–1000 μg/g of oil). However, the highest amount of α-tocopherol in vegetable oils was found in wheat germ oil (3200 μg/g of oil).

Tocopherols are unstable in the presence of oxygen. The oxidation is accelerated by exposure to ultraviolet light, metal ions, and peroxidizing fats. They are especially unstable in dehydrated foods (Harris, 1962).

Although natural grains often contain appreciable quantities of vitamin E, large losses of α-tocopherol occur during storage of processing of breakfast cereals (Herting and Drury, 1969). Dehydration caused 36–45% losses of α-tocopherol in beef and chicken (Thomas and Calloway, 1961). In a study by Livingston et al. (1968), losses of α-tocopherol during commercial-scale dehydration in alfalfa ranged from 5 to 33%, and larger losses (54–73%) occurred during storage.

The purpose of the present study was to determine the content of α-tocopherol in some fresh pepper varieties and its stability during dehydration and storage.

MATERIALS AND METHODS

Pepper fruits of variety Vandel, Gamba, and Mild California were picked at three ripening stages: green, green-red, and red. The fruits were cleaned, halved, and dehydrated at 55 °C to 4% moisture in a pilot tunnel-drier. A part of the halved fruits were freeze-dried to 2% moisture. The dehydrated fruits were crushed, pulverized, and passed through a 40-mesh sieve.

Paprika oleoresin was extracted from the dehydrated powder with petrol–ether (40:60), until the residue was colorless. The solvent was evaporated under vacuum, and the lipids were stored under nitrogen at −20 °C.

α-Tocopherol was determined by Emmerie-Engel's colorimetric method, using *dl*-α-tocopherol (after saponification of *dl*-α-tocopheryl acetate with a 93% recovery) as a standard for TLC and colorimetric reactions.

The recovery of α-tocopherol after saponification of a defined amount of α-tocopheryl acetate was done spectrophotometrically. A molar extinction coefficient in ethanol of 32.600 at 292 nm was adopted for calculation.

A 2-g sample of dried paprika or 0.5 g of oleoresin was saponified in 30 mL of absolute ethanol, to which was added 4 mL of 60% (w/v) aqueous potassium hydroxide and 10 mg of butylated hydroxytoluene. An inert atmosphere of nitrogen was maintained in the flask during reflux time (15 min). After refluxing, the flask was immediately cooled in an ice bath. The contents were quantitatively transferred with 40 mL of distilled water to a separatory funnel and 30 mL of petroleum ether (PE) was added. The upper layer was collected and the lower layer of water was reextracted two times more with PE. The three PE extracts were pooled and washed with distilled water. The washed PE extracts were dried by sodium sulfate and evaporated under vacuum, and the residue was dissolved in 25 mL of PE.

An aliquot of this solution was subjected to thin-layer chromatography (TLC). The conditions for TLC were as follows: plate, 0.5-mm thickness of Kieselgel HF_{254}; developing solvent, petroleum ether/ethyl ether, 8:2. The band of α-tocopherol detected by an ultraviolet lamp was scraped off in a lump from each plate and extracted with 5 mL of ethanol. An aliquot of the ethanolic extract (containing 10–20 μg of α-tocopherol) was transferred to a 15-mL test tube. The following reagents were added in ethanolic solutions: ethanol to a volume of 3.5 mL, 0.2 mL of 0.5% bathophenanthroline, and 0.2 mL of 0.07% ferric chloride. In exactly 60 s the color developing reaction was fixed by 0.5 mL of 0.1 M H_3PO_4 in ethanol. The color was

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0021-8561/79/1427-1316$01.00/0

Table I. α-Tocopherol Content of Some Selected Foods

sample	α-tocopherol content, μg/g: dry wt basis	fresh wt basis	references
vegetables			
kale, leaf	787		Brown (1953)
spinach	225	25	Booth and Bradford (1963)
asparagus	260	25	Booth and Bradford (1963)
tomato	60	3	Booth and Bradford (1963)
oils			
cottonseed, refined oil	366		Herting and Drury (1963)
safflower, crude oil	407		Herting and Drury (1963)
wheat germ, crude oil	1273		Herting and Drury (1963)

measured spectrophotometrically at 520 nm against a blank which contained ethanol and reagents.

All reagents and solvents were of analytical grade. The ethanol was treated with 1 g of sodium borohydride per liter, allowed to stand overnight, and distilled, discarding the first and last 5% of the distillate.

Petroleum ether (40–60) and ethyl ether (free of peroxides) were redistilled. The $FeCl_3$ solution was prepared weekly, or when the blank reading against water rose above 0.05 OD.

Carotene oxidation in samples incubated at 37 °C was determined by extracting the pigment with acetone and measuring the absorbance of the filtrate at 460 nm. Carotenoid destruction was expressed as percent decrease in absorbance. Water activity, a_w, of 0.01 and 0.75 was adjusted by holding thin layers of the samples in plastic dishes at 37 °C over $CaCl_2$ or saturated NaCl solution.

The oil content of pepper fruits was determined by extracting the lipids from the dry matter with petroleum ether in a Soxhlet instrument.

