Analysis of Antioxidant Compounds and Hydroxymethylfurfural in Processing Tomato Cultivars

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SUMMARY. Tomato (*Lycopersicon esculentum*) fruit is an important source of antioxidants, which are important in the prevention of many cancer types and cardiovascular diseases. In ripe tomato fruit, one of the most significant antioxidant compounds is lycopene. In this study, effects of the cultivars and the environmental conditions on the lycopene content were investigated. Temperature effect was characterized by calculating growing degree-day (GDD). Relationships among lycopene, total polyphenols, organic acid, and total antioxidant status (TAS) were analyzed. A variation of more than 30% was found among the lycopene levels of five processing tomato cultivars. Lycopene content ranged from 79.7 to 155.0 mg·kg⁻¹ fresh weight. There was a significant difference between the lycopene concentrations of fruit harvested at two different dates. The values were higher at the second harvest, probably due to the cooler weather conditions preceding that harvest. Total polyphenols correlated strongly with TAS but the other antioxidants did not have any relationship to TAS. GDD were strongly and positively correlated with hydroxymethylfurfural content in fresh tomatoes.

The nutrient value of tomato fruit is intimately connected with its composition in carbohydrates, organic acids, minerals, vitamins, and pigments (Helyes, 1999). In the last few years the consumption of tomatoes has played a great part in the prevention of many cancer types and cardiovascular diseases (Djuric and Powell, 2001; Levy et al., 1995; Micozzi et al., 1990; Takeoka et al., 2001). This protective effect is due to the antioxidant compounds in tomato fruit. These compounds are, among others, carotenoids, polyphenols, flavonoids, and ascorbic acid. The most essential carotenoid form is lycopene. Lycopene acts by quenching the singlet oxygen and scavenging peroxy radicals, which cause the oxidative modification of lipids, proteins, low density lipoprotein (LDL) particles, and DNA (Oshima et al., 1996; Tapiero et al., 2004). This antioxidant activity is related to its acyclic molecular structure involving 11 conjugated double bonds. Lycopene has the ability to regulate intercellular communication and cell growth. For

that reason it may enhance inhibition of tumor cell growth (Bertram et al., 1991; Trosko and Chang, 2001). The biosynthesis of lycopene is under genetic control (Hanson et al., 2004). It is formed from phytoene during four desaturation steps (Harker and Hirschberg, 1998). The lycopene content of tomato fruit is influenced by the cultivar and also by cultural practices and environmental conditions. Lycopene forms more readily at lower temperatures, 16 to 26 °C being the most favorable temperature range. Above 30 °C its biosynthesis is inhibited (Türk et al., 1994). According to Helyes et al. (2002) the lycopene content of tomato fruit cultivated in Hungary ranged from 39 to 171 mg·kg⁻¹. Tomato fruit is also rich in polyphenols, which amount to the largest part of the antioxidant capacity of the soluble phase. Thermal stress induces the accumulation of phenolic compounds like flavonoids and phenylpropanoids. At 35 °C, the polyphenol level is double that produced at 25 °C (George et al., 2004). This phenomenon could be considered an acclimation mechanism of the plant against heat stress (Rivero et al., 2001).

Polyphenols similar to lycopene have antioxidant capacity, which repeatedly exceed the capacity of ascorbic acid. George et al. (2004) measured large variations among the polyphenol (104–400 mg·kg⁻¹) and ascorbic acid (85–560 mg·kg⁻¹) content of different tomato cultivars. Light exposure enhances ascorbic acid accumulation in tomato fruit (Dumas et al., 2003). Tomatoes grown in greenhouses have lower ascorbic acid content than those grown in open fields (Lopez-Andreu et al., 1986). The quality and the chemical compounds of tomato change during thermal processing. High temperature treatment can promote a browning in processed tomatoes. In processed products, both enzymatic and non-enzymatic reactions are responsible for the development of brown compounds. The Maillard reaction belongs to the last group. Its intermediate compound is hydroxymethylfurfural (HMF), which is harmful to human health. HMF can be found not only in processed tomato foods but also in fresh fruit. According to Cámara et al. (2003), HMF content is directly proportional to the lycopene content of the processed tomato products. The aim of our study was to establish the effect of temperature on lycopene content in five processing tomato cultivars. The relationship between TAS and antioxidant molecules such as lycopene, total polyphenols, and ascorbic acid was also analyzed.

