

Quality parameters and total phenolic content in tomato fruits regarding cultivar and microclimatic conditions

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Abstract: The aim of this research was to evaluate the color, firmness, and total phenolic (TP) content in tomatoes according to cultivar and growing conditions. Cultivars with oval, elongated, round, and cherry-shaped fruits of determinate tomato were grown in Mediterranean (Dragonja Valley) and continental regions. Experiments in the continental region were conducted outdoors (Ljubljana-field) and under a low tunnel (Ljubljana-tunnel). Results indicated that the color and firmness were significantly influenced by the typology of the cultivars and by the maturity stage associated with the climatic conditions. Oval, elongated, and cherry fruits had darker and more intensely red fruit skins, with significantly higher a^* and lower L^* values than round fruits. The firmness of oval and elongated fruits was also higher than the firmness of round fruits. Fruits harvested in Dragonja Valley and the Ljubljana-tunnel location reached a higher level of maturity and were classified in the red maturity class ($a^*/b^* > 0.95$), compared to the fruits from the Ljubljana-field location, where tomatoes were classified in the light red maturity class ($0.65 > a^*/b^* > 0.95$). Variation in total phenol (TP) content was evaluated in regards to different microclimatic conditions of the Ljubljana locations, outdoors and under the low tunnel. TP content, expressed as chlorogenic acid, ranged from 1.89 mg 100 g⁻¹ to 3.28 mg 100 g⁻¹ fresh weight (fw) in field-grown tomatoes and from 2.31 mg 100 g⁻¹ to 4.90 mg 100 g⁻¹ in tunnel-grown tomatoes. Cherry tomato had a significantly higher content of TP, ranging from 8.60 mg 100 g⁻¹ fw in field-grown fruits to 10.39 mg 100 g⁻¹ fw in tunnel-grown fruits. Although the differences between TP content in tomato fruits, regarding the microclimatic environment, were not statistically significant, the increase in TP content in tunnel-grown tomato fruits could be a plant response mechanism to thermal stress.

Key words: Color, cultivars, firmness, growing conditions, tomato, total phenols

Introduction

In recent years, functional foods and nutraceuticals have attracted much attention, particularly with respect to protective dietary intake. Many epidemiological studies have described that

consumption of large quantities of vegetables and fruits reduces the risk of many types of human disease (Rao and Agarwal 2000; Levy and Sharoni 2004). It is well known that the antioxidant activity of fruits and vegetables differs with varieties and agronomic

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conditions (Veberic et al. 2005; Mikulic Petkovsek et al. 2007; Usenik et al. 2008). Among the vegetables, tomatoes represent the predominant source of antioxidants, and besides the carotenoids (lycopene, β -carotene, and lutein), the flavonoids have been confirmed as a group of polyphenols important in conferring antioxidant benefits (Stewart et al. 2000; Slimestad and Verheul 2005; Luthria et al. 2006). Tomato is a widespread species commonly grown either in the field or under greenhouse conditions. It has the highest average consumption among European countries because it is frequently consumed both fresh and in tomato-based products (Raffo et al. 2006). In recent years, several studies have already looked at the influence of genotypes (George et al. 2004) and levels of fruit maturity (Buta and Spaulding 1997), as well as agronomical practices, on the content of phenolic compounds in tomatoes (Dumas et al. 2003). Macheix et al. (1990) showed that, in addition to genetic control, which is the main factor in determining phenolic compound accumulation in vegetable foods, external factors may also have a significant effect. In cherry tomatoes, the increase in phenolic content is ascribed to an increase in solar radiation received by fruits (Wilkens et al. 1996; Raffo et al. 2006). Brandt et al. (1995) mentioned that the flavonol content in some plant species may be enhanced by the exposure of the plants to increased UV-B radiation. On the other hand, only limited or no data was found in the literature dealing with temperature as another important environmental factor influencing the content of phenolic compounds (Dumas et al. 2003). Rivero et al. (2001) reported that tomato plants grown at temperatures around 35 °C may develop an acclimated mechanism against thermal stress, which consists of the accumulation of phenolic compounds. In another study, Rivero et al. (2003) showed that the phenolic compounds were accumulated as a defense mechanism against temperature stress.

