Endogenous Levels of Tocopherols and Ascorbic Acid during Fruit Ripening of New Mexican-Type Chile (*Capsicum annuum* L.) Cultivars

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Endogenous levels of tocopherols and ascorbic acid were quantified by high-performance liquid chromatography (HPLC) during fruit ripening of New Mexican-type chile peppers (*Capsicum annuum* L.). γ-Tocopherol (γ-toc) was present in seeds, while α-tocopherol (α-toc) was present in the pericarp. γ-Tocopherol content reached its maximum concentration in seeds from red, succulent fruit and then declined. α-Tocopherol increased from the green to dry red maturity stages. Cultivars differed for α-toc but not for γ-toc contents. Ascorbic acid content increased after the green mature stage and peaked in red fruit with about 75% moisture content. Ascorbic acid was highest in the cultivars NuMex R Naky and Sandia.

Keywords: *Tocopherol; ascorbic acid; Capsicum annuum; HPLC; paprika; peppers*

INTRODUCTION

Chile peppers (*Capsicum annuum* L., New Mexican-type) are used as a vegetable and a spice. The mature green fruit are roasted and consumed whole, or added to enchiladas and salsas. The dried, red fruit are used as seasonings or natural red colorants. Chile pepper consumption is increasing and may be an important source of vitamins for world populations. In particular, the antioxidant vitamins (vitamins A, C, and E) are present in high concentrations in various pepper types. These vitamins may protect against cancer; therefore, food sources rich in antioxidants are being studied and promoted (Byers and Perry, 1992). Red chile peppers contain high amounts of carotenoids that have provitamin A activity (Mejia et al., 1988; Howard et al., 1994). The ascorbic acid (vitamin C) content has been reported between 46 and 243 mg/100 g fresh wt (Wimalasiri and Wills, 1983; Nisperos-Carriedos et al., 1992; Howard et al., 1994; Lee et al., 1995). Tocopherol (vitamin E) concentrations have ranged from 3.7 to 236 mg/100 g dry wt (Kanner et al., 1979; Daood et al., 1989; Biacs et al., 1992). Wide variations in vitamin levels have been attributed to differences in cultivars, maturity, growing practices, climates, postharvest handling, and analytical methods (Mozafar, 1994).

While carotenoids have been studied in depth, there are few reports describing changes in endogenous levels of ascorbic acid and tocopherols during chile pepper fruit ripening. Both compounds inhibit lipid oxidation, a cause of pigment degradation in chile fruit (Daood et al., 1989). Therefore, the nutritional value and carotenoid stability of chile pepper fruit may be improved through plant breeding. However, a determination of the optimum maturity stage for synthesis of ascorbic acid and tocopherols in the fruit is needed. The purpose of this study was to quantify the changes in ascorbic acid and tocopherols during ripening of four New Mexican-type chile cultivars.

MATERIALS AND METHODS

Materials. Healthy fruit from the second fruit set were harvested from New Mexican-type chile (*Capsicum annuum*) cultivars B-18, New Mexico 6−4, NuMex R Naky, and Sandia at five ripening stages (mature green, breaker, red succulent, red partially dry, and red fully dry). Fruit harvested at the mature green, breaker, and red succulent stages had 90.0 ± 2.0% moisture content. Red partially dry fruit had 70.0 ± 6.0% moisture content, and red fully dry fruit had 16.7 ± 1.6% moisture content. Fruit were harvested from five blocks in the field, washed, separated into pericarps and seeds, homogenized, and kept frozen at −18 °C. For each field block, 12 fruit were harvested from 12 different plants. The 12 fruit were homogenized into a compound sample that constituted a replication.