The dry matter content of pepper was determined by weighing samples before and after dehydration in a vacuum oven at 70 °C for 24 h.

RESULTS AND DISCUSSION

It was found that the pepper fruit is one of the richest sources of natural vitamin E in foods, in comparison with the most abundant sources of α-tocopherol in plants and natural products (Table I).

Our data (Table II) show that the three pepper varieties used in this study contained from 9000 to 10000 μg of α-tocopherol/g of oil (oleoresin). The oleoresin of paprika was found to be three times richer in α-tocopherol than is wheat germ oil. The dry matter of red pepper var. Mild was found to be almost rich in α-tocopherol as kale leaf, and the fresh fruits were found to be four times richer than fresh spinach and asparagus, which seem to be the richest sources of vitamin E in vegetables. Tomato, a solanaceous fruit like pepper, has only 60 μg of α-tocopherol/g of dry matter.

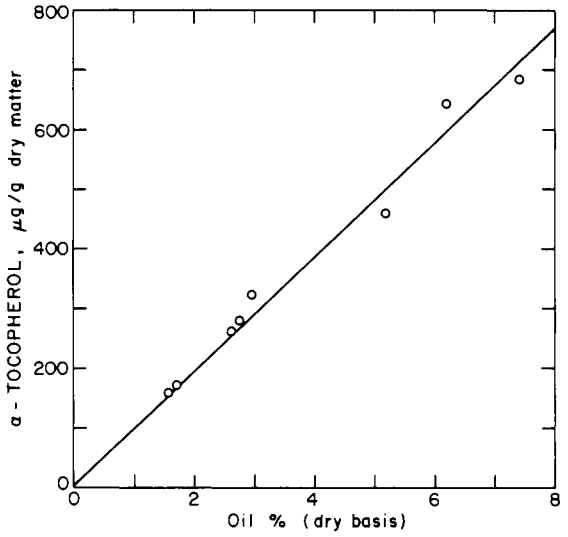

Figure 1. Relationship between the content of α-tocopherol and oil in the dry matter of pepper fruits (coefficient of variation between triplicates were 7.6%).

The content of α-tocopherol in the fresh pepper and its dry matter was found to depend on the content of lipids, which is dependent on the ripening stage and variety. The oil content rose during fruit ripening, from 1.5 to 3.0% in var. Vandel and var. Gamba, (bell-pepper varieties) and from 2.6 to 7.4% in var. Mild, a "sweet spice" variety.

The high correlation between oil content and α-tocopherol content in dry matter (Figure 1) is in agreement with the well-known observation that there is a strong correlation between the total tocopherols content of oils and their polyunsaturated fatty acids (PUFA) content (Harris, 1962). In pepper fruits more than 85% of the lipids are glycerides, which contain almost 70% unsaturated fatty acids (Szabo, 1970; Kinsella, 1971).

Our data on the quantity of α-tocopherol in a red sweet spice paprika variety are in agreement with the single previous report on α-tocopherol content in this fruit (Feldheim, 1957). However, the amount of α-tocopherol determined in the green fruits was higher in our varieties. This could be explained by a parallel higher accumulation of lipids.

Tocopherols are sensitive to oxygen and oxidative reactions, especially in the presence of catalysts such as ultraviolet light, metal ions, and peroxidizing unsaturated fatty acids. Paprika powder during processing and storage is exposed to one or more of these deleterious factors. It was found by us that during dehydration the loss of α-tocopherol in red pepper fruits is very low (less than 5%).

Figure 2 shows the oxidation of α-tocopherol in powdered paprika at low (0.01) and high (0.75) a_w. The tocopherol was found to be unstable in powdered paprika stored at low a_w, but very stable at high a_w. During 120 days at 37 °C, more than 90% of the vitamin was retained

Table II. α-Tocopherol Content of Pepper Fruits

variety	ripening state	dry matter, %	oil (dry basis), %	α-tocopherol, μg/g: oil	dry matter	fresh fruit
Vandel	green	6.3	1.5	10710	164.0	10.4
	red	10.0	2.7	10200	275.4	27.5
Gamba	green	6.0	1.7	9800	166.6	9.9
	red	9.8	2.9	11000	326.7	31.4
Mild	green	12.1	2.6	9850	262.0	31.8
	green-red	14.4	5.1	8890	460.5	66.2
	red	16.8	6.2	10350	644.8	108.4
	red, dried on the plant	79.9	7.4	9150	678.0	

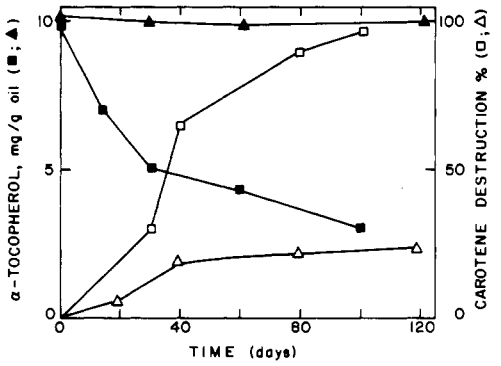

Figure 2. Oxidation of α-tocopherol and carotenoids during storage of paprika powder at 37 °C. α-Tocopherol, a_w = 0.01 (■), a_w = 0.75 (▲); and carotenoids, a_w = 0.01 (□), a_w = 0.75 (△).

