PLANT SCIENCES

MONITORING OF BIOACTIVE COMPOUNDS OF TOMATO CULTIVARS AS AFFECTED BY MULCHING FILM*

M. Valšíková[1], J. Mlček[2], L. Snopek[2], M. Rehuš[1], S. Škrovánková[2], T. Juríková[3], D. Sumczynski[2], O. Paulen[4]

[1]Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Department of Vegetable Production, Nitra, Slovakia
[2]Tomas Bata University in Zlín, Faculty of Technology, Department of Food Chemistry and Analysis, Zlín, Czech Republic
[3]Constantine the Philosopher University, Institute for Teacher Training, Nitra, Slovakia
[4]Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Department of Fruit Production, Viticulture and Enology, Nitra, Slovakia

The influence of varieties and the effect of mulching film on antioxidant capacity, polyphenol content, ascorbic acid content, and yield of tomato fruits were investigated. Results of two years (2012 and 2013) investigations were compared. The results proved a statistically significant effect of year, mulching film, and variety on the content of total polyphenols (0.92–1.49 g gallic acid equivalents per kg of fresh weight (FW), ascorbic acid (26.66–38.62 mg per 100 g FW), and antioxidant capacity (1.12–1.94 g ascorbic acid equivalents per kg FW), while the values were the highest in 2013 and in uncovered soil. Conversely, a higher yield was found in mulching film compared with uncovered soil, also in 2013 (48.65–120.38 t ha^{-1}). There was a negative correlation between the yield and the content of bioactive compounds (BC) and antioxidant capacity. The content of bioactive substances, antioxidant capacity, and yield of tomato fruits is dependent on the vintage, agronomical interventions, and genotype.

Solanum lycopersicum, varieties, mulching, yield, antioxidant capacity

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) fruit is produced year-round and its crop is the second most important vegetable crop, next to potato, worldwide. In 2016, world tomato production was 177 million t (FAOSTAT 2016, http://www.fao.org/faostat/en/#data). In terms of human consumption and health, tomato fruit is a major component of daily meals in many countries and an important source of minerals (potassium), vitamins, antioxidants, and ascorbic acid (Zushi, Matsuzoe, 2009; Vallverdu-Queralt et al., 2011). According to Leiva-Brondo et al. (2012) its nutritional and functional quality is determined mainly by the accumulation of antioxidant compounds. These antioxidants comprise, among other, carotenoids and phenolics (Marti et al., 2016). Tomato antioxidants include carotenoids such as β-carotene, a precursor of vitamin A, and mainly lycopene (Li et al., 2013). Main tomato phenolic compounds are hydroxycinnamic acids, flavanones, flavonols, and anthocyanins. In addition, flavonol glycosides like

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rutin and kaempferol-3-rutinoside are also present in tomato fruits (M a r t i et al., 2016). These compounds may play an important role in inhibiting reactive oxygen species responsible for many important diseases through free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways (P i n e l a et al., 2012; M l c e k et al., 2015).

Ascorbic acid is another important antioxidant compound presented in tomato. Although its content is moderate, its contribution to our diet is significant because of high consumption of tomatoes (K a u r et al., 2013).

The quantity and quality of phytochemicals detected in tomato fruit are known to vary in relation to genotype, but also depend on environmental and agronomic factors (I l a h y et al., 2011). As the antioxidant content of tomatoes depends on genetic factors, the choice of variety cultivated may affect the results of antioxidant activity of fruit. In recent decades, there has been a growing concern regarding the environment. This has caused an increase in the demand for food products produced with low-input or no agrochemicals in industrialized countries, what resulted in the use of natural fertilizers and adoption of ecological pest control such as cover crops and mulches (C a m p i g l i a et al., 2010). Mulching can be considered as a cultural practice that improves the global quality of fruits but reduces the post-harvest shelf life. The main advantages associated with mulching are: less water required for irrigation, earlier harvest, and a bigger size of plants (M e l g a r e j o et al., 2012).

According to our knowledge, there has been no research literature focused on the effect of cultural practices like mulching on the antioxidant activity and phenolic profile of tomatoes so far.