Methods. *Tocopherol Analysis.* The saponification and extraction procedures were adapted from Piironen et al. (1985). Samples from each field replication were analyzed. Pericarp or seed samples (5 g), ascorbic acid (0.25 g), distilled water (10 mL), and 100% ethanol (30 mL) were homogenized for 90 s and transferred to 50-mL test tubes. After 15 to 20 min, 6 mL of 50% KOH solution were added. The test tubes were flushed with nitrogen, screw-capped, and vortexed for 15−20 s. Samples were held overnight in a water bath at 30 °C, followed by 2 h at 50 °C. After cooling in a cold water bath, the saponified samples were filtered (315 VWR) and collected in 250-mL flasks. The slurry was rinsed with 30 mL of 50% ethanol and extracted three times with 30 mL of hexane. The combined hexane extract containing tocopherols was transferred to a separatory funnel and washed with distilled water. The hexane extract was transferred again to a 250-mL flask. The hexane was evaporated to dryness under a nitrogen stream, and the residue was redissolved in 10 mL of methanol. Samples were filtered through a VWR 0.22 μm membrane prior to HPLC injection.

A Hewlett-Packard 1090 series liquid chromatograph fitted with a Hewlett-Packard 1040 series II photodiode array detector was used to separate and quantify tocopherols. An octadecylsilane column (Phenomenex Prodigy ODS-2, 2 × 250 mm, 5-μm particle size) fitted with a guard column packed

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Table 1. γ- and α-Tocopherol Contents during Ripening of New Mexican-Type Chile Cultivars

	tocopherols (mg/100 g dry wt)	
ripening stage	γ-tocopherol	α-tocopherol
mature green	23.6 c[a]	3.9 e
breaker	35.3 b	7.6 d
red succulent	41.7 a	17.6 b
red partially dry	17.0 d	12.2 c
red fully dry	7.5 e	23.8 a

[a] The values represent the mean of 20 observations (4 cultivars and 5 replications per maturity stage). Means within columns having the same letter are not significantly different ($p > 0.05$).

with the same material was used for HPLC separations. The isocratic mobile phase was acetonitrile−methanol (85:15 v/v). The flow rate was 0.4 mL/min. Samples were run for 20 min with a postrun period of 10 min. A 20-μL sample was injected, and the detection was performed at 294 nm. Tocopherols were identified and quantified by comparing retention times, absorption spectra, and peak areas with those of authentic standards of α-tocopherol, γ-tocopherol, and δ-tocopherol obtained from Sigma (St. Louis, MO). For recovery tests, mixed standard solutions were added to sample aliquots prior to saponification.

Ascorbic Acid Analysis. L-Ascorbic acid was extracted using the method of Nisperos-Carriedos et al. (1992) with some modifications. Sample size was adjusted according to fruit moisture content and consisted of 4 g for fully dry pericarp, 8 g for partially dry pericarp, and 10 g for mature green, breaker, or red succulent pericarps. Samples were blended with 40 mL of 0.05 N H_3PO_4 for 3 min. The slurry was centrifuged for 10 min at 5000 rpm. The supernatant was filtered (541 Whatman), and the slurry was rinsed three times with 20 mL of the extraction solution to finally obtain 100 mL. The extract (4 mL) was passed through a C_{18} Sep-Pak cartridge (Waters Assoc., Milford, MA) that was preconditioned by flushing with 2 mL acetonitrile followed by 5 mL of doubly distilled water. The first 3 mL of the extract were discarded, and the last 1 mL was collected for HPLC analysis.

The HPLC analytical column was packed with octadecylsilane (Phenomenex Spherex 5 C_{18}, 2 × 250 mm, 5 μm particle size) and was preceded by a guard column packed with the same material. The isocratic mobile phase was 2% KH_2PO_4 (pH 2.3). The flow rate was 0.4 mL/min, and samples were run for 6 min with a postrun period of 4 min. A 5-μL sample was injected and the detection was performed at 254 nm for L-ascorbic acid. L-Ascorbic acid was identified and quantified by comparing retention times, absorption spectra, and peak areas with those of L-ascorbic acid standards obtained from Sigma. For recovery tests, standard solutions were added to samples before blending with the extraction solution.

