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Determination of antioxidant vitamins in tomatoes

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This study was conducted to investigate the antioxidant vitamin (vitamin E, vitamin C and β-carotene) content of one of the most important vegetables, tomato, using modern analytical techniques. High-performance liquid chromatographic procedures allowed the separation and quantification of these vitamins as well as their analogues in different cultivars.

Carotenoid extract could be fractionated into 14 components, including lycopene, β-carotene and lutein as the major ones. Paired-ion liquid chromatography provided excellent separation of ascorbic acid with high peak purity. In addition to different analogues of tocopherol, ubiquinone-10 could also be separated and sensitively detected by normal-phase chromatography and fluorescence detection.

The highest concentrations (3.15–3.98 $\mu g\ g^{-1}$) of total tocopherol (mainly α-analogue) were found in tomato fruits of Katinka, Gitana and Floriset cultivars. The vitamin C content was maximal (36–48 mg per 100 g) in DRW 3126, Primato, Tampo and Monika cultivars. The highest values for β-carotene were found in Monika, Ultimo and Falcato cultivars (3.5–3.9 $\mu g\ g^{-1}$). The dynamics of fruit ripening were also examined. © 1997 Elsevier Science Ltd

INTRODUCTION

In recent years great interest has been focused on antioxidant vitamins (vitamin E, vitamin C and β-carotene), particularly because of their likely role in the prevention of coronary heart disease and cancers (Gey & Puska, 1989; Simon, 1992; Gerster, 1991). Antioxidant vitamins can counteract the oxidizing effects of lipids by scavenging oxygen free radicals which have been found as major promoters of such diseases (Bruckdorfer, 1990; Sies, 1991).

The levels of the essential antioxidant vitamins, in contrast to other antioxidative defences, are determined mainly by their dietary supply. Fruit and vegetables are the main sources of antioxidant vitamins, making these foods essential to human health. Since tomatoes are major components of daily meals in many countries, it is becoming increasingly important to be able to assess their nutritional value in terms of antioxidant vitamin content. Furthermore, some environmental and varietal factors can initiate unfavourable changes in the chemical composition of plant products, which makes periodic analytical surveys important.

The objective of this work was to estimate, using modern analytical techniques, the antioxidant vitamin content of tomatoes cultivated in Hungary.

MATERIALS AND METHODS

Raw material

Tomato fruits from different cultivars were obtained from the experimental station of the Faculty of Horticulture, Kecskemét. The fruits were stored under refrigeration soon after harvest and at −20°C when not immediately analysed.

Extraction of carotenoids

The method described by Daood *et al.* (1989) was used for carotenoid extraction. Ten grams (random sample) of tomato fruit were taken, disintegrated with quartz sand in a mortar with pestle and mixed with 20 ml methanol. The mixture was mixed with 60 ml CCl_4–methanol (3:1) and mechanically shaken for 20 min.

The CCl_4 fraction was separated from the aqueous phase in a separatory funnel and dried over Na_2SO_4. The filtrate was evaporated to dryness under vacuum at 40°C.

Extraction and saponification of tocopherols

The method used for carotenoid extraction was also used for extraction of tocopherol. The extracted lipid fraction was saponified by refluxing with 4 ml of 30% methanolic KOH for 30 min at the boiling point of methanol in the presence of 0.5 g ascorbic acid. After cooling the flask, 15 ml of salted water were added and the analogues of tocopherol were extracted twice with 40 ml petroleum ether in a separatory funnel. The ether fractions were collected, washed twice with distilled water and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum at 30°C and the residues were redissolved in 5 ml of HPLC-grade hexane.

Extraction of organic acids (vitamin C)

Ten gram samples of tomatoes were randomly taken and diced in a crucible mortar with quartz sand. The macerate was then mixed with 50 ml of 2% metaphosphoric acid and transferred to a conical flask. Following mechanical shaking for 15 min, the mixture was filtered through a Rudfilter MN 640 d filter paper to obtain clear extracts, which were kept at −20°C until analysis.