The aim of this study was to determine the phenolic content level as well as the color parameters and firmness of fruits of 11 tomato cultivars, which were grown simultaneously in cultivation areas with different microclimatic conditions. Variations in Mediterranean and continental weather conditions, on one hand, and variations in microclimatic

environments in a low tunnel and outdoors in the continental area, on the other hand, can be expected to cause differences in the coloration and firmness of fruits associated with fruit maturation, which are important characteristics from the point of view of the processing industry as well as the consumer, and in the content of total phenols in tomatoes, which is important in terms of human health.

Materials and methods

Plant material

Eleven determinate tomato (*Lycopersicon esculentum* Mill.) cultivars, differing in fruit shape/typology (round, oval, elongated, and cherry fruit) and in country of origin (Italy, the Netherlands, Slovenia, and the USA) were tested in 3 experiments (Ljubljana-field, Ljubljana-tunnel, and Dragonja Valley-field), conducted June-September 2004, under 2 different sets of microclimatic conditions (continental in Ljubljana and Mediterranean in Dragonja Valley). Included in the experiments were 6 cultivars with round fruits ('Empire F1' (Petoseed, USA), 'Heinz 1370' (Semenarna, Ljubljana, Slovenia), 'Stormy F1' (Royal Sluis, the Netherlands), and 'Sun Chaser F1,' 'Sunjay F1,' and 'Super Red F1' (Asgrow, USA)), 2 cultivars with oval fruits ('Hypeel 347 F1' (Petoseed, USA) and 'Centurion F1' (Asgrow, USA)), 2 cultivars with elongated fruits ('Hypeel 108 F1' (Petoseed, USA) and 'San Marzano' (Zorzi Sementi, Italy)), and 1 cherry tomato cultivar ('GO 101' (SLO, Slovenia)).

The tomato transplants were greenhouse grown in a plug tray system, each in a 5 × 5 cm transplant cell filled with peat-based substrate. For each genotype, 7-week-old plants were transplanted into 2 rows in plots of 1.2 m in length, on raised beds of polyethylene mulch with within- and between-row spacing of 0.30 and 0.40 m, respectively. Each plot contained 8 plants. The plants were grown in heavy clay loam in the experimental field of the Biotechnical Faculty in Ljubljana (298 m ASL), and in sandy loam in a field in the Dragonja Valley (10 m ASL). To protect the plants from low night temperatures, rainfall, and consequent infections that are closely linked to humid growing conditions, the experiments in Ljubljana were conducted outdoors and in a 1 × 25 m (w × l) low

tunnel that was covered with transparent EVA film (0.20 mm thick, 91% transmittance of PAR (400-700 nm); Patilux D, P.A.T.I. S.p.A, Italy). In the Dragonja Valley, the experiment was conducted only in the open field. At each location, the experiments were laid out in a randomized complete block design for the 11 tomato cultivars as treatments. The treatments were replicated 3 times in each location. At both locations, 1 week before transplanting, the plots were fertilized with 1000 kg ha⁻¹ of 7 N: 20 P₂O₅: 30 K₂O. The remaining N (60 kg ha⁻¹) was applied via fertigation with Ca(NO₃)₂ in 6 equal 10-day intervals through irrigation water in Ljubljana, and 9 7-day intervals in the Dragonja Valley. Irrigation was applied as required through a drip tape (T-Tape TSX 500 Model, T-Systems International) beneath the plastic mulch. Diseases were managed with applications of Score, 0.03%, on July 24 (Ljubljana), and of Ridomil Gold MZ, 3 kg ha⁻¹, July 7 and 28 (Ljubljana and Dragonja Valley) and August 10 (Ljubljana).

Temperature measurement

Monthly meteorological data from May to September of 2004 from Ljubljana and Portorož Airport meteorological stations were used (Monthly 2004); inside the low tunnel, air temperatures during the growing period were measured using a thermograph (Casella, London, UK). Air temperature conditions in 2004 were close to the long-term average at both locations. The amount of precipitation in June, July, and August was greater than the long-term average in the Ljubljana region, but significantly lower in the Dragonja Valley (Table 1). Rainy weather in Ljubljana throughout the growing period, especially in June, July, and August, was the main reason for the first ripe tomatoes not being picked until August 23, about 2 weeks later than usual.

Tomato fruit harvest

The fruits were hand harvested at both locations, at the stages commonly marketed and suitable for the

Table 1. Monthly meteorological data from May to September 2004 from Ljubljana and Portorož Airport meteorological stations.