Materials and methods

PLANT MATERIALS. The experiment was conducted on the Experimental Farm of the Department of Horticultural Technologies at Szent István University, Gödöllő. Open-field experiments were conducted with five cultivars in 2001 ['Bonus' (Semini Hungária Vetőmag Kft., Budapest, Hungary), 'Early Fire' (United Genetics, Hollister, Calif.), 'Falcrosso' (Nunhems, Haalen, The Netherlands), and 'Nívó' and 'Korall' (Hungarian Vegetable Seed Co., Zöldségtermesztési Kutató Intézet, Kecskemét, Hungary)]; and one cultivar ('Early Fire') in 2002. The experimental design was

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Units

To convert U.S. to SI,
multiply by

0.3048

0.0929

1

(°F – 32) ÷ 1.8

U.S. unit

ft

ft²

ppm

°F

SI unit

m

m²

mg·kg⁻¹

°C

To convert SI to U.S.,
multiply by

3.2808

10.7639

1

(1.8 × °C) + 32

a randomized block, with four replications for each cultivar. Seeds were sown on 5 Apr. 2001 in the greenhouse and transplanted on 11 May 2001. Seedlings were planted in twin rows (0.4 m) with 0.4-m spacing inside the row and 1.4 m between adjacent twin rows, and individual plots were 80 m². During the growing season, nutrition supply, irrigation, and plant protection followed recommendations for commercial tomato production in the region (Helyes and Varga, 1994). Fruit samples were picked randomly by hand from the middle section of the plants. The sampling section was 5 m in each plot. This procedure was carried out twice: on 22 Aug. and 5 Sept. 2001. 'Early Fire' processing tomato also was cultivated in 2002. The growing method was identical to that followed in 2001. Sowing in the greenhouse was conducted on 10 Apr. and field transplanting on 16 May in 2002. Fruit were sampled three times (30 July, 21 Aug., and 4 Sept.) in 2002.

MEASUREMENT OF ENVIRONMENTAL PARAMETERS. Temperature and photosynthetically active radiation (PAR) were measured six times per hour (hourly mean were recorded and daily mean were calculated), using a Skye DataHog micrometeorological instrument, placed at 2-m height (Skye Instruments Ltd, Llandrindrod Wells, U.K.) (Fig. 1). Reduced ceiling method with 10 °C base and 32 °C ceiling temperature was used to calculate GDD in case of lycopene and 10 °C base and 24 °C ceiling temperature in case of HMF (Perry et al., 1997).

CHEMICAL ANALYSIS. Four fruit from each of four repetitions were washed, cut, and mixed using homogenizer (PT 3100; Kinematica Inc., Newark, N.J.) and the juice samples were refrigerated at -18 °C until analysis. Lycopene from homogenized tomato was extracted with n-hexane-methanol-acetone (2:1:1) mixture containing 0.05% butylated hydroxytoluene (BHT). Water-free sodium sulphate (Na₂SO₄) was used to remove water traces from the upper part. Optical density of the hexane extract was measured spectrophotometrically at 502 nm, compared with the hexane blank (Sadler et al., 1990) using a ultraviolet-visible spectrophotometer (Lambda 3B; PerkinElmer Life and Analytical Sciences, Inc., Wellesley, Mass.). The concentration of lycopene was calculated using the

specific extinction coefficient $E_{1\text{cm}}^{1\%}$ 3150 (Merck and Co., Inc., 1989). Total polyphenols were determined using the Folin-Denis method according to the Association of Official Analytical Chemists (AOAC) official method of analysis 952.03 (AOAC, 1990). Ascorbic acid content was measured chromatographically using high performance liquid chromatography (Dong and Pace, 1996).

The determination of TAS was carried out using the Randox diagnostic kit (Randox Laboratories Ltd., Crumlin, U.K.) according to the procedure de-

veloped by Miller et al., (1993). HMF was quantified using the colorimetric method based on the thiobarbituric acid color reaction measured at 443 nm (Guzmán et al., 1986).