MATERIAL AND METHODS

Plant material and growth conditions

A small experimental plot with tomatoes was established in the Botanical garden of the Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Department of Vegetable Production. The town of Nitra lies in the southwest of Slovakia, between the Danube River and the Tríbeč Mts. (48°18’ N, 1814°› E). Six varieties of tomatoes of Czech origin were used: Darinka F1, Denar, Diana, Orange, Pavlina, and Šejk, all intended for open field cultivation.

Experimental plots with tomato as a fore crop were fertilized during the autumn using manure at a dose of 40 t ha^{-1}. In spring (on April 24th, 2012 and April 22nd, 2013) the land was fertilized before planting using ammonium sulfate at a dose of 300 kg ha^{-1}. Then the soil was levelled and mulched using a brown polyethylene mulching film. Seedlings were planted on May 14th, 2012 and May 15th, 2013. The experiment was conducted in two variants: with uncovered soil (W), and using a mulching film (F).

For the years 2012 and 2013, the annual average temperature in the area of the Botanical Garden in Nitra was 11.16°C and 11.0°C and the annual average precipitations were 470 mm and 654 mm, respectively.

There were four harvests of tomatoes at the stage of consumer (red) maturity. Tomato fruits were taken for analysis (total content of polyphenols and antioxidant capacity) at the second harvest on August 6th, 2012 and August 2nd, 2013.. The analysis of ascorbic acid content and determination of the yield were carried out in four harvests. In 2012, tomatoes were harvested on July 19th, August 6th and 27th, and September 17th and in 2013, on July 16th, August 2nd and 26th, and September 16th. When harvested, 10 pieces of tomato fruits were taken from each variety and variant composing an average sample for analysis.

Extraction of samples

In this study, the extraction method based on K i m et al. (2003) was used. The samples were stored at 4°C for subsequent analyses.

Total phenolic content (TPC)

The TPC of each extract was determined in duplicate using the Folin–Ciocalteu procedures. The results were expressed as g gallic acid equivalents per kg of fresh weight (g GAE kg^{-1} FW) (K i m et al., 2003).

Total antioxidant capacity (TAC)

The TAC of the tomato extracts was measured using a DPPH method described by T h a i p o n g et al. (2006) using free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The absorbance results were converted using a calibration curve of the standard and expressed as g ascorbic acid equivalents per kg of fresh weight (g AAE kg^{-1} FW) (R u p a s i n g h e et al., 2006).

Determination of ascorbic acid (AA) content

The AA content was determined according to the method by M i k i (1981) modified by R o p et al. (2010). It was calculated as mg 100 g^{-1} FW.

Statistical analysis

Each experiment was performed in triplicate. The data were analyzed using Adstat software (Version 1.25) (TriloByte) and expressed as means ± standard deviations. Significant differences between samples were determined by one-way analysis of variance,

Table 1. Total polyphenol content (g GAE/kg FW) and total antioxidant capacity (g AAE/kg FW)

Year	2012		2012		2013		2013	
Cultivar	GAE	SD	AAE	SD	GAE	SD	AAE	SD
Denár (W)	1.07[a]	±0.05	1.37[c]	±0.18	1.12[a]	±0.04	1.41[c]	±0.20
Denár (F)	0.92[a]	±0.04	1.13	±0.04	0.94[a]	±0.04	1.18	±0.03
Šejk (W)	1.35	±0.04	1.89	±0.03	1.49	±0.02	1.94	±0.03
Šejk (F)	1.11	±0.03	1.38	±0.07	1.2	±0.09	1.64	±0.13
Darinka (W)	1.27[a]	±0.03	1.77[c]	±0.07	1.28[a]	±0.03	1.78[c]	±0.06
Darinka (F)	0.95[a]	±0.02	1.12[c]	±0.02	0.96[a]	±0.02	1.13[c]	±0.02
Diana (W)	1.26[a]	±0.07	1.73[c]	±0.18	1.29[a]	±0.06	1.72[c]	±0.18
Diana (F)	0.95[a]	±0.02	1.13[c]	±0.05	0.97[a]	±0.02	1.16[c]	±0.03
Pavlína (W)	1.11[a,b]	±0.19	1.45[c,d]	±0.32	1.14[a,b]	±0.18	1.57[c]	±0.28
Pavlína (F)	1.02[b]	±0.03	1.29[d]	±0.03	1.13[b]	±0.12	1.34	±0.04
Orange (W)	1.16	±0.05	1.48	±0.08	1.29	±0.09	1.57	±0.09
Orange (F)	1.08	±0.04	1.38	±0.05	1.21	±0.10	1.46	±0.04