Month		TS	TOD	TX	TM	MSR	RR	RO
May	Lj-field	14.0	- 0.6	19.5	8.5	19.3	109	90
	Lj-tunnel	15.8		23.2	12.0			
	Dragonja Valley	14.9	- 1.3	20.5	9.6	22.3	92	112
June	Lj-field	18.8	1.0	24.2	13.8	18.6	172	111
	Lj-tunnel	21.2		29.4	14.6			
	Dragonja Valley	20.7	0.6	26.3	14.5	24.3	40	43
July	Lj-field	20.9	1.0	24.2	14.7	21.6	125	103
	Lj-tunnel	23.2		29.4	16.2			
	Dragonja Valley	22.5	0.1	28.8	16.0	25.5	74	93
August	Lj-field	20.7	1.6	27.1	15.2	18.4	164	114
	Lj-tunnel	22.6		29.8	17.3			
	Dragonja Valley	22.3	1.2	28.8	16.9	21.8	41	41
September	Lj-field	15.6	0.1	21.2	11.2	12.6	117	90
	Lj-tunnel	19.2		23.1	12.2			
	Dragonja Valley	18.7	1.2	25.2	13.6	15.5	64	57

TS, mean monthly air temperature (°C); TOD, temperature deviation (°C), i.e. deviation from 1961-1990 average; TX, mean daily temperature maximum for the month (°C); TM, mean daily temperature minimum for the month (°C); MSR, mean daily solar radiation (MJ m⁻²); RR, precipitation amount (mm); RO, deviation of monthly values of precipitation (%) from the 1961-1990 average.

processing industry. In the Ljubljana field, we were afraid that infection by *Phytophthora infestans* could lead to a reduced quality of fruit, so we harvested there 3 times: once in August and twice in September. In the Dragonja Valley there were no problems with infection by *P. infestans*, so the fruits were harvested all together, in September, which is the common practice of local growers. At both locations, after harvesting in September, for each cultivar and each of 3 replications, 8-12 tomatoes were randomly selected from among the marketable and undamaged fruits. Variation of carpometric characteristics (firmness and color) and the content of total phenols were studied for these samples.

Fruit color measurement

Immediately after harvesting, the tomato fruits were washed and wiped to dryness, and color measurements were performed on the opposite sides of the equatorial section of the fruit. Skin color was measured using a Minolta CR 300 Chroma portable colorimeter (Minolta Co., Osaka, Japan) with C illuminant. Fruit chromaticity was expressed in L^* , a^* , and b^* color space coordinates (CIELAB). The colorimeter was calibrated with a white standard calibration plate ($Y = 93.9$, $x = 0.3134$, $y = 0.3208$) before use. L^* corresponds to a dark/light scale (0 = black, 100 = white) and represents the relative lightness of colors, being low for dark colors and high for light colors (McGuire 1992; Lancaster et al. 1997).

Firmness measurements of fruits

Flesh firmness was measured immediately after the color measurements, using a Chatillion DFG-50 penetrometer with an 11 mm plunger (Chatillion & Sons, USA). Before using the penetrometer, the tomato skin was removed from opposite sides of the equatorial section of each fruit. When the plunger reached a predetermined depth marked by an incision on the piston, the display value was noted. This value represented the resistance of the pericarp to the sinking of the plunger. Two measurements were taken for each fruit, with the resultant means calculated and expressed in newtons (N).

Sample preparation

When color and firmness measurements had been performed, each fruit was chopped and stored at -20 °C. For detection of dry weight (dw), 2 g of frozen sample was freeze-dried for 22 h in a Gamma 2-20

lyophilizer (Christ, Germany). Water content (%) was calculated from the difference in the masses before and after lyophilization. Samples were then stored at -20 °C until analyzed as described below.

Extraction and determination of total phenolic content

In a dark bottle, 5 mL of 50% methanol was added to 100 mg of lyophilized sample of tomato fruit, and the bottle was then tightly closed. The extraction proceeded for 24 h at 60 °C with frequent stirring. After 24 h, the bottle was cooled to room temperature and opened, and the contents were centrifuged for 5 min at 13,000 rpm in an Eppendorf 5415C centrifuge. The clear supernatant was used for total phenolic determination. Total phenolics were determined according to the method of Waterman and Mole (1994), with Folin-Ciocalteu reagent (Fluka, Switzerland) diluted with deionized water in a ratio of 2:1 ($v v^{-1}$). Chlorogenic acid (Fluka) solution (1 mmol L^{-1}) was used for the construction of the calibration curve. Total phenolics were determined in 0.15 mL of the supernatant obtained after centrifugation of the extract. First, 2.6 mL of the diluted Folin-Ciocalteu reagent was added. The solution was mixed well, and after 5 min, 0.5 mL of a 20% solution of Na_2CO_3 was added. The solution was again mixed and left to stand for 90 min, when absorbance at 746 nm was measured. Each determination was repeated 3 times. The concentration of total phenolics was obtained from the calibration curve. Results were expressed as milligrams of chlorogenic acid per 100 grams, fresh weight.