HPLC analysis

Apparatus

A Beckman series liquid chromatograph was used, consisting of a Model 114 solvent delivery pump, a Model 421 controller, a Model 165 variable wavelength UV/visible detector (for carotenoids and organic acids) and a Shimadzu fluorescence detector (for tocopherols). The detector signals were recorded by a Model C-R2A Shimadzu integrator. For photodiode-array detection, a Waters Model 990 liquid chromatograph was used.

The data were stored and processed by means of a NEC.APCIV power meta 2 IBM computing system. The absorption spectra of carotenoids were recorded between 190 and 700 nm (for carotenoids) and 190–350 nm (for organic acids) at the rate of two spectra per second. Separation conditions for the antioxidant vitamins are listed in Table 1.

Identification of peaks

Carotenoids The peaks of β-carotene on the chromatogram were identified by comparing their retention times and spectra with that of pure standard (Sigma, St Louis, USA).

Identification of *cis*-isomers of carotenoids was based on the appearance of extra maxima between 320 and 360 nm in the absorption spectrum of the individual peaks (Chandler & Schwartz, 1987).

Tocopherols For identification of peaks, the retention times and maximum absorption spectra of tocopherols were compared with those of standard materials (Sigma), which were also used for quantification.

Ascorbic acid Chromatographic peaks were identified by comparing both retention time and absorbance spectra with those of standards (Fluka, Switzerland). Cochromatography of the standards with the samples was also used to identify peaks with close retention times.

RESULTS AND DISCUSSION

HPLC of tocopherols

Figure 1 show the HPLC separation of tocopherol analogues extracted from ripe tomato fruits. The normal phase system provided an excellent separation of α-, β- and γ-tocopherols as well as their tocotrienes. The fluorescence detector allowed monitoring of ubiquinone derivatives, which can perform antioxidation functions as actively as tocopherols do in biological systems. Such a simultaneous detection cannot be achieved with UV detectors which are much less sensitive than

Table 1. Conditions for HPLC separation of antioxidant vitamins

Parameters	Conditions		
	Carotenoids	Organic acids	Tocopherols
Column	Chromsil C-18 6 μm, 46 mm×250 mm	Lichrosorb C-18 10 μm, 46 mm×250 mm	Lichrosorb 10 μm, 46 mm×250 mm
Eluent	Acetonitrile–isopropanol–methanol–water (39:52:5:4)	0.1 M KH_2PO_4–methanol–TBAOH (97:3:0.05)	Hexane–ethanol (99.5:0.5)
Flow rate	0.9–1.2 ml min^{-1}	1 ml min^{-1}	1.2 ml min^{-1}
Detection	Visible 440	UV 225 nm	Fluorescence: excitation 295 nm, emission 295 nm
Reference	Biacs & Daood, 1994	Daood *et al.*, 1994	Speek *et al.*, 1985

TBAOH, tetrabutylammonium hydroxide.

fluorescence detectors. Reproducibility of the analytical method was tested by repeating (five times) the measurement for the same well-homogenized sample. The coefficient of variation (CV%) was calculated to be 3.5 for tocopherols, indicating that the analytical procedure used is highly accurate.

HPLC of vitamin C

By ion-pair chromatography, ascorbic acid could be separated from other organic acids existing in the extract of tomato fruit (Fig. 2). To check whether the ascorbic acid peak contains any interfering materials, a peak purity test was displayed by the chromatographic software and showed an organic acid with absorption maxima of 190 nm and 208 nm overlapping with ascorbic acid. Fortunately such interfering materials did not affect the quantitation of ascorbic acid since their absorption at the monitoring wavelength used (225 nm) is negligible.