RESULTS AND DISCUSSION

Tocopherol Content. Tocopherols were detected at 294 nm, and the retention times for γ-toc and α-toc were 14.17 and 16.35 min, respectively. The response was linear for γ-toc standards between 50 and 200 ppm and for α-toc standards between 125 and 500 ppm. The detection limit for tocopherols using our method and conditions was 0.07 μg, and the recovery for α-toc was 90.7%.

γ-Tocopherol was present in seeds and α-toc in the pericarp. Differences ($p \leq 0.05$) among the ripening stages were detected for both tocopherols (Table 1). γ-Tocopherol reached its maximum in seeds of fruit at the red succulent stage, and then declined (Figure 1). No significant differences for γ-toc in seeds were detected among cultivars within ripening stages. α-Tocopherol increased during ripening from the mature green to red fully dry stages (Figure 2). Significant differences ($p \leq 0.05$) were detected among cultivars in the red succulent and red fully dry stages. The content of α-toc in chile pericarp is dependent on the lipid content, which varies according to ripening stage and variety (Kanner et al., 1979). Percent oil content was highest in red, dried fruit with 80% dry matter (Kanner et al., 1979). It is unclear why the γ-toc declined in seeds as fruit dried. It may be that the lipid content is highest in seeds from red, succulent fruit.

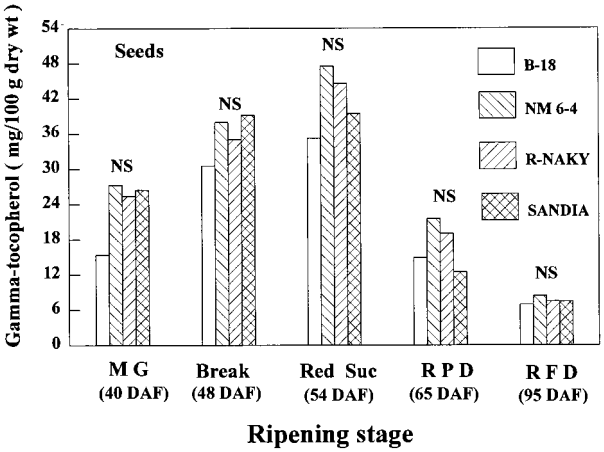

Figure 1. γ-Tocopherol content of chile cultivars during ripening. Each bar is the mean of five replications. Cultivar means within ripening stages are not significantly different ($p > 0.05$). Abbreviations: MG, mature green; break, breaker; Red Suc, red succulent; RPD, red partially dry; RFD, red fully dry; DAF, days after flowering.

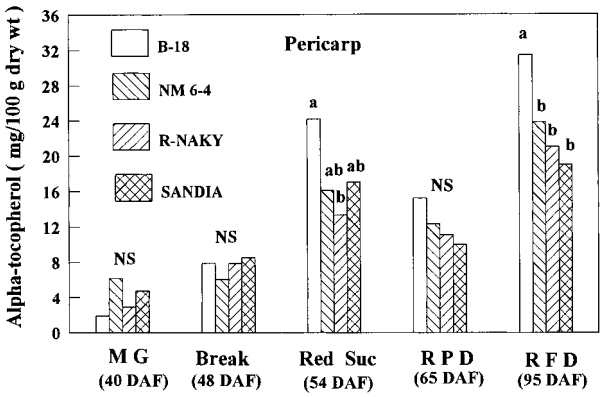

Figure 2. α-Tocopherol content of chile cultivars during ripening. Each bar is the mean of five replications. Cultivar means within ripening stages having the same letter are not significantly different ($p > 0.05$). Abbreviations: MG, mature green; break, breaker; Red Suc, red succulent; RPD, red partially dry; RFD, red fully dry; DAF, days after flowering.

In a study by Kanner et al. (1979), α-toc increased in chile fruit from the green to red stages from 16.4 to 67.8 mg/100 g dry wt. Our results exhibit a similar trend, but lower values. The α-toc content increased from 3.9 mg/100 g dry wt in mature green fruit to 23.8 mg/100 g dry wt in red fully dry fruit. In addition, Daood et al. (1989) and Biacs et al. (1992) reported tocopherol concentrations from 3.7 to 9.3 mg/100 g dry wt for γ-toc in seeds and from 14.2 to 236.0 mg/100 g dry wt for α-toc in pericarp. Our values were much higher for γ-toc and lower for α-toc.