Statistical analysis

The results were analyzed by the method of least squares using a GLM procedure (SAS Software, 1999). The statistical model for the analyzed instrumental color and firmness and total phenolics included the effects of group (G - typology of tomato fruits), cultivars (C), and growing conditions (GC):

$$y_{ijk} = m + C_i + GC_j + e_{ijk} \quad (\text{model 1})$$

$$y_{ijk} = m + G_i + GC_j + e_{ijk} \quad (\text{model 2})$$

where y_{ijk} = the ijk^{th} observation, μ = general mean value, G_i = effect of the i^{th} group, C_i = effect of the i^{th} cultivar, GC_j = effect of j^{th} growing conditions (Ljubljana-field, Ljubljana-tunnel, Dragonja Valley), and e_{ijk} = residual random term with variance σ_e^2 . All of the data are presented as least square means (LSM).

Results

Fruit color

The statistical analysis showed that the average values for L^* ($P < 0.0002$) and a^* ($P < 0.0018$) color parameters were significantly affected by fruit typology as well as by growing location ($P < 0.0001$), with no significant interaction between them (Table 2). When averaged over growing location, the lightness factor L^* of oval, elongated, and cherry fruits was significantly lower and the a^* values were significantly higher when compared to the round fruits. When averaged over the cultivars, the L^* parameter for fruits picked from Dragonja Valley was significantly lower compared to the fruits from Ljubljana (tunnel and field) ($P < 0.0001$), and the L^* values for the fruits from the last 2 locations differed significantly, too. The average a^* value for the fruits picked from Ljubljana-tunnel was significantly lower compared to the fruits from Ljubljana-field and Dragonja Valley, and between the last 2 locations, the difference was not significant (Table 2).

The color index, which corresponds to the saturation or vividness of the color, is chroma (C^*), and as C^* increases, color becomes more intense. In our study, chroma was significantly affected by the typology of fruits ($P < 0.0001$) and growing locations ($P < 0.0001$), with no significant interaction between them (Table 2). More intense color was recorded from oval, elongated, and cherry fruits and from fruits from

Dragonja Valley and Ljubljana-field, compared to round fruits and fruits harvested from Ljubljana-tunnel.

We expressed chromometric parameters as the ratio of a^* to b^* (Table 3), and the statistical analysis showed significant interaction between cultivar and growing location ($P < 0.0001$). At the Ljubljana-field location, the lowest a^* to b^* ratio among the cultivars with round-shaped fruits was recorded for 'Stormy,' and this was significantly lower than the a^* to b^* ratio of all other cultivars of that group. Among the cultivars with oval and elongated fruits, 'San Marzano' had the highest a^* to b^* ratio in all 3 locations, except the 'Hypeel 347' picked from Dragonja Valley. At the Ljubljana-tunnel location, significant differences in the a^* to b^* ratio were recorded only for cultivars with oval and elongated fruit. The highest a^* to b^* ratio was observed for 'San Marzano,' which was significantly higher than the a^* to b^* ratio of 'Hypeel 108.' At the Dragonja Valley location, there were no significant differences in the a^* to b^* ratios among the cultivars.

Fruit firmness

The average firmness of fruits of all 11 cultivars harvested at the 3 locations is presented in Table 3. Statistical analysis showed significant interaction between cultivars and growing locations ($P < 0.0001$). For individual tomato varieties, there were significant differences in average firmness on the basis of the growing location. The oval and elongated fruits

Table 2. Least square means and standard errors (LSM \pm SE) of color parameters (L^* , a^* , b^*) and indexes (a^*/b^* , h , C) for fruit groups (oval, elongated, cherry, round) and growing locations (Ljubljana-field, Ljubljana-tunnel, Dragonja Valley).