STATISTICAL ANALYSIS. Two-way analysis of variance (ANOVA) was used and the means separated according to Student's test at $P = 0.05$ to determine significant differences among cultivars, harvesting dates, and years. The correlation analysis was done by the linear regression-analysis module. All statistical analyses were performed using the Microsoft® Excel 2002

Fig. 1. Daily temperature (Temp), photosynthetically active radiation (PAR), and rainfall (Rain) during tomato fruit development in (A) 2001 and (B) 2002; $(1.8 \times ^\circ\text{C}) + 32 = ^\circ\text{F}$; 1 mm = 0.0394 inch; 1 m² = 10.7639 ft².

Analysis Toolpak (Microsoft Corp., Redmond, Wash.).

Results and discussion

There were significant differences in lycopene contents of the five processing tomato cultivars harvested in September, but not in August (Table 1). Lycopene content of the cultivars ranged from 79.7 to 155.0 mg·kg⁻¹, 'Nívó' having the highest content (155.0 mg·kg⁻¹), and 'Early Fire' second with a very similar value (140.5 mg·kg⁻¹). The lycopene content of 'Falcrosso' and 'Korall' differed significantly from that of 'Nívó' and 'Early Fire' at the second harvesting date. The lycopene content of fruit differed between the two harvest dates. The lycopene content of the fruit harvested on 22 Aug. ranged from 79.7 to 101.0 mg·kg⁻¹, whereas the lycopene concentrations of the fruit harvested on 5 Sept. ranged from 111.3 to 155.0 mg·kg⁻¹ in 2001 (Table 1). The amount of lycopene in the second harvest was generally higher than that in the first harvest. The average lycopene content of the samples picked on 5 Sept. was 45% higher than in samples harvested earlier. In the 10 d preceding the 22 Aug. harvest, there was more than 20 mm rainfall and the maximum temperature was over 30 °C on five occasions (Fig. 1). These conditions probably inhibited lycopene production and could explain why the lycopene content was lower by 40.7 mg·kg⁻¹ than the average. On the other hand, the week preceding the second harvest was rainy and cold. During this week, the minimum temperature was 10 °C on 5 of 7 d and the maximum values were hardly higher than 20 °C. It is likely that the low temperatures activated the biosynthesis of lycopene. Each tomato plant was supplied with the same fertilizer and the same amount of water so the large difference in the lycopene content between the two harvests was probably caused by weather conditions.

Ascorbic acid level was highly variable, ranging from 161 to 447 mg·kg⁻¹. In the samples picked on 22 Aug. it was generally higher than in those picked on 5 Sept. (Table 1). Ascorbic acid content was inversely proportional to the lycopene level. The ascorbic acid content decreased by more than 50% at the second harvest date. Ascorbic acid is synthesized from glucose and its accumulation has been shown to

increase under high solar radiation conditions (Dumas et al. 2003; Winsor and Adams, 1976) 10 d preceding the first harvest. As mentioned earlier, weather conditions before the second harvest were wet and cloudy.

Lycopene analysis was repeated in 2002 with cultivar Early Fire and data analysis showed no year by treatment interaction. We compared the average lycopene content in the harvests of 22 Aug., and 5 Sept. in 2001, with the average lycopene content in the harvests of 21 Aug. and 4 Sept. in 2002. We could not find any significant difference between the 2 years at $P = 0.05$.

The last 10 d of the ripening phase may play a key role in the formation of lycopene. Lycopene accumulates mainly in the last period of ripening (Brandt et al., 2006). The average daily tem-

perature during the 14 d before harvest (August) rose steadily from 17 to 26 °C in both years, which can be considered roughly as the optimum temperature for tomato plants. The maximum temperature, however, exceeded six times in 2001 and twice in 2002 the critical value of 30 °C. Temperature prior to the second harvest varied from 12 to 22 °C in 2001 and 19 to 21 °C in 2002. Maximum temperature did not exceed 30 °C during this period. The cooler weather conditions may have caused the higher lycopene level of tomato fruit in the second harvest. For this reason, only the heat units accumulated during the 10 d preceding the harvest were used. There was significant negative correlation (significant at $P = 0.001$) between GDD and lycopene content of fruit (Fig. 2).

Table 1. Lycopene and ascorbic acid content of five tomato cultivars at two harvest dates in 2001 (mean $\pm$ SD, n = 4).