Levels of tocopherols in seeds may be related to color retention of dried ground paprika (Biacs et al., 1989, 1992), but conflicting reports exist. Wall et al. (1994) found that for B-18 and NuMex Sweet paprika, color

retention during storage was improved by the addition of seeds, especially at ambient temperatures. However, Biacs et al. (1989, 1992) stated that color stability was not improved when at least 15% seeds were added to powder of Hungarian paprika cultivars. The amounts of γ-toc in seeds of New Mexican cultivars were much higher (7.5−41.7 mg/100 g dry wt) than in Hungarian cultivars (3.7−9.3 mg/100 g dry wt). This may explain why the color retention of Hungarian cultivars was not improved by the addition of seeds.

For human nutrition, our results confirm that chile peppers are rich sources of vitamin E. Vitamin E includes several tocopherols and tocotrienols. However, α-toc is commonly referred to as vitamin E, because it is the most biologically active and widely distributed form in nature. Red, dry chile had α-toc levels comparable to those for spinach (22.5 mg/100 g) and asparagus (26 mg/100 g) on a dry weight basis, but 4 times higher than tomatoes (6 mg/100 g) (Booth and Bradford, 1963; Kanner et al., 1979). Seed oils are one of the highest sources of tocopherols, but 100 g dry red chile had twice as much α-toc as 100 g of soybean oil (10.7 mg), but only one-third the amount in 100 g of sunflower oil (78.3 mg) (Speek et al., 1985). On a dry weight basis, 100 g of red fruit would exceed the RDA (8−10 mg) for the average adult (NRC, 1989).

Ascorbic Acid Content. L-Ascorbic acid was detected at 254 nm and the retention time was 3.2 min. The minimum detection level was 0.11 μg, and the recovery was 90%. We were unable to analyze L-dehydroascorbic acid (DHA) concentrations with our method, even at a 214-nm detection wavelength. In a report by Wimalasiri and Wills (1983), DHA was below detectable limits (<1 mg/100 g) in pepper fruit. Using the same method, Howard et al. (1994) reported DHA levels of 3.4 mg/100 g for green and red chile fruit (cultivar New Mexico-6). Nisperos-Carriedo et al. (1992) noted that oxalic acid coelutes with DHA when using a reversed-phase column. They coupled a reversed-phase column with an organic acid column to separate these compounds, but had absorption by interfering compounds at 215 nm. They quantified DHA by obtaining the difference in absorbance between 215 and 260 nm. With that method, pepper fruit contained 12 mg/100 g of DHA (Nisperos-Carriedo et al., 1992).

In our study, the levels of L-ascorbic acid varied from 14.8 to 276.6 mg/100 g fresh wt. Differences ($p \leq 0.05$) were detected among cultivars. Ascorbic acid increased during the ripening of pericarp tissue (Figure 3), and peaked at the red succulent stage for Sandia and B-18, and at the red partially dry stage for NuMex R Naky and New Mexico 6−4. These differences can be attributed to variation in moisture content. NuMex R Naky and New Mexico 6−4 had about 75% moisture at the red partially dry stage, whereas Sandia and B-18 had 64% moisture content at that stage. Ascorbic acid is a water-soluble compound that can be expected to decline as the fruit dehydrate on the plant. According to Biacs et al. (1994), ascorbic acid degradation is greatest when the water content decreases below 30%. In our study, red fully dry fruit had 15−18% moisture and the lowest levels of ascorbic acid among maturity stages.