Parameter	Oval, elongated, and cherry fruits			Round fruits			P-value		
	Ljubljana-field	Ljubljana-tunnel	Dragonja Valley	Ljubljana-field	Ljubljana-tunnel	Dragonja Valley	G*GC	G	GC
L^*	44.7 \pm 0.5 a	42.8 \pm 0.5 b	42.0 \pm 0.5 b	45.5 \pm 0.4 a	45.0 \pm 0.4 a	43.0 \pm 0.4 b	0.2708	0.0002	<0.0001
a^*	26.6 \pm 0.6 a	26.7 \pm 0.6 a	26.6 \pm 0.6 a	22.0 \pm 0.5 c	19.8 \pm 0.5 d	24.0 \pm 0.5 b	0.0009	0.0018	<0.0001
b^*	29.5 \pm 0.7 a	27.6 \pm 0.6 b	26.6 \pm 0.6 b	27.4 \pm 0.6 b	26.3 \pm 0.5 b	27.0 \pm 0.5 b	0.0999	0.0086	0.0406
a^*/b^*	0.9 \pm 0.0 b	1.0 \pm 0.0 a	1.0 \pm 0.0 a	0.8 \pm 0.0 c	0.8 \pm 0.0 c	0.9 \pm 0.0 b	0.0329	<0.0001	<0.0001
h	47.8 \pm 0.8 bc	45.8 \pm 0.8 cd	44.9 \pm 0.8 d	51.3 \pm 0.7 a	53.2 \pm 0.6 a	48.4 \pm 0.6 b	0.0123	<0.0001	<0.0001
C	30.2 \pm 0.7 c	32.7 \pm 0.7 ab	33.9 \pm 0.7 a	27.7 \pm 0.6 d	26.9 \pm 0.6 d	30.9 \pm 0.5 bc	0.0320	<0.0001	<0.0001

G*GC, interaction group*growing conditions; G, group; GC, growing conditions. Significance: statistically not significant, $P > 0.05$; statistically significant, $P \leq 0.05$ and $P \leq 0.01$; highly statistically significant, $P \leq 0.001$; a,b,c,d groups with a different letter within rows differ significantly ($P \leq 0.05$).

Table 3. Least square means and standard errors (LSM \pm SE) of color index a^*/b^* and firmness (N) for all 11 cultivars and all 3 locations.

Parameter	a^*/b^* value			Firmness (N)		
	Ljubljana-field	Ljubljana-tunnel	Dragonja Valley	Ljubljana-field	Ljubljana-tunnel	Dragonja Valley
Cultivar						
<i>Oval fruits</i>						
Centurion	0.93 \pm 0.02 xy*	0.99 \pm 0.02 xy	0.99 \pm 0.02 x	21.6 \pm 1.0 ax	16.9 \pm 1.0 bx	17.6 \pm 1.0 bx
Hypeel 347	0.87 \pm 0.03 by	0.96 \pm 0.03 axy	1.03 \pm 0.03 ax	23.9 \pm 1.4 ax	19.7 \pm 1.2 bx	16.1 \pm 1.2 bx
<i>Elongated fruits</i>						
Hypeel 108	0.85 \pm 0.03 by	0.92 \pm 0.03 aby	0.97 \pm 0.03 ax	24.5 \pm 1.2 ax	18.8 \pm 1.2 bx	18.5 \pm 1.3 bx
San Marzano	0.99 \pm 0.02 x	1.03 \pm 0.02 x	1.03 \pm 0.02 x	11.3 \pm 1.0 ay	9.3 \pm 1.0 aby	6.9 \pm 1.0 by
P-value _{GC}	<0.0001			<0.0001		
P-value _C	<0.0001			<0.0001		
P-value _{C*GC}	0.3336			0.3029		
<i>Round fruits</i>						
Empire	0.77 \pm 0.06 x	0.73 \pm 0.05 xy	0.82 \pm 0.05 y	10.1 \pm 1.1 awz	10.5 \pm 0.9 ayw	5.5 \pm 0.9 bz
Heinz 1370	0.84 \pm 0.05 ax	0.66 \pm 0.05 by	0.94 \pm 0.05 ax	10.2 \pm 1.8 wz	12.3 \pm 1.8 yw	6.6 \pm 1.8 wz
Stormy	0.54 \pm 0.05 by	0.65 \pm 0.04 by	0.89 \pm 0.04 axy	24.9 \pm 2.5 ax	19.9 \pm 1.8 ax	12.0 \pm 1.8 bx
Sun Chaser	0.84 \pm 0.03 x	0.91 \pm 0.03 x	0.90 \pm 0.03 xy	17.3 \pm 1.1 ay	15.8 \pm 1.1 axy	7.3 \pm 1.1 bw
Sunjay	0.86 \pm 0.06 x	0.77 \pm 0.05 xy	0.84 \pm 0.04 xy	8.0 \pm 1.5 z	8.2 \pm 1.2 w	6.6 \pm 0.9 wz
Super red	0.86 \pm 0.03 x	0.88 \pm 0.03 xy	0.94 \pm 0.03 x	14.2 \pm 1.8 yw	14.6 \pm 1.5 xy	9.9 \pm 1.5 y
P-value _{GC}	<0.0001			<0.0001		
P-value _C	<0.0001			<0.0001		
P-value _{C*GC}	0.0791			0.0603		
<i>cherry tomato</i>	0.87 \pm 0.08	0.81 \pm 0.08	0.91 \pm 0.07	7.8 \pm 0.7	7.6 \pm 0.7	7.6 \pm 0.6
P-value _{GC}	0.6343			0.9621		

