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ARTICLE

Lipophilic Antioxidants and Some Carpometric Characteristics of Fruits of Ten Processing Tomato Varieties, Grown in Different Climatic Conditions

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Microclimatic conditions and fruit maturity can affect the health-promoting compounds in tomato fruits. The aim of this study was to evaluate the influence of Mediterranean and continental weather conditions, on fruit firmness and coloration as well as on the concentrations of lycopene, α- and β-carotene and lutein and on α-, δ- and γ-tocopherol, in fruits of 10 processing tomato varieties. Compositional data analysis was carried out in order to determine the composition of carotenoids and tocopherols. Mediterranean weather conditions associated with agricultural practice have contributed to faster fruit ripening, which significantly improved the coloration with all varieties, and significantly increased the concentration of total carotenoids and lycopene with varieties with oval and elongated fruits. Environmental conditions in a low tunnel increased the red coloration, but did not significantly increase the content of either total carotenoids or tocopherols. The cultivar and the location of cultivation had a strong effect on the composition of the analyzed lipophilic antioxidants.

KEYWORDS: Tomato; *Lycopersicon esculentum*; varieties; lipophilic antioxidants; carotenoids; tocopherols; compositional data analysis

INTRODUCTION

Tomato is a commercially important vegetable throughout the world, both for the fresh-fruit market and for the processed food industry. Processing tomato varieties are grown in a wide range of climates, usually outdoors, but in locations where unsuitable environmental conditions hinder growth and maturity, they are grown in low tunnels. In the continental part of Slovenia, where lower air temperatures, frequent rainfall and consequent infections shortened tomato growing season, processing varieties are cultivated in low tunnels, otherwise their production is linked to the Mediterranean region (*1*).

The content of antioxidant compounds has today become an important quality parameter of fruits and vegetables. As reported by many authors (*2*, *3*), the antioxidant activity of various fruits and vegetables may differ with varieties and agronomic conditions. Tomato is known as an important source of antioxidants, especially carotenoids. Among them, lycopene is predominant and plays an important role in reducing cardiovascular diseases and digestive tract tumors, as the most efficient singlet oxygen quencher (*4*). Another important lipophilic antioxidant in tomato fruit is vitamin E, which has been proved to be important in reducing the risk of cardiovascular diseases, enhancing immune status and modulating important degenerative conditions associated with aging (*5*). It consists of four tocopherols (α-, β-, δ- and γ-tocopherol), of which α-tocopherol is the most biologically active form. Tomato, spinach and broccoli have been reported to be significant sources of α-tocopherol for the US consumer (*6*). It has also been observed that the beneficial effects associated with the consumption of tomatoes are attributed to the synergistic effects of the tomato compounds, especially lycopene and α-tocopherol, which have been shown to inhibit prostate carcinoma cell proliferation, HL-60 leukemic cell differentiation and low-density lipoprotein (LDL) oxidation (*5*).

Carotenoid biosynthesis in plants is nowadays well-characterized (*7*, *8*), and over the past 10 years many studies have been made to improve the carotenoid content in tomato fruits through genetic modification or modification of cultivation practice. Osmotic stress induced by high electrical conductivity in irrigation water can improve the organoleptic quality and lycopene content in tomato fruit (*9*, *10*). It has also been reported that biosynthesis of lycopene is strongly influenced by environmental factors, especially daily air temperature (the optimum is between 22 and 25 °C) and exposure to sunlight (*11*). However, with field grown plants, when the fruits were exposed to direct solar radiation, the temperatures of the fruits may exceed 35 °C, especially with plants with insufficiently dense foliage and, consequently, lycopene accumulation is inhibited, mostly because of the conversion of lycopene into β-carotene (*11*).

As many authors have reported, various biotic and abiotic factors, such as genotype (*12*), fruit maturity level (*11*), cultivation practice (*13*), and environmental conditions (*14*, *15*), influence the

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Table 1. Varieties Included in the Experiments

variety	typology/shape of fruit	seed company	country of origin
Epire F1	round	Petoseed	USA
Heinz 1379	round	Semenarna Ljubljana	SI
Stormy F1	round	Royal Sluis	NL
Sun chaser F1	round	Asgrow	USA
Sunjay F1	round	Asgrow	USA
Super red F1	round	Asgrow	USA
Centurion F1	oval	Asgrow	USA
Hypeel 347 F1	oval	Petoseed	USA
Hypeel 108 F1	elongated	Petoseed	USA
San Marzano	elongated	Zorzi Sementi	IT

content of carotenoids and tocopherols in tomato fruit. Some of these studies have been based on samples of tomatoes purchased in a local or selected market or on fruits sampled from experiments carried out in hydroponic systems. Only limited or no data have been found in the literature dealing with the content and composition of carotenoids and tocopherols in tomato fruits as a response to the variety and, simultaneously, the growing conditions, particularly variations in temperature and solar radiation.

The aim of this study was to determine the content and the composition of carotenoids and tocopherols, as well as the color parameters and firmness of fruits, of 10 tomato varieties, which were grown simultaneously on cultivation areas with different microclimatic conditions. Compositional data analysis was carried out in order to determine the composition of carotenoids and tocopherols. Variations in Mediterranean and continental weather conditions, on the one hand, and variations in microclimatic environments in 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, which are important characteristics from the point of view of the processing industry as well as the consumer, and in the content and composition of the carotenoids and tocopherols in fruits, which is important in terms of human health.

MATERIALS AND METHODS

Plant Materials. Fruits of tomato varieties were collected in 2004, from three parallel experiments that were conducted from June to September, two on the experimental field (298 m asl) of the Biotechnical Faculty in Ljubljana (continental region), and one on a field in the Dragonja Valley (10 m asl) (Mediterranean region). Variety descriptions (name, shape of fruit, seed company and country of origin) and list of practical work and characteristics relating to the experiments are given in **Tables 1** and **2**. To protect the tomato plants from low night temperatures and rainfall and consequent slow growth and infection with diseases, the experiments in Ljubljana were conducted on an open field and in two, 1 m wide × 25 m long low tunnels made from 2.2 m plexiglass bars which were fixed at 1.5 m intervals and covered with transparent EVA film (0.20 mm thickness, 91% transmittance of PAR (400−700 nm), Patilux D, P.A.T.I. S.p.A, Italy) and kept open at both sides. In the Dragonja Valley, because of the dry, warm and windy weather conditions, the experiment was conducted only in the open field.