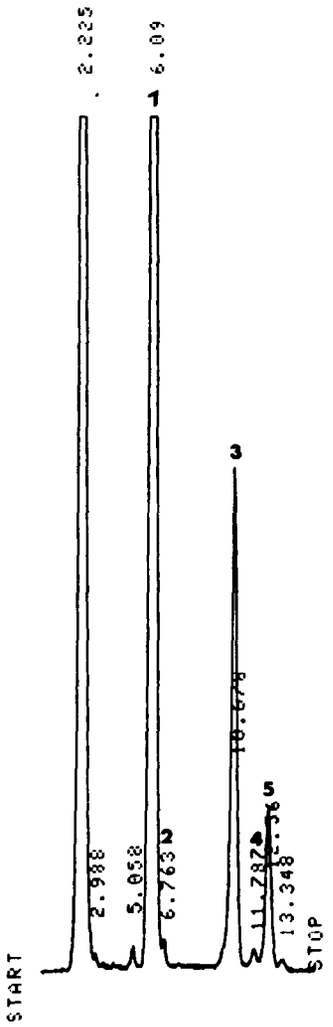

Fig. 1. Separation of tocopherol analogues from tomato by normal-phase HPLC using hexane–ethanol, 99.5:0.5, as the mobile phase and fluorescence detection (excitation 295 nm, emission 320 nm). 1, α-tocopherol; 2, tocotriene; 3, ubiquinone-10; 4, β-tocopherol; 5, γ-tocopherol.

In addition to the high sensitivity of this method (maximum detectable concentration was 3–4 μg ascorbic acid), it showed high reproducibility (CV% of 2–3 when five measurements were performed on the same sample).

HPLC of carotenoids

The diversity of the carotenoid extract of some plant products such as tomato makes it difficult to separate the individual carotenoids by a one-step procedure. However, the recently elaborated HPLC method (Biacs & Daood, 1994) provided a good isocratic separation of the carotenoid complex extracted from plant products.

Shown in Fig. 3 is the separation profile of tomato pigment. The pigment was fractionated into 14 components of which lutein, β-cryptoxanthin, lycoxanthin, lycopene, neolycopene, γ-carotene and β-carotene were the major carotenoids, with lycopene being the main red-coloured one accounting for more than 75% of the total carotenoid content.

Among carotenoids separated by HPLC, β-carotene is of special interest because it is the only one that has antioxidant properties in the human and animal body (Burton & Ingold, 1984). Furthermore, we focused only on the all-*trans* form of β-carotene which has been reported to be the biologically most active analogue of

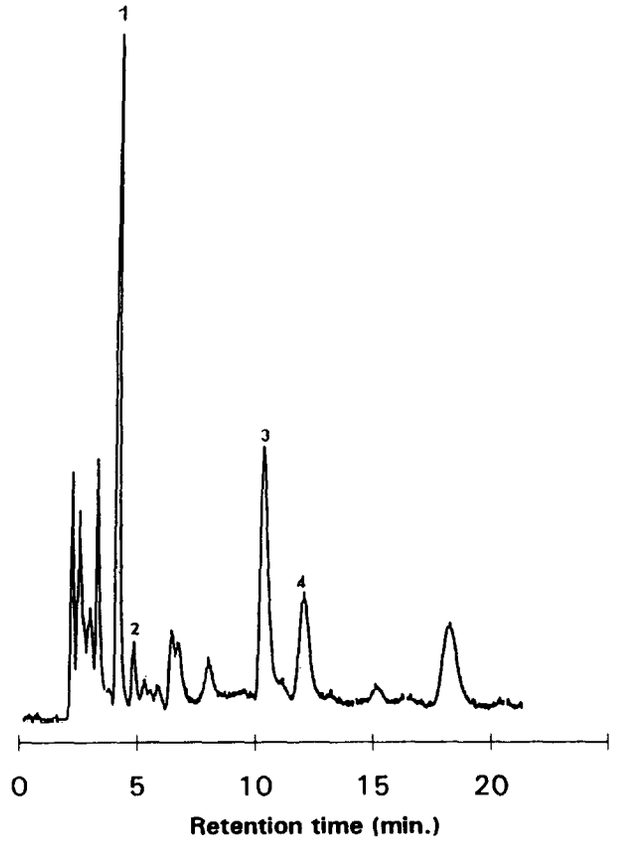

Fig. 2. HPLC profile of organic acids and vitamin C from ripe tomato fruit. For conditions see text. 1, ascorbic acid; 2, malic acid; 3, citric acid; 4, fumaric acid.

carotene; its conversion to the *cis*-form substantially reduces its antioxidant activity (Sies, 1991).