G*GC, interaction group*growing conditions; G, group; GC, growing conditions. Significance: statistically not significant, $P > 0.05$; statistically significant, $P \leq 0.05$ and $P \leq 0.01$; highly statistically significant; $P \leq 0.001$; * a,b,c groups with a different letter within rows differ significantly ($P \leq 0.05$); x,y,w,z cultivars with a different letter within a column differ significantly ($P \leq 0.05$).

picked from Ljubljana-field had significantly higher firmness than the same cultivars picked from the other 2 locations. Round fruits picked from Ljubljana-field and Ljubljana-tunnel had significantly higher firmness than the same cultivars picked from Dragonja Valley. Otherwise, among the cultivars with oval and elongated fruits, higher firmness was detected in 'Centurion,' 'Hypeel 347,' and 'Hypeel 108,' compared to fruits of 'San Marzano' at all experimental locations. Among the cultivars with round fruits, the differences in fruit firmness were not the same at all 3 locations. The exception was 'Stormy,' with the highest average firmness in all 3 locations, while the lowest average firmness was recorded for the 'Sunjay' fruits picked from Ljubljana-field and Ljubljana-tunnel, and for the 'Empire' picked from Dragonja Valley.

Total phenolic (TP) content in tomato fruits

The average total phenolic (TP) content found in fruits of 11 tomato cultivars grown in Ljubljana-field and Ljubljana-tunnel are presented in Table 4. The statistical analysis for oval and elongated fruits showed significant differences between growing conditions ($P < 0.0133$), and for round-shaped fruits, differences were significant ($P < 0.0003$) for cultivars. For individual tomato cultivars, there were no significant differences in average TP content on the basis of the growing conditions, except for 'Hypeel 347' among the cultivars with oval and elongated fruits, and 'Heinz 1370' among the cultivars with round fruits. Both cultivars ('Hypeel 347' and 'Heinz 1370') had a significantly higher average TP content in fruits picked from the tunnel compared to the fruits picked from the field (outdoors). Under the same

Table 4. Least square means and standard errors (LSM \pm SE) of total phenolic content in fruits (mg 100 g⁻¹ fw) of 11 tomato cultivars, grown in different growing conditions (Ljubljana-field and Ljubljana-tunnel).

Growing conditions	Ljubljana-field	Ljubljana-tunnel
Cultivar		
<i>Oval fruits</i>		
Centurion	2.78 \pm 0.31	2.85 \pm 0.24
Hypeel 347	2.25 \pm 0.13 b*	3.26 \pm 0.16 a
<i>Elongated fruits</i>		
Hypeel 108	2.56 \pm 0.19	3.06 \pm 0.11
San Marzano	3.08 \pm 0.41	3.71 \pm 0.42
P-value _{GC}	0.0133	
P-value _C	0.0933	
P-value _{C*GC}	0.3514	
<i>Round fruits</i>		
Empire	2.95 \pm 0.46 xy	2.31 \pm 0.19 y
Heinz 1370	3.17 \pm 0.31 bx	4.89 \pm 0.67 ax
Stormy	1.89 \pm 0.25 y	2.38 \pm 0.26 y
Sun Chaser	2.41 \pm 0.14 xy	2.47 \pm 0.26 y
Sunjay	2.44 \pm 0.56 xy	2.33 \pm 0.38 y
Super Red	2.11 \pm 0.56 xy	2.36 \pm 0.09 y
P-value _{GC}	0.1915	
P-value _C	0.0003	
P-value _{C*GC}	0.0768	
Cherry tomato (GO101)	8.60 \pm 0.62	10.39 \pm 0.63
P-value _{GC}	0.1186	