Note: Variant with uncovered soil (W), and variant with mulching film (F), standart deviation (SD), statistically nonsignificant differences between years (a – GAE, c - AAE) and soil treatment (b – GAE, d - AAE) (p>0,05), same letter superscripts

considering differences significant at $P < 0.05$. This statistical analysis was performed with STATISTICA (Version1.25) (StatSoft).

RESULTS AND DISCUSSION

The TPC and TAC values, of the six tomato cultivars (cvs) investigated are shown in Table 1. The values ranged from 0.92 g GAE kg^{-1} FW (cv. Denár, 2012) to 1.49 g GAE kg^{-1} FW (cv. Šejk, 2013) and from 1.12 g AAE kg^{-1} FW (cv. Darinka F1, 2012) to 1.94 g AAE kg^{-1} FW (cv. Šejk, 2013). The TPC of cv. Šejk was the highest among all cvs while the cvs Denár and Pavlína reached the lowest values in the monitored periods 2012 and 2013. The same cvs showed the highest and the lowest antioxidant capacity, too. These conclusions were obtained for the varieties cultivated without mulching film.

Among the varieties cultivated with mulching film, cv. Šejk had the greatest TAC in 2013 together with cv. Orange in 2012. The lowest values of TAC were found in cvs Darinka, Denár, and Diana. Cv. Šejk showed the highest phenolic content in 2012 and cv. Orange in 2013. However the lowest content of phenolics was found out in cv. Denár (both 2012 and 2013).

The present results are similar to those by Zushi, Matsuzoe (2009). Ilahy et al. (2011) and Kalogeropoulos et al. (2012) stated the total polyphenols content to be about half or lower. The TPC range of 0.26–1.42 g GAE kg^{-1} FW was published by Kaur et al. (2013), 1.49–1.96 g GAE kg^{-1} FW by Vinha et al. (2014).

Overall, higher levels of both TPC and TAC were achieved in all cvs in 2013 (excluding cv. Diana – TAC) and in the variant without mulching film. Also Melgarejo et al. (2012) stated lower levels of TPC and antioxidants in Japanese plum grown under a polyethylene film. Some authors (Arakawa, 1988; Iglesias, Alegre, 2009; Sackey et al., 2015) suppose that this is because the PE film shows a lower sunlight reflection and also in consequence of obtaining less coloured fruits. A lower content of natural pigments such as carotenoids or anthocyanins has a direct influence on the level of TAC (Gardner et al., 2000).

For TPC and TAC the effect of year was statistically significant both in plots with and without mulching film ($P < 0.05$) as well as the effect of soil treatment in different years ($P < 0.05$), except for some measurements (Table 1).

High levels of AA in tomato fruits provide health benefits for humans and also play an important role in several aspects of plant life. Agronomical conditions, light, temperature, and varietal differences may account for significant variations in AA. The content ranged from 26.66 mg 100 g^{-1} (Orange, mulching film, 2012) to 38.62 mg 100 g^{-1} (Diana, without mulching film, 2013).

Overall, higher values were achieved in 2013 compared to 2012 for both variants, with or without mulching film (with the exception of cv. Pavlína, in which a higher AA content was achieved in 2012 with mulching film). Regarding the differences between the variants with or without mulching film, higher levels of AA were achieved without mulching film in all

Table 2. Content of ascorbic acid in fresh tomato fruit in 2012 (mg/100g)