in the powder. The effect of a_w on the stabilization of the tocopherol in powdered paprika was found to be similar to that found for carotenoids (Kanner et al., 1978). This effect was found to be mainly indirect, via solubilization of ascorbic acid and copper salts, which together form an antioxidant system. This view was supported by the fact that in an oleoresin–cellulose model, an increase in a_w from 0.01 to 0.75 in the absence of additives prolonged the induction period from 6 to 12 days, but when the system contained ascorbic acid and copper ions, the induction period was increased from 10 to 120 days. There is not enough evidence available to determine whether the prolonged induction period at high a_w in the inhibition of carotenoids and tocopherol oxidation was obtained due to the activity of ascorbic acid only, or to an inhibitory synergistic effect of α-tocopherol and ascorbic acid on lipid oxidation. At low a_w the oxidation of α-tocopherol is faster than that reported for carotenoids (Kanner et al., 1978). This indicates that at low water content α-tocopherol acts as an antioxidant and does not seem to be protected or regenerated by ascorbic acid.

The average α-tocopherol intake per capita in the United States was found to be about 14 mg/day, of which over 50% was contributed by oils and fats (Harris et al., 1950). In 1965 Bunnell et al. found that the daily intake of α-tocopherol was 2.6–15.4 mg, with an overall daily average of 7.4 mg. The current vitamin E daily allowance for man as recommended in the 1974 NAS–NRC Food and Nutrition Board publication is 5 mg for infants, 7–10 mg for children, and 15 mg for adults. A ratio of 0.6 mg of α-tocopherol to 1 g of PUFA is recommended. Most fruits and vegetables, particularly processed, do not contribute a significant amount of α-tocopherol to the diet (Bunnell et al., 1965). However, the large amount of α-tocopherol which was found by us in the fresh ripe pepper fruits (ca. 3–10 mg/100 g), and the excellent ratio of 15 between the vitamin and PUFA, indicates that this vegetable could become an important source of vitamin E in the human diet. As is well known, the ripe fruit is also very rich in ascorbic acid (ca. 200 mg/100 g). The synergistic effect of ascorbic acid and phenolic antioxidants, including α-tocopherol, in inhibiting lipid oxidation was established and summarized by Klaui (1971). In peppers the relation between these two vitamins is well balanced naturally, a fact of great importance in nutritional and technological considerations.

LITERATURE CITED

Bauernfeind, J. C., *Crit. Rev. Food Sci. Nutr.* **9**, 337 (1977).
Booth, V. H., Bradford, M. P., *Br. J. Nutr.* **17**, 575 (1963).
Brown, F., *J. Sci. Food Agric.* **4**, 161 (1953).
Bunnell, R. H., Keating, J., Quaresimo, A., Farman, G. K., *Am. J. Clin. Nutr.* **17**, 1 (1965).
Draper, H. H., in "Fat-Soluble Vitamins", International Encyclopedia of Food and Nutrition, Vol. 9, R. A. Morton, Ed., Pergamon Press, London, 1970, p 333.
Feldheim, W., *Z. Lebensm. Untersuch. Forsch.* **106**, 116 (1957).
Green, J., in "Fat-Soluble Vitamins", International Encyclopedia of Food and Nutrition, Vol. 9, R. A. Morton, Ed., Pergamon Press, London, 1970, p 71.
Harris, P. L., Quaife, M. L., Swanson, W. J., *J. Nutr.* **40**, 368 (1950).
Harris, R. S., *Vitamins Hormones* **20**, 603 (1962).
Herting, D. C., Drury, E. J. E., *J. Nutr.* **81**, 335 (1963).
Herting, D. C., Drury, E. J. E., *J. Agric. Food Chem.* **17**, 785 (1969).
Kanner, J., Mendel, H., Budowski, P., *J. Food Sci.* **41**, 183 (1976).
Kanner, J., Mendel, H., Budowski, P., *J. Food Sci.* **42**, 1549 (1977).
Kanner, J., Mendel, H., Bodowski, P., *J. Food Sci.* **43**, 709 (1978).
Kinsella, J. E., *J. Food Sci.* **36**, 865 (1971).
Klaui, H., "The Functional (Technical) Uses of Vitamins", Stein, M., Ed., University of Nottingham Seminar Vitamins, London, England, 1971, p 110.
Livingston, A. L., Nelson, J. W., Kohler, G. O., *J. Agric. Food Chem.* **16**, 492 (1968).
NAS–NRC Recommended Dietary Allowances, 8th ed, National Academy of Sciences, Washington, DC, 1974.
Thomas, M. H., Calloway, D. H., *J. Am. Diet. Assoc.* **39**, 105 (1961).
Szabo, P., *Am. Perfum. Cosmet.* **85**, 39 (1970).

Received for review December 6, 1978. Accepted May 30, 1979.