In previous reports using HPLC, ascorbic acid concentrations of mature green chile fruit ranged from 121.8 to 146.5 mg/100 g fresh wt, and red succulent fruit had 233.3 mg/100 g (Howard et al., 1994; Lee et al., 1995). The cultivars used in our study had lower levels of ascorbic acid in mature green fruit, but values were similar to other reports for ripe fruit. In our study, chile fruit (100 g) at the breaker, red succulent, or red partially dry stages contained enough ascorbic acid to meet or exceed the adult RDA (60 mg) for vitamin C (NRC, 1989).

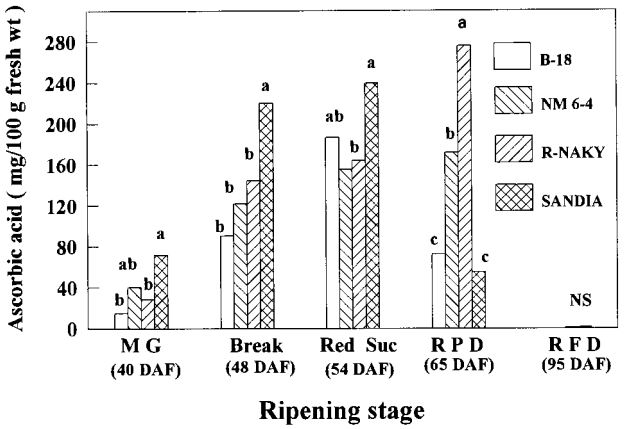

Figure 3. Ascorbic acid content of chile cultivars during ripening. Each bar is the mean of five replications. Cultivar means within ripening stages having the same letter are not significantly different ($p > 0.05$). Abbreviations: MG, mature green; break, breaker; Red Suc, red succulent; RPD, red partially dry; RFD, red fully dry; DAF, days after flowering.

The exact mechanism for ascorbic acid accumulation in fruit is not fully understood, but the concentration is likely associated with carbohydrate metabolism (Lantz, 1943; Mozafar, 1994) and senescence (Leshem, 1988). In green tissues, most ascorbic acid is located in the chloroplasts where it acts as a free radical scavenger. Light intensity increases the concentration of ascorbic acid and glucose, the precursor to ascorbic acid. As such, ascorbic acid levels in fruit are influenced by the availability of light to the crop and to individual fruit, and by diurnal fluctuations in temperature and light (Mozafar, 1994). Ascorbic acid can be transported from leaves to fruit along with carbohydrates during fruit ripening. Total and reducing sugars are at maximum levels in red succulent chile fruit (Wall and Biles, 1993).

In advanced ripening stages, the respiration rate decreases and oxygen accumulates in chile fruit (Biacs et al., 1994). Oxygen species can damage cellular function and hasten senescence (Leshem, 1988). Ascorbic acid is an antioxidant that acts to slow senescence and to maintain biological integrity during ripening. In the process, ascorbic acid is oxidized to DHA, and several oxidases may be involved in ascorbic acid degradation. Ascorbate peroxidase uses two molecules of ascorbic acid to reduce H_2O_2 to water (Noctor and Foyer, 1998). Peroxidases increase in ripening chile fruit (Biles et al., 1997). Conceivably, other oxidases increase with ripening and contribute to ascorbic acid degradation, but no evidence exists for chile fruit.

The data presented here are useful to plant breeders and food scientists working to maximize the nutritional content and carotenoid stability of fresh, processed, and dehydrated chile peppers. In breeding for high nutrient content, selection should be done at the red succulent or red partially dry stages when natural antioxidants are present in high levels.

Chiles are nutritionally balanced with respect to the antioxidant vitamins and could become an important source of vitamins E, C, and A in the human diet.

Currently, green chiles are promoted as high in vitamin C. According to our results, red succulent fruit are a much better nutritional source, because they contain at least three times more vitamin C and four times more vitamin E than green fruit. We suggest that new uses for fresh, red chile fruit be developed.

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LITERATURE CITED

Biacs, P.; Czinkotai, B.; Hoschke, A. Factors affecting stability of colored substances in paprika powders. *J. Agric. Food Chem*. **1992**, *40*, 363−367.