Changes during ripening

During the ripening process many vegetables undergo characteristic changes that, in general, lead to an increase in nutritive value and consumer acceptance. However, some important micronutrients tend to decrease when the fruits become fully ripe. This depends upon the mechanism of the ripening process (climacteric or non-climacteric).

To characterize the dynamics of ripening, the fruits of Floriset cultivar were harvested at four ripening stages: green, colour break-I (yellow), colour break-II (pink) and red. Figure 4 shows the changes that occurred in the vitamin C content of tomato fruits during ripening stages. The maximum concentration of ascorbic acid was estimated in tomato fruits that turned yellow in colour (colour break-I); however, the advanced ripeness caused the ascorbic acid content to decrease, most likely due to its antioxidant function when the ripening cells absorb high amounts of oxygen as a result of increasing rate of cell respiration, the characteristic physiological change in climacteric fruits and vegetables at ripeness (Tünk *et al.*, 1993).

As for β-carotene, its concentration increased in proportion to the advanced ripeness in accordance with the rapid accumulation of red pigment (fruit coloration) (Fig. 5). Such a change tendency does not agree with that observed by Biacs *et al.* (1987), who found that β-carotene approached its maximum level in yellow-colored fruits of Ventura cultivar (tomato for processing) and then declined. This variation is probaly due to the effect of some varietal factors on carotenogenesis in tomato fruit.

The change in tocopherol analogues as a function of climacteric ripeness is depicted in Fig. 6. α- and β-forms increased proportionally to the advanced ripeness, while γ-tocopherol approached its maximum level at colour break-I stage (yellow fruits), then declined showing a change similar to that observed with ascorbic acid.

The changes in γ-tocopherol and ascorbic acid revealed their important role as first oxidation barriers in the lipophilic and hydrophilic phases of the foods. Despite the high antioxidative activity of γ-tocopherol

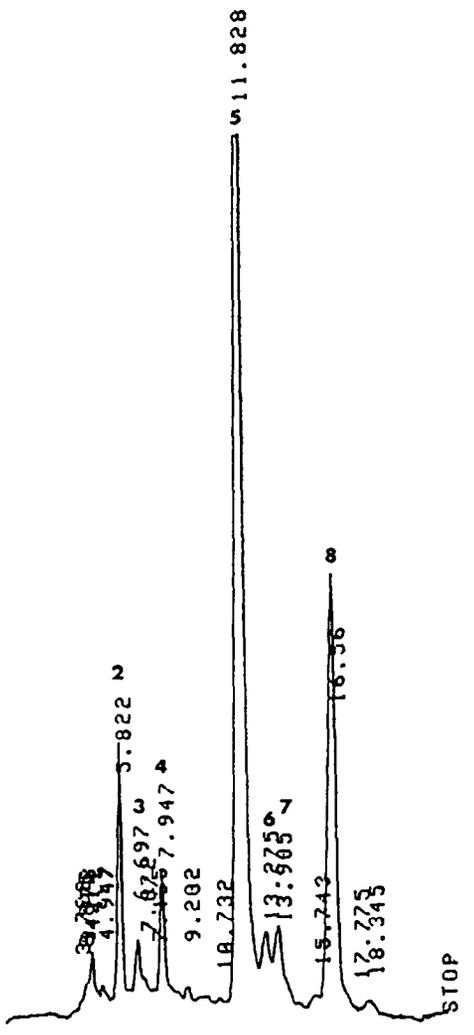

Fig. 3. HPLC profile of carotenoid pigments extracted from ripe tomato fruit. For conditions see text. 1, violaxanthin; 2, lutein; 3, β-cryptoxanthin; 4, lycoxanthin; 5, lycopene; 6, neo-lycopene; 7, γ-carotene; 8, β-carotene.