Tomato variety	Soil treatment	Ascorbic acid (mg/100g)										
		1st harvest		2nd harvest		3rd harvest		4th harvest		Average		
		M	SD	M	SD	M	SD	M	SD	M	SD	p
Denár	W	24.00	0.55	32.10	1.08	39.60	1.32	32.95	0.56	32.16	1.6	*
	F	21.05	0.45	24.30	0.57	34.00	0.44	31.60	0.29	27.73	1.0	
Šejk	W	26.12	0.34	36.82	1.12	36.91	0.54	33.29	0.64	33.28	1.8	*
	F	24.58	0.77	27.94	0.53	32.61	0.57	25.91	0.39	27.76	1.5	
Darinka	W	30.72	0.46	39.45	0.83	40.19	0.93	33.63	0.70	35.99	2.1	NS
	F	33.80	0.53	31.61	0.71	34.76	0.64	30.27	0.60	32.61	1.6	
Diana	W	27.66	0.73	42.84	0.76	40.81	0.61	36.69	0.43	37.10	2.0	NS
	F	24.58	0.71	38.75	0.70	36.48	0.37	34.99	0.40	33.70	1.5	
Pavlína	W	24.58	0.22	33.42	0.52	33.55	0.16	32.61	0.30	31.04	1.5	NS
	F	34.04	0.53	33.41	0.93	34.60	0.53	26.54	0.65	32.14	1.3	
Orange	W	34.04	1.04	31.70	0.56	34.73	0.42	31.25	0.37	32.93	1.4	**
	F	28.04	0.86	24.10	0.51	33.31	0.78	27.86	0.55	26.66	1.2	

Note: Variant with uncovered soil (W), and variant with mulching film (F), statistically significant differences between soil treatment *p<0,05, **p<0,01, NS - not significant

Table 3. Content of ascorbic acid in fresh tomato fruit in 2013 (mg/100g)

Tomato variety	Soil treatment	Ascorbic acid (mg/100g)										
		1st harvest		2nd harvest		3rd harvest		4th harvest		Average		
		M	SD	M	SD	M	SD	M	SD	M	SD	p
Denár	W	26.00	0.79	32.00	0.77	40.86	0.57	34.15	0.54	33.25	1.6	*
	F	23.25	0.83	24.30	0.39	34.00	0.67	31.60	0.65	28.29	1.2	
Šejk	W	27.10	0.39	35.85	0.81	39.92	0.97	35.83	0.59	34.68	2.1	*
	F	25.80	0.50	27.94	0.61	32.61	0.59	30.91	0.97	29.32	1.6	
Darinka	W	32.45	0.93	40.50	0.59	40.32	0.45	35.36	0.64	37.16	2.6	NS
	F	35.80	0.66	31.61	0.49	34.76	0.18	32.27	0.56	33.61	2.0	
Diana	W	28.72	0.68	45.20	0.90	42.85	0.56	37.70	0.63	38.62	3.1	NS
	F	27.45	0.44	38.75	0.52	36.48	0.76	34.99	0.98	34.42	2.5	
Pavlína	W	26.00	0.43	30.40	0.51	35.65	0.48	34.55	0.39	31.65	2.0	NS
	F	20.03	0.56	33.41	0.80	34.60	0.54	28.54	0.52	29.15	1.3	
Orange	W	34.20	0.63	32.85	0.37	37.75	0.59	33.35	0.86	34.54	1.7	*
	F	25.04	0.52	24.10	0.11	37.30	0.51	34.85	0.74	30.32	1.3	

Note: Variant with uncovered soil (W), and variant with mulching film (F), statistically significant differences between soil treatment *p<0,05, **p<0,01, NS - not significant

cases, except cv. Pavlína, in which a higher content was achieved in 2012 with mulching film (Tables 2, 3).

Similar results for AA were presented by Zushi, Matsuzoe (2009), Ilahy et al. (2011), Kaur et al. (2013), and Vinha et al. (2014). Lower values of AA (10.86–18.56 mg 100 g^{-1}) were reported by Pinela et al. (2012). Kotikova et al. (2011) stated the highest content of AA to be at the stage of full maturity. Our results follow the time sequence of harvesting at the stage of consumer maturity in four quartiles. It was observed that in the third harvest the tomatoes were highest in the content of AA. As stated by many authors (e.g. Dumas et al., 2003; Gautier et al. 2009), solar irradiance can directly influence the AA content,

Table 4. Total tomato yield in 2012 (t/ha)