G*GC, interaction group*growing conditions; G, group; GC, growing conditions. Significance: statistically not significant, $P > 0.05$; statistically significant, $P \leq 0.05$ and $P \leq 0.01$; highly statistically significant, $P \leq 0.001$; *a,b groups with a different letter within rows differ significantly ($P \leq 0.05$); x,y cultivars with a different letter within a column differ significantly ($P \leq 0.05$).

growing conditions, the differences in average TP content were significant only between cultivars with round fruits ($P < 0.0001$). In both growing conditions (field and tunnel), the highest average TP content was recorded in fruits of 'Heinz 1370,' which was significantly different from the average TP content recorded in fruits of 'Stormy' when the fruits were picked in the field. Fruits of 'Heinz 1370' had the highest TP content among the cultivars while fruits of 'Empire' had the lowest, when the fruits were picked from the tunnel. The TP content in the cherry tomato fruits ('GO 101') was considerably higher compared to all other tested cultivars.

Discussion

Tomato is known as an excellent source of different antioxidants and secondary metabolites such as carotenoids and phenolics compounds (Luthria et al. 2006). The chemical constituents in tomatoes are often reported with limited information about the growing conditions of the plant. Some of the reported studies have been based on samples of tomatoes purchased in a local or selected market (Martinez-Valverde et al. 2002) or samples from plants grown in hydroponic systems (Moraru et al. 2004). Description of growing conditions is particularly important, as it has been confirmed that genotype and environmental factors have significant effects on the content of the secondary metabolites in tomato fruits (Dumas et al. 2003; George et al. 2004; Toor et al. 2006). Therefore, our experiments were performed with different tomato cultivars at locations with different weather, Mediterranean (in Dragonja Valley) and continental (in Ljubljana) climates, as well as under different microclimatic conditions (outdoors and under a low tunnel). In our study, some quality parameters (color and firmness) especially important for the processing industry as well as for the consumer were evaluated regarding variations in genotype and environmental conditions. According to the literature, the color of tomato fruits affects the grade and appearance of the end processing products and is a result of the presence of different pigments, particularly lycopene, the expression of which is influenced by physical factors, such as surface topography and shape of fruit (Lancaster et al. 1997), as well as the rate of fruit maturity (Batu 2004) and environmental factors, above all temperature and solar radiation (Brandt et al. 2006). The color indexes in our study, expressed in the CIELAB system, showed, on one hand, that the oval and elongated fruits had darker and more intensely red skin compared to the round fruits (higher a^* and lower L^* values), and, on the other hand, that growing conditions significantly influenced fruit coloration. Namely, oval and elongated fruits picked from the plants grown in more suitable microclimatic conditions (Dragonja Valley and Ljubljana-tunnel) were found to be in the red maturity stage ($a^*/b^* > 0.95$), considering the a^* to b^* ratio as a reference parameter for the ripening stage (Raffo et al. 2002; Batu 2004), while the round fruits were found to be in the light red stage

($0.65 < a^*/b^* < 0.95$) in all 3 experiments, except the fruits of 'Stormy' picked in Ljubljana-field; those fruits were found to be at the pink ripe stage ($a^*/b^* = 0.54$). It has been reported that tomatoes that reached the red color stage, according to the USDA color classification (USDA 1997), might have ripened on the vine too long (Batu 2004) or might have had a long overall storage time. As we analyzed the fruits immediately after harvest, it can be assumed that a higher a^* to b^* ratio was due to increasing maturity, which was achieved through agricultural practices typical for the Mediterranean region (harvesting fruits all at once at the end of the season), and at the same time associated with more appropriate environmental conditions during the ripening period in the Dragonja Valley and in Ljubljana-tunnel. At both locations, the mean monthly air temperatures, and in Dragonja Valley, the mean daily solar radiation during August and September, were higher (Table 1) than in Ljubljana-field, on average by 2-3 °C and 3.8 MJ m⁻², respectively, and this presumably contributed to the faster maturation of fruits.