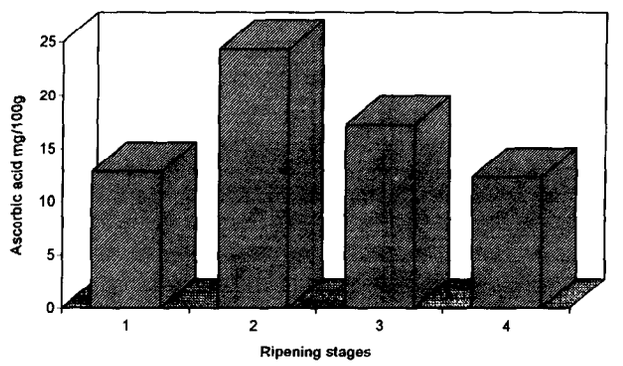

Fig. 4. Changes in ascorbic acid content during ripening stages of tomato fruit (Floriset cultivar).

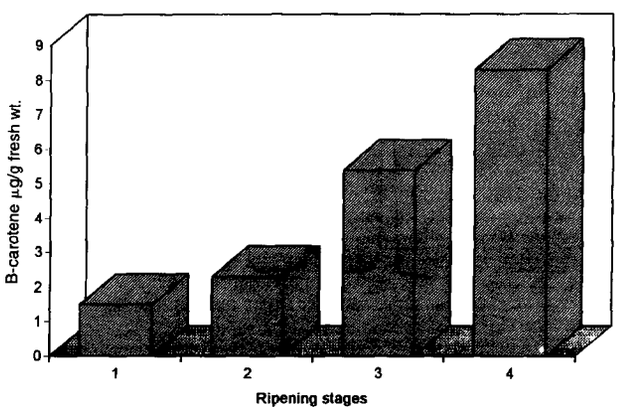

Fig. 5. Changes in β-carotene content during ripening stages of tomato fruit (Floriset cultivar).

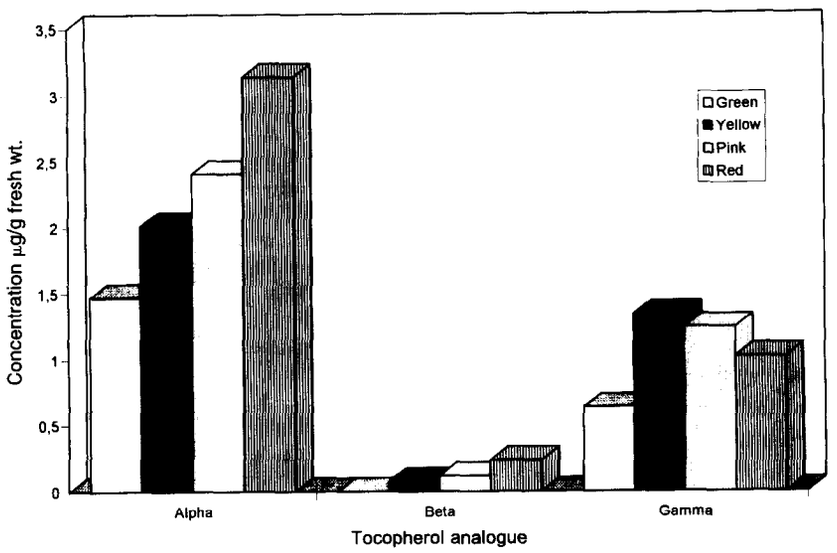

Fig. 6. Changes in tocopherol content of tomato as a function of ripeness.

in food systems, it is of no importance from the nutritional point of view because of being biologically inactive as vitamin E in the human body. As a highly reactive antioxidant, it is very important in protection of foods against oxidative deterioration taking place during ripening, processing and storage.

Effect of varietal factors

Table 2 shows the results obtained from the analysis of antioxidant vitamins in different cultivars of tomato. The highest values with regard to α-tocopherol (3.15 $\mu g\ g^{-1}$) was obtained with Katinka, while the lowest values were estimated in Tampo, Pankor, DRW 3126 and Falcato. Other cultivars contained medium levels of vitamin E.