The fruits were hand harvested at both locations at the stages commonly marketed and suitable for processing industry. In the Ljubljana field, we were afraid that infection with *Phythophthora infestans* could lead to a reduced quality of the fruits, so we harvested them 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 three replications, ten tomatoes were randomly selected from among marketable and undamaged fruits. Variation of carpometric characteristics (firmness and color) and the content of carotenoids and tocopherol were studied on these samples.

Measurement of Minolta (a^*/b^*) Color Values. Skin color parameters were measured using a Minolta CR 300 Chroma portable

Table 2. List and Description of the Experimental Work Relating to the Experiments of Our Study

location of the experiment	Ljubljana field, tunnel	Dragonja Valley field
date of transplanting	June 4	June 6
experimental design	Randomized complete block design 10 treatments, 3 replicates	
soil texture	heavy clay loam	sandy loam
fertilization		
basic aplication	May 28/1000 kg ha^{-1} NPK (7:20:30)	
fertigation	6 times/every 10 days with WSF $Ca(NO_3)_2$	
plant protection score (0.03%)	June 24	−
Ridomil Gold MZ (3 kg ha^{-1})	July 7	July 28
	August 10	−
harvest: date (DAT: day after transplanting)	Sep 8 (96 DAT)	Sep 6 (92 DAT)

colorimeter (Minolta Co., Osaka, Japan) with C illuminant. Fruit chromaticity was expressed in L^*, a^*, 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. The parameters a^* and b^* scales extend from −60 to +60 where a^* is negative for green and positive for red and b^* is negative for blue and positive for yellow. Color was described by parameter lightness (L^*) and two indexes: ratio a^*/b^* and chroma ($C^* = (a^2 + b^2)^{1/2}$). C^* is an index analogous to color saturation or intensity (*16*). The color parameters were measured on 10 fruits from each treatment and each replication. Four measurements on the equatorial region of the fruits were performed for each fruit and an average was calculated.

Firmness Measurement. Flesh firmness was measured immediately after harvesting the fruit, in the pulp of 10 fruits, by removing 1 cm^2 of the fruit skin, on opposite sides using a Chatillion penetrometer (model DFG 50), equipped with an 11 mm diameter round stainless steel probe with flat end (John Chatillion & Sons, U.S.A.). The firmness was measured on 10 fruits from each treatment and each replication. Two measurements of the force needed to penetrate the mesocarp tissue were taken for each fruit, and an average was calculated and expressed in newtons (N).

Sample Preparation. When color and firmness measurements had been performed, all ten tomatoes were chopped, frozen in liquid nitrogen and stored at −20 °C. For detection of dry weight (DW), 2 g of the frozen sample was freeze-dried for 22 h in a Gamma 2-20 lyophilizer (Christ, Germany). Water content (%) was calculated from the difference between the masses before and after the lyophilization.

For analysis of lipophilic antioxidants, the frozen samples were ground to a fine powder and stored at −20 °C in humidity proof plastic containers.

Analysis of Lipophilic Antioxidants. Carotenoids and tocopherols were determined using the method described in Šircelj et al. (*17*). Pigments and tocopherols were extracted from the dry fruit powder with ice-cold acetone. All extraction procedures were performed in dim light. Acetone extracts were subjected to HPLC gradient analysis (column Spherisorb S5 ODS-2 250 × 4.6 mm with precolumn S5 ODS-2 50 × 4.6 mm). Tocopherols were separated using methanol as solvent. Tocopherols were detected directly by fluorometry (excitation 295, emission 325). Pigments were separated using the following solvents: solvent A, acetonitrile/methanol/water (100/10/5, v/v/v); solvent B, acetone/ethyl acetate (2/1, v/v), at a flow rate of 1 mL·min^{-1}, linear gradient from 10% solvent B to 70% solvent B in 18 min was applied, run time 30 min, photometric detection at 440 nm.

Weather Measurement. Monthly meteorological data from May to September 2004 from Ljubljana and Portorož meteorological stations were used (*18*), **Table 3**, and air temperatures during the growing period were measured inside the low tunnels using a thermograph (Casella, London, U.K.). Air temperature conditions in 2004 were close to the long-term average at both locations; the amount of precipitation in June,

Table 3. Monthly Meteorological Data from May to September 2004 from Ljubljana and Portorož (near the Experimental Location in Dragonja Valley) Meteorological Station (*19*)[a]

	TS			TOD		TX			TM			MSR		RR		RO	
month	Lj-f	Lj-t	Dv	Lj-f	Dv	Lj-f	Lj-t	Dv	Lj-f	Lj-t	Dv	Lj-f	Dv	Lj-f	Dv	Lj-f	Dv
May	14.0	15.8	14.9	−0.6	−1.3	19.5	23.2	20.5	8.5	12.0	9.6	19.3	22.3	109	92	90	112
June	18.8	21.2	20.7	1.0	0.6	24.2	29.4	26.3	13.8	14.6	14.5	18.6	24.3	172	40	111	43
July	20.9	23.2	22.5	1.0	0.1	27.1	31.3	28.8	14.7	16.2	16.0	21.6	25.5	125	74	103	93
Aug	20.7	22.6	22.3	1.6	1.2	27.1	29.8	28.8	15.2	17.3	16.9	18.4	21.8	164	41	114	41
Sep	15.6	19.2	18.7	0.1	1.2	21.2	23.1	25.2	11.2	12.2	13.6	12.6	15.5	117	64	90	57

[a] Lj-f, Ljubljana field; Lj-t, Ljubljana tunnel; Dv, Dragonja Valley. TS, mean monthly air temperature (°C); TOD, temperature deviation from 1961−1990 average (°C); TX, mean daily temperature maximum for month (°C); TM, mean daily temperature minimum for month (°C); MSR, mean daily solar radiation (MJ/m^2); RR, precipitation amount (mm); RO, relative deviation of monthly precipitations amount from 1961−1990 average (%).