Biacs, P.; Daood, H.; Huszka, T. Biochemical and varietal perspective on the color loss in spice red pepper (paprika). *Hung. Agric. Res*. **1994**, *3*, 32−37.

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

Biles, C. L.; Kuehn, G. D.; Wall, M. M.; Bruton, B. D.; Wann, E. 1997. Characterization of chile pepper fruit peroxidases during ripening. *Plant Physiol. Biochem*. **1997**, *35*, 273−280.

Booth, V. H.; Bradford, M. P. Tocopherol contents of vegetables and fruits. *Brit. J. Nutr*. **1963**, *17*, 575−581.

Byers, T.; Perry, G. Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. *Annu. Rev. Nutr*. **1992**, *12*, 139−159.

Daood, H. G.; Biacs, P. A.; Kiss-Kutz, N.; Hajdu, F.; Czinkotai, B. Lipid and antioxidant content of red pepper. In *Biological Role of Plant Lipids*; Biacs, P. A., Gruiz, K., Kremmer, T., Eds.; Plenum: New York, 1989; pp 491−494.

Howard, L. R.; Smith, R. T.; Wagner, A. B.; Villalon, B.; Burns, E. E. Provitamin A and ascorbic acid content of fresh pepper cultivars (*Capsicum annuum*) and processed jalapeños. *J. Food Sci*. **1994**, *59*, 362−365.

Kanner, J.; Harel, S.; Mendel, H. Content and stability of α-tocopherol in fresh and dehydrated pepper fruits (*Capsicum annuum L.*). *J. Agric. Food Chem*. **1979**, *27*, 1316−1318.

Lantz, E. M. The carotene and ascorbic acid content of peppers. New Mexico State University Agric. Exp. Sta. Bul. 306; New Mexico State University: Las Cruces, NM, 1943.

Lee, Y.; Howard, L. R.; Villalon, B. Flavonoids and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars. *J. Food. Sci*. **1995**, *60*, 473−476.

Leshem, Y. Y. Plant senescence processes and free radicals. In *Free Radical Biology and Medicine;* Pergamon Press: Oxford, U.K., 1988; pp 39−49.

Mejia, L. A.; Hudson, E.; Gonzalez de Mejia, E.; Vasquez, F. Carotenoid content and vitamin A activity of some common cultivars of Mexican peppers (*Capsicum annuum*) as determined by HPLC. *J. Food Sci*. **1988**, *53*, 1448−1451.

Mozafar, A. *Plant Vitamins: Agronomic, Physiological and Nutritional Aspects*; CRC Press: Boca Raton, FL, 1994.

National Research Council (NRC), Food and Dietary Board. *Recommended Dietary Allowances*; National Academy of Sciences: Wash. D.C., 1989.

Nisperos-Carriedos, M. O.; Buslig, B. S.; Shaw, P. E. Simultaneous detection of dehydroascorbic, ascorbic and some organic acids in fruits and vegetables by HPLC. *J. Agric. Food Chem*. **1992**, *40*, 1127−1130.

Noctor, G.; Foyer, C. H. Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **1998**, *49*, 249−279.

Piironen, V.; Syvaoja, E. L.; Varo, P.; Salminen, K.; Koivistoinen, E. Tocopherols and tocotrienols in Finnish foods: meat and meat products. *J. Agric. Food Chem*. **1985**, *33*, 1215−1218.

Speek, A. J.; Schrijver, J.; Schreurs, W. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric detection. *J. Food Sci*. **1985**, *50*, 121−124.

Wall, M. M.; Biles, C. L. Alternaria fruit rot of ripening chile peppers. *Phytopathology* **1993**, *83*, 324−328.

Wall, M.; Bosland, P.; Waddell, C. Postharvest color analyses of dehydrated red chile. Proc. 12th Natl. Pepper Conf.; Las Cruces, NM, 1994.

Wimalasiri, P.; Wills, R. B. H. Simultaneous analysis of ascorbic acid and dehydroascorbic acid in fruits and vegetables by high-performance liquid chromatography. *J. Chromatogr*. **1983**, *256*, 368−371.

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