Fruit firmness is another important parameter that contributes to internal fruit quality. In our study, the round fruits had a lower average firmness than the oval and elongated fruits. The exception was the 'Stormy' fruits harvested in Ljubljana-field, with the highest average firmness (24.9 N) among the investigated varieties. According to both characteristics of the fruits of 'Stormy' (the highest firmness and the previously mentioned low average a^*/b^* index of 0.54), we assumed that this cultivar was maturing more slowly than others, since it has been reported that fruit firmness varies with maturation and decreases with the ripening of fruits (Batu 2004). Differences in average firmness between round and oval or elongated fruits were expected, bearing in mind that fruit firmness depends on skin toughness, flesh firmness, and the pericarp to locular material ratio (Jackman and Stanley 1995). A thicker pericarp, smaller locular cavity, and higher solid and pectin content are characteristics of that type of tomato (Moraru et al. 2004), which was also recorded for fruits in our study (data not shown). Besides the maturity stage, Jackman and Stanley (1995) mentioned that cultivation practice and environmental events, such as the degree of sun

exposure, drought, salinization, and water stress, also affect tomato texture. The impact of environmental factors on fruit firmness was confirmed in our study, too, since the highest average firmness was recorded for the oval and elongated fruits that were harvested in cold, less suitable climatic conditions (Ljubljana-field). We assume that cultivars with oval and elongated fruits need higher temperatures during the maturation period to reach the higher maturity stage.

The influence of growing conditions on the content of total phenolics has also been reported by Davies and Hobson (1981), who found that more flavonoids (quercetin and kaempferol) were synthesized in field-grown than in greenhouse-grown tomatoes. A similar trend was confirmed in a recent study by Luthria et al. (2006), which showed that when tomatoes were grown under a covering material that allowed the transmission of solar UV radiation up to 400 nm, there was a higher content of TP of about 10%-16% in the fruits than in those grown under UV-exclusion conditions. According to the aforementioned statements, a higher TP content was expected in the tomato fruits harvested outdoors, but our results showed a higher content of TP in the fruits produced under the low tunnel, although the differences were not significant, except for 'Hypeel 347' and 'Heinz 1370,' grown under the low tunnel and showing a content of TP significantly higher than that of fruits from outdoors. As already noted, our results are somewhat contradictory to the published data. Although a limited number of studies described the influence of environmental temperature on TP content, Rivero et al. (2003) reported that higher air temperature (35 °C) increased the concentration of TP in tomato plants due to the activation of the enzyme phenylalanine ammonia-lyase (PAL). They assumed that phenolic compounds accumulate as an apparent defense mechanism against temperature stress. Taking into account that during the ripening period (in July and August) in our experiment, the maximum temperatures in the tunnel often exceeded 35 °C (data not shown) and the average maximum temperatures during the experimental period were 2-3 °C higher than maximum average temperatures outdoors, we assume that our results of higher TP content could be explained with the findings observed by Rivero et al. (2001), describing the metabolic

synthesis of phenols as a response to heat stress and reporting that phenolic compounds in tomato were accumulated as a result of an acclimatization mechanism to overcome heat stress.

In our study, the TP amount in cherry tomato ('GO 101') was considerably higher than that detected in oval, elongated, and round fruits, ranging from 8.60 mg 100 g⁻¹ fw (in field-grown fruits) to 10.39 mg 100 g⁻¹ fw (in tunnel-grown fruits). These data are in agreement with those of Giovanelli et al. (1999), who found variations in TP content in cherry tomatoes ranging from 5.0 to 15.0 mg 100 g⁻¹ fw, and those reported by George et al. (2004), who pointed out that the content of phenolic compounds in the pulp of 3 cherry tomato cultivars (expressed as catechin) was the highest among 12 other analyzed tomato varieties with larger fruits, ranging from 22 to 27.0 mg 100 g⁻¹ fw. As explained by Stewart et al. (2000), higher levels of TP content in cherry tomatoes, compared to cultivars with larger fruits, are largely due to the higher skin to volume ratio of these varieties, which

could enhance their phenolic content, particularly flavonols, since these compounds occur within the skin of the fruit.

Based on our results, areas with higher amounts of solar radiation, higher average air temperatures, and low precipitation amounts (in our case, the Mediterranean region of Dragonja Valley), as well as areas with less suitable growing conditions, e.g. the protected cultivation under the low tunnel, are recommended for the production of tomatoes with better coloration and a higher content of total phenolics, which are important parameters for the processing industry as well the consumer.

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