Table 4. The Averages () and Standard Deviations (SD) of Color Parameter and Indexes: L^*, a^*/b^* and C^* for All Ten Varieties and Three Locations[a]

	L^*						a^*/b^*						C^*					
	Lj-f		Lj-t		Dv		Lj-f		Lj-t		Dv		Lj-f		Lj-t		Dv	
cultivar		SD		SD		SD		SD		SD		SD		SD		SD		SD
oval fruits																		
Centurion	45.8	1.2	44.5	1.2	43.8	0.5	0.87	0.07	0.97	0.01	1.01	0.05	42.9	2.2	43.5	1.7	40.7	1.0
Hypeel 347	46.8	3.2	43.9	2.7	41.7	1.8	0.86	0.19	0.98	0.15	1.05	0.11	41.7	2.3	39.8	3.1	38.1	3.7
enlongated fruits																		
Hypeel 108	46.5	0.9	46.3	1.6	43.4	1.1	0.80	0.02	0.85	0.10	0.97	0.03	40.9	1.1	40.7	2.6	40.5	0.3
San Marzano	42.0	1.0	38.8	1.3	38.5	1.0	0.96	0.06	1.02	0.04	1.07	0.07	35.5	2.9	31.4	1.7	30.3	5.6
round fruits																		
Stormy	54.7	1.0	48.6	2.0	42.3	1.2	0.50	0.07	0.62	0.13	0.88	0.03	34.5	0.6	32.9	3.6	33.8	1.8
Sun chaser	44.6	1.3	42.6	2.9	43.9	2.5	0.87	0.10	0.90	0.16	0.87	0.15	37.9	1.2	34.3	2.0	36.9	1.9
Sunjay	45.6	2.3	44.1	5.5	43.1	2.0	0.85	0.10	0.79	0.22	0.85	0.06	38.4	2.4	31.5	2.3	37.3	3.4
Empire	46.8	3.7	42.0	0.5	42.7	0.2	0.73	0.12	0.88	0.09	0.87	0.05	37.6	0.3	33.9	3.0	37.3	1.4
Heinz 1370	43.5	2.9	47.6	4.7	43.5	2.0	0.89	0.08	0.62	0.15	0.88	0.09	34.8	6.9	31.9	2.8	36.1	3.8
Super red	47.7	3.1	47.3	1.3	42.0	0.4	0.84	0.16	0.79	0.10	0.93	0.00	38.3	2.7	40.0	1.3	36.4	0.2

[a] Lj-f, Ljubljana field; Lj-t, Ljubljana tunnel; Dv, Dragonja Valley.

July and August was greater than the long-term average in the Ljubljana region, but significantly lower in the Dragonja Valley (**Table 3**). Rainy weather in Ljubljana throughout the growing period, especially in June, July and August, was the main reason that we harvested the tomato fruits as soon as they became red.

Statistical Analysis. In the statistical analysis, the experiment was treated as a two factor randomized block experiment design. The first factor (cultivar) had ten levels, i.e., ten different tomato varieties; and the second factor (location) had three levels, i.e., three different climatic/microclimatic conditions. The interaction between the two factors (cultivar*location) was also analyzed. Each of 30 treatments was replicated three times (three randomized blocks). The data of color parameters (L^*, a^*/b^* and C^*), firmness, total carotenoids and total tocopherols were analyzed by analysis of variance (ANOVA) and Duncan's or Bonferroni's multiple comparison test at a significance level of 0.05. In cases in which the assumption of homogeneity of variances among treatments was not met, generalized linear models (GLM) assuming different variance among treatments were used instead of ANOVA.

The average structure of carotenoids and tocopherols was analyzed using compositional data analysis (*19*, *20*). The geometric mean was used as the measure of the central tendency for four components of carotenoid composition and three components of tocopherol composition. The data were transformed with additive log ratio (alr) transformation before ANOVA.

RESULTS AND DISCUSSION

Fruit Color. In general, the color parameter (L^*) and indexes (a^*/b^*, C^*) showed different average values according to the varieties and growing locations (**Table 4**). The GLM model showed statistically significant interaction between cultivar and location for the relative lightness of colors (L^*) ($p < 0.0001$). For individual tomato varieties there were no significant differences in average L^* based on the growing location (Bonferroni test, $\alpha = 0.05$) except for Stormy (54.7) picked from the Ljubljana field location. At the same location the average L^* for Stormy was significantly higher than that for all other varieties except Super Red. In Ljubljana tunnel, the lowest average L^* was recorded for San Marzano (38.8), and it was significantly different from the average L^* measured for Heinz 1370, Stormy and Super Red. In the Dragonja Valley, the average L^* ranged between 38.5 (for San Marzano) and 43.9 (for Sun Chaser) but the differences between them were not significant. Our results are in accordance with those reported by Moraru et al. (*21*), who described L^* values for different hydroponically grown varieties of determinate tomato between 41.7 and 43.8; and with results given by other authors (*22*, *23*), who reported similar L^* values of eight processing tomato varieties soil-grown in Italy, between 37 and 44, and five tomato varieties grown in Tenerife, between 39.7 and 46.9.

The average a^*/b^* ratio was significantly affected by varieties ($p < 0.0001$) and growing location ($p < 0.0001$), with no significant interaction between them. When averaged over varieties, the a^*/b^* ratio of fruits picked from Dragonja Valley was significant higher (0.94) compared to fruits from the Ljubljana field (0.82) and Ljubljana tunnel (0.84), but the difference between the last two locations was not significant. When averaged over the growing location, a^*/b^* values were more variable, and according to the Bonferroni test, a significantly lower a^*/b^* ratio, ranging between 0.67 and 0.83, was recorded for Stormy, Heinz 1370, Empire and Sunjay, compared to San Marzano, Hypeel 347, Centurion, Sun Chaser, Hypeel 108 and Super Red (a^*/b^* values ranging between 0.85 and 1.01). Our results were a little lower than those found by Moraru et al. (*21*), who reported an average a^*/b^* ratio for ten hydroponically grown processing tomatoes between 1.22 and 1.37 and much lower than that reported by investigators (*24*) in relation to soil-grown processing tomato varieties, with values ranging between 2.11 and 2.32.

Chroma (C^*) is the index calculated from a^* and b^* and corresponds to the saturation or vividness of the color. As C^* increases, the color becomes more intense (*16*). In our study C^* was significantly affected by varieties ($p < 0.0001$) and growing location ($p < 0.001$), with no significant interaction between them. When averaged over growing location, significantly higher C^* values, ranging between 38.2 and 42.2, were recorded for Centurion, Hypeel 108, Hypeel 347 and Super Red, compared to San Marzano, Stormy, Heinz 1370, Sunjay, Empire and Sun Chaser (C^* values ranging from 32.4 to 36.4). When averaged over the varieties, the chroma value measured for fruits picked from the Ljubljana field was significantly higher (38.2) than the values measured for fruits picked from the Ljubljana tunnel (36.0) and Dragonja Valley (36.7), but the difference between the last two locations was not significant.