Cultivars ^y	Lycopene (mg·kg ⁻¹) ^z		Ascorbic acid (mg·kg ⁻¹) ^z	
	22 Aug.	5 Sept.	22 Aug.	5 Sept.
Early Fire	101.0 $\pm$ 20.9	140.5 $\pm$ 17.0 ab	286.5 $\pm$ 10.7 a	163.0 $\pm$ 35.2
Bonus	85.2 $\pm$ 2.9	127.0 $\pm$ 27.9 ab	334.0 $\pm$ 60.0 ab	161.0 $\pm$ 29.7
Falcrosso	79.7 $\pm$ 17.2	111.3 $\pm$ 28.3 b	415.3 $\pm$ 49.6 b	162.3 $\pm$ 24.8
Korall	81.0 $\pm$ 14.8	113.0 $\pm$ 7.0 b	446.5 $\pm$ 105.5 b	191.0 $\pm$ 28.9
Nívó	97.0 $\pm$ 18.3	155.0 $\pm$ 14.0 a	441.5 $\pm$ 28.2 b	192.5 $\pm$ 54.5
Average	88.7	129.4	348.8	174.0

^z1 mg·kg⁻¹ = 1 ppm.

^yData in the same column bearing the same letter are not significantly different at the 5% level (tested by Student's test).

Fig. 2. Correlation between lycopene content of 'Early Fire' tomato fruit and growing degree-day (GDD) in 2001–02 (n = 20). The vertical bar represents the standard error of the regression. 1 mg·kg⁻¹ = 1 ppm; (1.8 $\times$ °C) + 32 = °F.

Fig. 3. Correlation between antioxidants (total polyphenols, ascorbic acid, lycopene) and total antioxidant status (TAS) of tomato fruit in 2002 ($n = 12$). The vertical bars represent the SE of regressions; $1 \text{ mg}\cdot\text{kg}^{-1} = 1 \text{ ppm}$, $1 \text{ L} = 0.2642 \text{ gal}$.

Table 2. Lycopene and hydroxymethylfurfural (HMF) content of 'Early Fire' tomato at three harvest dates in 2002 (mean $\pm$ SD, $n = 4$).^z

		30 July	21 Aug.	4 Sept.
Lycopene ($\text{mg}\cdot\text{kg}^{-1}$) ^y	Average	102.2 ± 18.9	100.5 ± 3.1	110.4 ± 13.6
HMF ($\text{mg}\cdot\text{kg}^{-1}$) ^y	Average	12.8 ± 1.5	14.3 ± 2.6	11.3 ± 1.7

^zDifferences among harvest dates were nonsignificant.

^y $1 \text{ mg}\cdot\text{kg}^{-1} = 1 \text{ ppm}$.

Fig. 4. Correlation between hydroxymethylfurfural (HMF) content of tomato fruit and growing degree-day (GDD) in 2002 ($n = 12$). The vertical bar represents the SE of regression; $1 \text{ mg}\cdot\text{kg}^{-1} = 1 \text{ ppm}$; $(1.8 \times ^\circ\text{C}) + 32 = ^\circ\text{F}$.

A strong correlation ($r = 0.82$) was obtained between the total polyphenols level and TAS (Fig. 3) but lycopene ($r = 0.24$) and ascorbic acid ($r = 0.13$) did not correlate with TAS. The average total polyphenol contents of three harvesting dates ranged from 78.6 to $115.4 \text{ mg}\cdot\text{kg}^{-1}$.

According to Cámara et al. (2003), HMF content is in direct proportion to the lycopene content of the processed tomato. HMF can form not only during industrial processing, but also is synthesized during different physiological processes (photosynthesis, transpiration) in the plant. The aim of our experiments was to determine the HMF level in fresh tomato fruit and the relationship between GDD and HMF. The differences between the lycopene and HMF content of the three harvests were not significantly different (Table 2). Correlation analysis suggested a strong positive relation ($r = 0.95$) between GDD and HMF (Fig. 4) in fresh tomatoes.

Lycopene content is determined fundamentally by the genetic nature of cultivars, but is also strongly affected by environmental factors. The lycopene content is greatly reduced above 30°C . A close negative relation was found between GDD and lycopene content and a strong positive connection between GDD and HMF in fresh tomatoes. The antioxidant content of fruit of the processing tomato studied depended mostly on environmental conditions (mainly temperature) and genetic factors.

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