Table 2. Antioxidant vitamin content of different tomatoes cultivated in 1995, Kecskemét, Hungary

Cultivar	Carotenoid ($\mu g\ g^{-1}$)		Tocopherol ($\mu g\ g^{-1}$)		Ascorbic acid ($mg\ g^{-1}$)
	β-Carotene	Total	α-Tocopherol	Total	
DRW 3042	1.66	28.03	2.16	2.37	0.29
Primato	2.73	41.39	1.46	1.88	0.37
Floriset	1.52	26.57	2.25	3.15	0.26
Katinka	3.62	56.48	3.15	3.98	0.32
Selma	1.14	25.83	1.40	1.40	0.26
Revido	1.40	18.45	1.73	2.78	0.25
DRW 3126	1.45	30.23	1.07	1.57	0.48
Gitana	3.74	52.62	2.10	3.36	0.34
Ultimo	2.86	45.45	1.45	1.84	0.26
Relento	2.46	36.50	2.23	2.69	0.26
Pankor	1.70	18.69	0.98	1.32	0.22
Delfino	3.13	39.25	1.77	2.36	0.33
Tampo	1.13	27.80	0.96	1.17	0.38
Monika	3.04	60.75	1.78	2.52	0.36
Falcato	1.82	30.88	1.31	1.64	0.31

The values represent means of 3–4 replications with coefficients of variation (CV%) ranging from 4 to 10 for carotenoids, 6 to 10 for tocopherols and 5 to 8 for ascorbic acid.

DRW 3126, Tampo, Primato and Monika were evaluated as cultivars with high vitamin C levels (36–48 mg per 100 g). The lowest values (21–26 mg per 100 g) were recorded for Pankor, Revido, Ultimo, Selma and Floriset. In the other cultivars, ascorbic acid concentration ranged between 29 and 34 mg per 100 g. In comparison with the values reported in the literature, the vitamin C level in Hungarian salad tomatoes is high. Bajaj *et al.* (1990) reported a range of 6.13–17.56 mg per 100 g for ascorbic acid in 35 varieties, while a value of 21–23 mg per 100 g has been recorded for ascorbic acid content in other cultivars (De Serrano *et al.*, 1993).

Although carotenoid content was maximal in Monika (60.8 $\mu g\ g^{-1}$), followed by Katinka and Gitana, the highest values for β-carotene were found in Gitana, Katinka and Delfino (3.13–3.79 $\mu g\ g^{-1}$). The lowest levels of β-carotene were in Tampo and Selma. These data indicate that varietal factors can affect (to a considerable extent) the overall biosynthesis of carotenoids, particularly β-carotene formation via cyclization of lycopene. The β-carotene content of Hungarian cultivars seems to be very close to that of some Japanese (Johjima, 1994) and American (Tonucci *et al.*, 1995) tomatoes, but much less than that found in Brazilian cultivars such as Santa Cruz, which contains about 5.1 µg of β-carotene per 1 g of fresh fruit (Tavares & Rodriguez-Amaya, 1994).

CONCLUSION

From these results it can be concluded that, although not a rich source of vitamin E and provitamin A, tomatoes merit attention because of their year-round availability and accessible prices, which makes their

average daily consumption by man much higher than that of all other fruits and vegetables. Moreover, tomato products supply human diets with non-provitamin A carotenoids such as lycopene, which has been found to be a more efficient antioxidant (singlet oxygen quencher) than β-carotene, α-carotene, α-tocopherol or albumin-bound bilirubin (Di Mascto *et al.*, 1989; Sies, 1991).

To put the estimated values of antioxidant vitamins in context with respect to the recommended daily intake of each, it is noteworthy that a comprehensive evaluation of several cross-cultural European epidemiological studies revealed 60–100 mg vitamin C, 25–37 mg vitamin E and 6–10 mg carotene as the recommended daily intake to provide good health and a reduction in risk of some diseases (Lachance, 1988; National Research Council, 1989; Germann, 1990) Accordingly, one serving of 100 g of tomato can contribute 24–48%; 0.5–0.8% and 2.7–4.0% the recommended daily intake of vitamin C, vitamin E and β-carotene, respectively.

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