From the point of view of the processing industry and the consumer, the color of the tomato fruit is an important quality attribute, as it affects grade and appearance of the end processing product. Red color in the tomatoes is the result of chlorophyll degradation as well as synthesis of lycopene and other carotenoids, as chloroplasts are converted into chromoplasts (*7,8*). The expression of pigment color is influenced by physical factors (*16*), as well as the rate of fruit ripening (*25*), as well as environmental factors, above all temperature and solar radiation (*26*). Considering a^*/b^* ratio as a reference parameter for ripening stage (*25,27*), we can see that at both locations the harvested fruits were found to be the same maturity stage (light red), although the a^*/b^* values for fruits harvested from Dragonja Valley were close to the separation values between the light red and red stages (0.95). It has been reported that tomatoes which reached the red color stage, according to the USDA color classification (*28*), might have ripened on the vine too long (*25*) or might have had a long overall storage time. Since we analyzed the fruits immediately after the harvest and the number of days from transplanting to harvesting (**Table 2**) was almost the same at both locations (96 in Ljubljana and 92 in Dragonja Valley, respectively), we assumed that their higher a^*/b^* ratio is due to the agricultural practices characteristic of the Mediterranean region, associated with suitable environmental conditions during the ripening period, which together contributed to faster fruit ripening.

Firmness of Tomato Fruits. The average firmness values measured on fruits of all ten varieties harvested at the three locations are represented in **Figure 1**.

The average firmness of tomatoes ranged from 8.3 to 25.4 N, and average values differed for both varieties and growing locations. ANOVA showed statistically significant interaction between varieties and locations ($p < 0.0001$). Namely, varieties with round fruits had lower average firmness than varieties with oval and elongated fruits. The exception was the Stormy harvested in Ljubljana, with the highest average firmness (25.4 N) among the investigated varieties. On the basis of the high average firmness and the previously mentioned low average a^*/b^* index (0.5) and the high average L^* value (54.7) for fruits of Stormy, we assumed that fruits of this cultivar were harvested in Ljubljana field too soon to be mature or Stormy is perhaps a variety of slower ripening fruits, since it has been reported (*25*) that fruit firmness varies with maturation and decreases with the ripening of fruits.

Differences in average firmness between round, on the one hand, and oval or elongated shaped fruits, on the other, were expected, bearing in mind that fruit firmness depends on skin toughness, flesh firmness and the pericarp/locular material ratio (*29*). The highest firmness is characteristic of tomato varieties

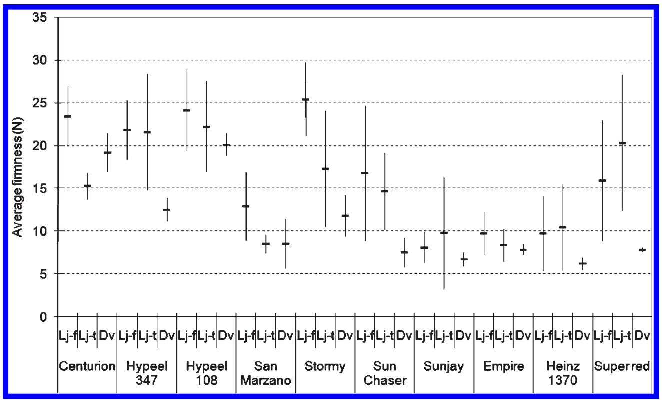

Figure 1. The average firmness with the intervals $\pm$ SD of tomato fruits for all ten varieties and three locations.

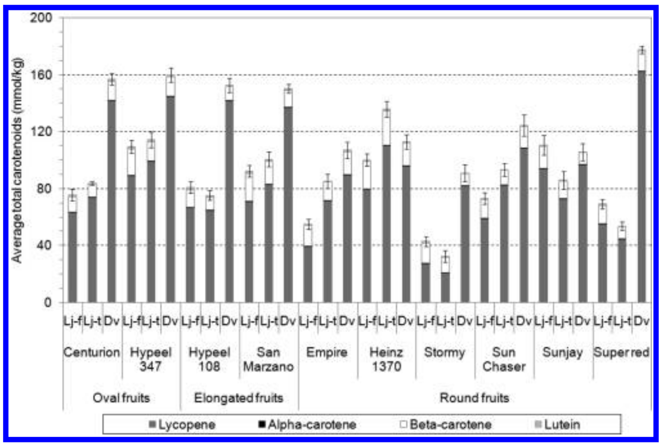

Figure 2. Average total amount of carotenoids (with SE bars) and its average structure for ten tomato varieties at three locations (Lj-f, Ljubljana field; Lj-t, Ljubljana tunnel; Dv, Dragonja Valley).

with oval, elongated or oblong fruits, due to the thicker pericarp, smaller locular cavity, higher solid and pectin content, which are specific to that type of tomato (*21*), and this was confirmed in our study (data not shown). Other factors affecting tomato texture have also been reported (*30*) and include maturity at harvest, cultivation practice, and environmental events, such as the degree of sun exposure, drought, salinization, water, freezing and chilling stress. We observed that with varieties with oval and elongated fruits, microclimatic conditions greatly affected the firmness of the fruits, since with these varieties the highest average firmness was observed with fruits harvested in cold, less suitable climatic conditions (Ljubljana field location). We assume that tomatoes harvested in Ljubljana field were less mature at harvest or perhaps varieties with oval and elongated fruits need higher temperatures during the maturation period to red maturity stage .

Lipophilic Antioxidants in Tomato Fruit. *Total Carotenoid Content*. The average content of total carotenoids and the average composition of them are presented in **Figure 2**.

ANOVA for the total amount of carotenoid (TC) shows a highly significant interaction between locations and varieties ($p < 0.0001$). The differences between average TC amounts of ten tomato varieties were not the same in all three locations. The averages of the TC amount for seven tomato varieties (Centurion, Hypeel 347, Hypeel 108, San Marzano, Empire, Sun Chaser and Super Red) ranging between 90.81 mmol/kg and 177.49 mmol/kg were significantly higher in the fruits from the Dragonja Valley (Dv) than for the same varieties grown in the Ljubljana field (Lj-f), where values ranged between 42.72 mmol/kg and 109.47 mmol/kg.

Table 5. The Average Percentages (%) of Individual Carotenoids (Lycopene, α-Carotene, β-Carotene and Lutein), Which Were Established in the 10 Varieties of Tomatoes, Grown in Three Experimental Locations (Ljubljana Field, Ljubljana Tunnel and Dragonja Valley)[a]

	Ljubljana field					Ljubljana tunnel					Dragonja Valley					
cultivar	lyc	α-car	β-car	lut	Lyc/β	lyc	α-car	β-car	lut	Lyc/β	Lyc	α-car	β-car	lut	Lyc/β	
oval fruits																
Centurion	83.7	0.3	14.0	2.0	6.0	88.2	0.3	10.2	1.3	8.7	90.5	0.2	8.1	1.2	11.2	c
Hypeel 347	81.5	0.3	16.7	1.5	4.9	87.1	0.1	11.7	1.1	7.4	90.5	0.1	8.5	0.9	10.6	c
elongated fruits																
Hypeel 108	82.7	0.4	14.9	2.1	5.6	86.1	0.3	11.6	1.9	7.4	93.0	0.2	6.1	0.7	15.2	c
San Marzano	76.8	0.2	21.0	2.0	3.7	82.8	0.1	15.5	1.6	5.4	91.1	0.1	7.9	0.9	11.6	bc
round fruits																
Empire	71.7	0.3	25.9	2.0	2.8	83.8	0.1	15.3	0.8	5.5	84.1	0.2	14.6	1.1	5.7	a
Heinz 1370	79.8	0.2	19.0	1.1	4.2	81.2	0.2	17.6	1.0	4.6	85.1	0.2	14.0	0.8	6.1	ab
Stormy	64.9	0.2	32.6	2.4	2.0	64.6	0.2	32.4	2.8	2.0	90.6	0.1	8.9	0.4	10.1	a
Sun Chaser	81.1	0.2	17.5	1.2	4.6	88.3	0.1	11.0	0.6	8.0	87.0	0.2	12.0	0.9	7.3	bc
Sunjay	85.1	0.2	13.7	1.1	6.2	84.7	0.2	14.1	1.1	6.0	91.7	0.1	7.8	0.4	11.7	c
Super red	80.1	0.2	18.2	1.5	4.4	83.1	0.2	15.1	1.6	5.5	91.6	0.1	7.8	0.5	11.7	bc

[a] Statistical significant differences between the average ratios Lyc/β of ten varieties are assigned with different letters (a, b, c) in the last column.

The differences between averages of TC amount for tomatoes growing in the Dragonja Valley and the Ljubljana tunnel were smaller and statistically significant only for four tomato varieties (Centurion, Hypeel 108, San Marzano and Super Red). There were no significant differences between the average TC amounts for all ten varieties grown in the two locations in Ljubljana (field and tunnel). A higher content of TC in fruits from the Dragonja Valley compared to fruits from the Ljubljana field was expected, namely, better coloration was already confirmed for these fruits, and red color in tomatoes is due to the presence of lycopene and other carotenoids (*16*). Since it was confirmed that the concentration of carotenoids increases during ripening between 10- to 14-fold, due mainly to accumulation of lycopene, (*7*, *8*, *27*), we assumed that higher content of TC is a result above all of the increasing maturity, reached due to the harvesting fruits all at once at the end of the season. Such agricultural practice is possible in areas where environmental conditions allow simultaneous ripening. In our research that was in the Mediterranean region, where the mean monthly air temperatures and the mean daily solar radiation during August and September were higher (**Table 3**) than in the Ljubljana field, on average by 2−3 °C and 3.8 MJ/m^2, respectively these conditions presumably contributed to the faster maturation of fruits. On the other hand, the small differences between mean monthly air temperature in the Dragonja Valley and the Ljubljana tunnel caused statistically significant differences in levels of TC in fruits only with 4 varieties, three of them with oval or elongated fruits. We assume that, for varieties with oval and elongated fruits, the improved microclimatic conditions induced by the low tunnel were not enough to reach the levels of TC comparable to the levels determined in tomatoes from the Mediterranean region. This suggests that these varieties need, in addition to higher temperature during the ripening period, also enough solar radiation, in order to synthesize carotenoids in considerable levels, since Dumas et al. (*11*) reported that the content of carotenoids in tomato fruits is greatly affected by environmental factors, especially air temperature and solar radiation.

The cultivar proved to be another important factor that influenced the content of TC in tomato fruits. At all three locations, Stormy had the smallest average TC (42.72 mmol/kg in Lj-f, 32.39 mmol/kg in the Ljubljana tunnel and 90.81 mmol/kg in the Dragonja Valley). Among the varieties harvested in the Ljubljana field, Sunjay had the highest average TC (110.47 mmol/kg), and there was no significant difference between it and Heinz 1370 and Hypeel 347. Among the varieties harvested in the Ljubljana tunnel, Heinz 1370 had the highest average TC (135.92 mmol/kg), and there was no significant difference between this cultivar and Sunjay, Sun Chaser, San Marzano and Hypeel 347. Among the fruits harvested in the Dragonja Valley, Super Red (177.49 mmol/kg) had the highest average TC, but there was no significant difference between it and San Marzano, Hypeel 108, Centurion and Hypeel 347. The levels of TC detected in our samples are comparable to the values reported in the literature. In 15 Hungarian industrial tomato varieties, the levels of TC ranged from 68 to 132 mg/kg (126.6 to 245.9 mmol/kg) (*13*); while for different typologies of tomato, the amount of TC was 6.4 mg/kg (11.9 mmol/kg) (for salad type), 26 mg/kg (48.4 mmol/kg) and 92 mg/kg (171.4 mmol/kg) (for elongated and cluster type tomatoes, respectively) (*12*)

Composition of Carotenoids. Four carotenoids were detected in our samples: lycopene, α-carotene, and β-carotene and lutein. The mean compositions of carotenoids for 10 varieties of tomato fruits harvested at three locations are presented in **Table 5**. The geometric mean is calculated as a representative measure of the central tendency. Lycopene was by far the main component of the carotenoid fraction, ranging from 27.71 to 93.98 mmol/kg, which was 64.4 to 83.6% of the TC in fruits harvested in the Ljubljana field, from 20.92 to 110.34 mmol/kg (75.3 to 88.7% of the TC) in fruits harvested in the Ljubljana tunnel and from 82.25 to 162.51 mmol/kg (83.4 to 93.0% of the TC) in fruits from the Dragonja Valley, which is in accordance with previously published data. In cherry tomato varieties (*14*), lycopene varied in relation to seasonal fluctuations, from 76% (in fruits harvested in January) to 85% of the TC (in fruits harvested in July); in salad and processing tomato varieties cultivated in Hungary (*13*), lycopene ranged from 96.5 to 216.1 mmol/kg (75.6 to 86.7% of the TC), while in different types of tomato (*12*), it varied from 18.6 mmol/kg (38% of the TC) in elongated types to 147.1 mmol/kg (85% of the TC) in cluster types. The second main component of the carotenoid fraction determined in our study was β-carotene, ranging from 10.56 to 19.45 mmol/kg (14.0 to 32.9% of the TC) in fruits from the Ljubljana field, from 8.12 to 23.93 mmol/kg (9.7 to 22.5% of the TC) in fruits from the Ljubljana tunnel and from 8.10 to 15.78 mmol/kg (6.0 to 15.2% of the TC) in fruits from the Dragonja Valley. The fractions of β-carotene detected in our samples, are higher than those reported in the literature, when the presented absolute values are converted into relative fractions. For some Hungarian processing varieties (*13*), the fraction of β-carotene ranged from 3.9 to 7.4 mmol/kg (2 to 5.6% of the TC). In fruits harvested in the Dragonja Valley, the fraction of

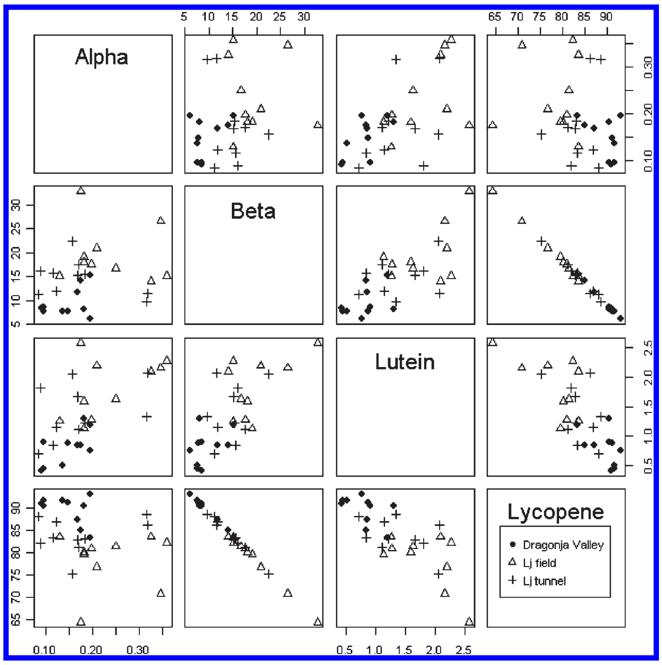

Figure 3. The scatterplot for geometric means of carotenoid components (%) for ten tomato varieties at three locations (Lj-f, Ljubljana field; Lj-t, Ljubljana tunnel; Dv, Dragonja Valley).

β-carotene seems to be a little closer to that reported for salad and elongated types of tomato 1.5 mmol/kg (12.5% of the TC) and 5.4 mmol/kg (11.3% of the TC), respectively, and 9.1 mmol/kg (5.3% of the TC) for the cluster type (*12*).

Although lycopene and β-carotene represent the most abundant carotenoids in tomato, we also measured the amount of α-carotene, which is known as a pro-vitamin A, and lutein, which is an important nutrient for human vision. The proportions of α-carotene were the smallest among the detected carotenoid fractions and in fruits from the Ljubljana field represent only 0.1 to 0.4% of TC (0.13 to 0.28 mmol/kg), in fruits from the Ljubljana tunnel, 0.1 to 0.3% (0.09 to 0.24 mmol/kg), and in fruits from the Dragonja Valley, 0.1 to 0.2% (0.09 to 0.28 mg/kg). The proportions of lutein were a little bit higher, but still low, ranging between 1.05 to 1.88 mmol/kg (1.3 and 2.6% of the TC) in fruits from the Ljubljana field, between 0.60 to 1.65 mmol/kg (0.7 and 2.1% of the TC) in fruits from the Ljubljana tunnel and between 0.37 to 1.95 mmol/kg (0.4 and 1.3% of the TC) in fruits from the Dragonja Valley. According to the literature, higher values for lutein, ranging between 1.34 and 7.54 mmol/kg (1% and 5% of TC), were found in 15 industrial tomato varieties cultivated in Hungary (*13*), while only trace amounts (0.1−0.3% of the TC) or no lutein content was detected in various other types of tomato (*12*).

Relationship between Lycopene and β-Carotene. In our study a strong linear correlation (**Figure 3**) between the mean content of lycopene and its cyclization product β-carotene was found. For a more detailed analysis of the impact of varieties and location of cultivation on the structure of carotenoids, we analyzed the relationship between lycopene and β-carotene (lyc/β-car), and no significant interaction was recorded between cultivar and the two location factors. The maximum ratio of lyc/β-car was found in fruits gathered from the Dragonja Valley (10.3), followed by the Ljubljana tunnel (6.3), then the Ljubljana field (4.3). The higher amount of lycopene detected in fruits from the Dragonja Valley is probably the result of later harvesting of fruits on that location and associated with the longer exposure of plants and fruits to sunlight and suitable temperatures for fruit maturation, which together may markedly affect the biosynthesis of lycopene. It has been reported (*11*) that the biosynthesis of lycopene greatly depends on the temperature range, and at favorable temperatures, between 22 and 25 °C, the rates of lycopene and carotene synthesis can be increased by illuminating tomato plants, especially during the ripening period. As was mentioned above, the mean solar radiation in the Dragonja Valley was on average 3.8 MJ/m^2 higher than at the Ljubljana field, throughout the experimental period (**Table 3**). On the other hand, Brandt et al. (*26*) reported that lycopene production is inhibited when fruits are directly exposed to strong solar radiation and, consequently, their temperature rises above 35 °C. However, in all three experiments, we did not observe a high temperature increase of tomato fruits, since the plants had sufficiently dense foliage to protect the fruit from direct exposure to the sun.

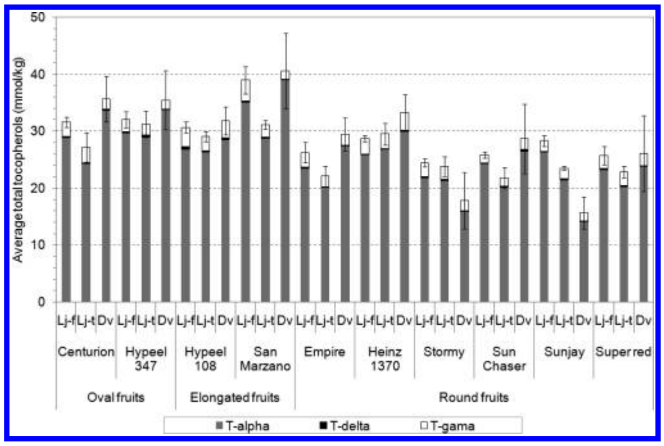

Figure 4. Average amounts of tocopherols (with SE bars) and theirs average structure for ten tomato varieties, grown in three different microclimatic locations (Lj-f, Ljubljana field; Lj-t, Ljubljana tunnel; Dv, Dragonja Valley).

In addition to the influence of the environmental factors, an impact of varieties on the variation in the lyc/β-car ratio was also found. On average, the smallest ratio was calculated for Stormy, Empire and Heinz 1370, in which the first two varieties significantly differed from all the other tested varieties, while Heinz 1370 differed only from Sunjay, Hypeel 347, Centurion and Hypeel 108. Differences between absolute values of lycopene and β-carotene content among tomato varieties had been previously reported for Hungarian processing varieties (*13*); for 40 field and greenhouse grown tomato varieties (*15*), for different tomato typologies (*12*) and for tomato cultivars grown with different cultivation methods (intensive, organic and hydroponic) (*23*), although the compositional data analysis for determining the relationship between lycopene and β-carotene content in tomato fruits presented in our study has not been previously published.

Tocopherols (α-, δ- and γ-Tocopherol). Three tocopherols were detected (α-, δ- and γ-tocopherol), and the results as the average amount of total tocopherols (TT) in 10 tomato varieties grown under different microclimatic conditions are shown in **Figure 4**; see also **Table 6**.

Statistical analysis showed that the interaction between varieties and locations was statistically significant. The average TT amount does not vary as much among the three locations as the average total carotenoid amount varied. Significant differences in average TT amount exist only for the varieties Sunjay, Sun Chaser, Empire and San Marzano (**Figure 4**: different letters in the columns indicate significant differences).

Among the varieties harvested in the Ljubljana field, the average amount of TT in fruits of Stormy, Super Red and

Table 6. The Average Percentages (%) of Individual Tocopherols (α-, δ- and γ-Tocopherols), Which Were Analyzed in 10 Varieties of Tomatoes, Grown in Three Experimental Locations (Ljubljana Field, Ljubljana Tunnel and Dragonja Valley)

	Ljubljana field				Ljubljana tunnel				Dragonja Valley			
cultivar	T-α	T-δ	T-γ	α/γ	T-α	T-δ	T-γ	α/γ	T-α	T-δ	T-γ	α/γ
oval fruits												
Centurion	91.1	0.7	8.1	11.2	89.2	0.8	10.0	9.0	94.4	0.4	5.2	18.1
Hypeel 348	92.5	1.0	6.5	14.3	92.5	1.3	6.2	14.9	95.3	0.2	4.5	21.2
elongated fruits												
Hypeel 109	87.3	1.7	11.0	7.9	90.5	1.0	8.5	10.7	89.6	1.0	9.4	9.5
San Marzano	90.0	0.5	9.5	9.4	92.6	0.7	6.7	13.8	96.3	0.3	3.4	28.0
round fruits												
Empire	89.5	0.8	9.7	9.3	91.1	0.6	8.3	11.0	93.1	0.3	6.6	14.1
Heinz 1371	89.8	0.6	9.6	9.4	90.7	0.7	8.6	10.5	89.8	0.6	9.6	9.4
Stormy	89.0	1.1	9.9	9.0	89.4	1.0	9.6	9.4	89.7	0.7	9.6	9.4
Sun Chaser	93.9	0.4	5.7	16.4	92.4	1.2	6.4	14.5	92.0	1.3	6.6	13.9
Sunjay	92.8	0.5	6.6	14.0	91.4	0.7	7.8	11.6	90.7	0.6	8.7	10.4
Super red	90.3	0.8	8.9	10.2	88.6	0.8	10.6	8.4	91.1	0.5	8.4	10.9

Sunchaser was significantly lower, ranging between 24.45 and 25.85 mmol/kg fw, than that of Sunjay, Heinz 1370, Hypeel 108, Centurion, Hypeel 347 and San Marzano, ranging between 28.30 and 38.95 mmol/kg fw. San Marzano had the highest average TT (38.95 mmol/kg fw), and this value was also significantly higher than those of Centurion (31.58 mmol/kg fw) and Hypeel 347 (31.99 mmol/kg fw). Among the tomatoes grown in the Ljubljana tunnel were the varieties (Sun Chaser, Empire, Super Red, Sunjay and Stormy) with significantly lower average TT, ranging between 21.75 and 23.80 mmol/kg, compared to Centurion, Hypeel, Heinz 1370, San Marzano and Hypeel 347, for which the average TT ranged between 27.15 and 31.22 mmol/kg fw. In the Dragonja Valley, only two varieties (Sunjay and Stormy) had a significantly lower (15.63 and 17.76 mmol/kg fw) average TT than all other varieties, whereby the average amount of TT ranged from 26.06 mmol/kg fw for Super Red to 40.52 mmol/kg fw for San Marzano. The amount of α-tocopherol obtained in the varieties tested in our research appeared to be higher than the results reported by Abushita et al. (*13*), who found amounts of TT in salad tomato varieties ranging from 2.81 to 7.56 mmol/kg fw, and by Raffo et al. (*14*) who found 0.96 mmol/kg fw in cherry tomato harvested in December and 27.84 mmol/kg fw in fruits harvested in June. The same authors (*13*) in later experiments also reported TT contents in different salad tomato varieties ranging from 8.93 to 34.22 mmol/kg fw and in processing tomato varieties ranging from 14.97 to 52.22 mmol/kg fw. They pointed out that fruits of processing tomato varieties generally had a higher content of TT than salad tomatoes varieties.

The relationship among three tocopherols detected in our study is shown in **Table 4**. As can be seen, α-tocopherol was the main component, ranging from 88.5 to 96.5%, and δ-tocopherol had the lowest amounts, ranging from 0.0 to 1.5%. Analysis of variance showed that the interaction was not statistically significant. There were also no significant differences in relation to the location of cultivation. Differences were only significant between the varieties Hypeel 108, Stormy, Super Red and Heinz 1370 compared to Sunchaser, San Marzano and Hypeel 347, which coincides with the findings of a study by Fanasca et al. (*8*), who reported that the concentration of α-tocopherol in tomato fruits was significantly affected by the tomato varieties. Little data has been reported in the literature on the qualitative composition of tocopherols in tomato fruits, since the contribution of tocopherols to the antioxidant properties of tomato is of minor relevance. Mainly absolute values of α-tocopherol in tomato fruits have been reported (*8*, *14*, *15*), although it has been pointed out that in tomato fruits only the seeds, which are not digested by humans, mainly contain vitamin E.

According to our results, the areas with higher amount of solar radiation, higher average air temperature and low precipitation amount (in our case that was in the Mediterranean region in Dragonja Valley), especially during the ripening period, as well as tomato varieties with oval and elongated fruits, are recommended for the production of tomatoes with higher content of lipophilic antioxidants and better coloration, which are important parameters for the processing industry, as well as in terms of the consumer.

LITERATURE CITED

(1) Kacjan Maršić, N.; Osvald, J; Jakše, M. Evaluation of ten cultivars of determinate tomato (Lycopersicum esculentum Mill.), grown under different climatic conditions. *Acta Agric. Slov.* **2005**, *85* (2), 321–328.

(2) Usenik, V.; Fabčič, J.; Štampar, F. Sugar, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). *Food Chem.* **2008**, *107*, 185–192.

(3) Veberic, R.; Trobec, M.; Herbinger, K.; Hofer, M.; Grill, D.; Stampar, F. Phenolic compounds in some apple (*Malus domestica* Borkh.) cultivars of organic and integrated production. *J. Sci. Food Agric.* **2005**, *85*, 1687–1694.

(4) Levy, J.; Sharoni, Y. The functions of tomato lycopene and its role in human health. *HerbalGram* **2004**, *62*, 49–56.

(5) Amir, H.; Karas, M.; Giat, J.; Danilenko, M.; Levy, R.; Yermiahu, T.; Levy, J.; Sharoni, Y. Lycopene and 1,25-dihidroxyvitamin D_3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. *Nutr. Cancer* **1999**, *33*, 105–112.

(6) Chun, J.; Lee, J.; Ye, L.; Exler, J.; Eitenmiller, R. R. Tocopherol and tocotrienol contents of raw and processed fruits and vegetables in the United Stated diet. *J. Food Compos. Anal.* **2006**, *19*, 196–204.

(7) Hirschberg, J. Carotenoid biosynthesis in flowering plants. *Curr. Opin. Plant Biol.* **2001**, *4*, 210–218.

(8) Bramley, P. M. Regulation of carotenoid formation during tomato fruit ripening and development. *J. Exp. Bot.* **2002**, *53*, 2107–2113.

(9) Fanasca, S.; Colla, G.; Maiani, G.; Venneria, E.; Rouphale, Y.; Azzini, E.; Saccardo, F. Changes in antioxidant content of tomato fruits in response to cultivar and nutrient solution composition. *J. Agric. Food Chem.* **2006**, *54*, 4319–4325.

(10) Wu, M.; Kubota, C. Effects of electrical conductivity of hydroponic nutrient solution on leaf gas exchange of five greenhouse tomato cultivars. *HortTechnology* **2008**, *18*, 196–319.

(11) Dumas, Y.; Dadomo, M.; Di Lucca, G.; Grolier, P. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J. Sci. Food Agric.* **2003**, *83*, 369–382.

(12) Leonardi, C.; Ambrosino, P.; Esposito, F.; Fogliano, V. Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. *J. Agric. Food Chem.* **2000**, *48*, 4723–4727.

(13) Abushita, A. A.; Daood, H. G.; Biacs, P. A. Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *J. Agric. Food Chem.* **2000**, *48*, 2075–2081.
(14) Raffo, A.; La Malfa, G.; Fogliano, V.; Maiani, G.; Quaglia, G. Seasonal variations in antioxidant components of cherry tomatoes (*Lacopersicon esculentum* cv. Naomi F1. *J. Food Compos. Anal.* **2006**, *19*, 11–19.
(15) Kuti, J. O.; Konuru, H. B. Effects of genotype and cultivation environment on lycopene content in red-ripe tomatoes. *J. Sci Food Agric.* **2005**, *85* (12), 2021–2026.
(16) Lancaster, J. E.; Lister, C. E.; Reay, P. F.; Triggs., C. M. Influence of pigment composition on skin color in a wide range of fruit and vegetables. *J. Am. Hort. Sci.* **1997**, *122* (4), 594–598.
(17) Šircelj, H.; Batič, F. Evaluation of selected nutritional factors in *Aposeris foetida* (L.) Less. during the harvesting period. *J. Appl. Bot. Food Qual.* **2007**, *81*, 121–125.
(18) Monthly meteorological reports of the Environmental Agency of the Republic of Slovenia.[Online]. **2008**. Available: http://www.arso.sov.si/o_agenciji/knjiz~nica/publikacije/bilten.htm. [Accessed 20 January 2008].
(19) Pawlowsky-Glahn, V.; Egozcue, J. J. Geometric approach to statistical analysis on the simplex. *Stochastic Environ. Res. Risk Asses.* (*SERRA*) **2001**, *15* (5), 384–398.
(20) Aitchinson, J. *The statistical analysis of compositional data*; The Blackburn press: Caldwell, NJ, 2003; 408 pp.
(21) Moraru, C.; Logendra, L.; Lee, T. C.; Janes, H. Characterictics of 10 processing tomato cultivars grown hydroponically for the NASA Advances Life (ALS) Support Program. *J. Food Compos. Anal.* **2004**, *17*, 141–154.
(22) Pagliarini, E.; Ratti, S. Relationship between objective measurements and sensory attributes of quality of eight tomato cultivars. *Acta Hortic.* **1999**, *487*, 349–532.
(23) Hernández, M; Rodríguez, E.; Díaz, C. Free Hydroxycinnamic acids, lycopene, and colour parameters in tomato cultivars. *J. Agric. Food Chem.* **2007**, *55*, 8604–8615.
(24) Macua, J. I.; Garnica, J.; Merino, J.; Gutierrez, M. Processing tomato variety choice in the Ebro Valley. *Acta Hortic.* **1999**, *487*, 339–342.
(25) Batu, A. Determination of acceptable firmness and colour values of tomatoes. *J. Food Eng.* **2004**, *61*, 471–475.
(26) Brandt, A.; Pék, Z.; Barna, É.; Lugasi, A.; Helyes, L. Lycopene content and colour of ripening tomatoes as affected by environmental conditions. *J. Sci. Food Agric.* **2006**, *86*, 568–572.
(27) Raffo, A.; Leonardi, C.; Fogliano, V.; Ambrosino, P.; Salucci, M.; Gennaro, L.; Bugianesi, R.; Giuffrida, F.; Quaglia, G. Nutritional value of cherry tomatoes (*Lycopersicon esculentum* Cv. Naomi F1) harvested at different ripening stages. *J. Agric. Food Chem.* **2002**, *50*, 6550–6556.
(28) USDA. *United States standards for grades of fresh tomatoes*; US Department Agric., Mktg. Serv., Washington, DC, 1997; pp 1−10.
(29) Barrett, D. M.; Anthon, G. Lycopene content of California-grown tomato varieties. *Acta Hortic.* **2001**, *542*, 165–173.
(30) Jackman, R. J.; Stanley, D. W. Creep behaviour of tomato pericarp tissue as influenced by ambient temperature ripening and chilled storage. *J. Texture Stud.* **1995**, *26* (5), 537–552.

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