Tomato variety	Soil treatment	Yield – uncovered soil (t/ha)					
		1st harvest	2nd harvest	3rd harvest	4th harvest	Total	Difference of totals
Denár	W	1.70	4.71	27.33	14.91	48.65	18.00
	F	1.70	3.95	24.78	36.22	66.65	
Šejk	W	3.71	9.34	33.24	27.94	74.23	24.65
	F	5.71	12.82	46.85	33.50	98.88	
Darinka	W	2.86	7.50	30.57	19.53	60.46	39.66
	F	5.02	9.96	48.24	36.90	100.12	
Diana	W	6.88	10.51	29.49	20.46	67.34	35.42
	F	13.51	16.21	30.51	42.53	102.76	
Pavlína	W	5.64	7.20	30.87	26.79	70.5	34.17
	F	6.25	3.25	41.99	53.18	104.67	
Orange	W	10.50	13.97	25.79	15.37	65.63	9.88
	F	14.56	20.45	35.90	40.50	75.51	

Note: Variant with uncovered soil (W), and variant with mulching film (F)

Table 5. Total tomato yield in 2013 (t/ha)

Tomato variety	Soil treatment	Yield – uncovered soil (t/ha)					
		1st harvest	2nd harvest	3rd harvest	4th harvest	Total	Difference of totals
Denár	W	1.90	3.91	29.65	16.30	51.76	24,37
	F	2.74	5.90	28.29	39.20	76.13	
Šejk	W	4.65	11.24	50.00	29.68	95.57	11,97
	F	7.95	14.56	49.33	35.70	107.54	
Darinka	W	3.60	9.50	31.54	22.33	66.97	44,61
	F	6.85	12.73	52.20	39.80	111.58	
Diana	W	10.98	15.81	30.75	24.35	81.89	32,56
	F	13.50	20.25	34.50	46.20	114.45	
Pavlína	W	6.50	9.30	30.77	28.25	74.82	45,56
	F	8.28	7.20	45.80	59.10	120.38	
Orange	W	12.52	16.50	28.84	28.32	86.18	25,33
	F	12.66	20.40	37.30	41.15	111.51	

Note: Variant with uncovered soil (W), and variant with mulching film

however, sanitary and nutritional status of the plant and changes in water availability are important factors as well (R a f f o et al., 2006).

The effect of vintage (with or without mulching film) as well as of variants on the AA content was statistically significant ($P < 0.05$) in both years.

The average yields values of the fruits from the variants were converted to crops in t ha^{-1}. Tomato yields in 2012 and 2013 (Tables 4, 5) ranged from 48.65 t ha–1 (cv. Denár, 2012) to 120.38 t ha^{-1} (cv. Pavlína, 2013). This corresponds with the statements of many authors (e.g. Favati et al. 2009; Ren et al. 2010). Contrary to BC (total polyphenols, ascorbic acid) and total antioxidants, the tomato yields were higher in the mulching film variant. Furthermore, in 2013 the yields were higher in both variants. A positive influence of different types of mulches (hairy vetch, subclover, and hairy vetch/oat) on the yields was stated by Campiglia et al. (2010).

The effect of the year (with or without mulching film) as well as of soil treatment in each year on the yield was statistically significant ($P < 0.05$).

The results show that the highest yields were always achieved in varieties grown with mulching film, either in 2012 or 2013, compared to cvs grown without it (uncovered soil). When comparing the two years, the yields were higher in 2013, when the average annual temperature was lower, but the precipitations were higher.

The results are completely opposite in the total polyphenols, antioxidants, and AA, as their content was almost in all cases lower in the plot with mulching film (except the AA content in variety Pavlína in 2012). When comparing the years, 2013 seemed to be more convenient regarding the content of BC mentioned above and the antioxidant capacity. Overall, there was a negative correlation between yields and the content of bioactive compounds ($r = 0.407$, $y = –1.1469x + 56.485$). Many authors reported a negative correlation between BC and fruit size (Connor et al. 2005; Iamjud et al. 2016). The influence of fruit size on BC was not observed in our work. Results also differ among cultivars. Therefore it can be stated that the quantity and quality of phytochemicals as well as yields of tomato fruits are known to depend greatly on environmental condition, agronomic interventions, and genotype.

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Corresponding Author:

Doc. Ing Jiří M l č e k , Ph.D., Tomas Bata University in Zlín, Faculty of Technology, Department of Food Chemistry and Analysis, Zlín, Czech Republic, phone: +420 576 033 030, e-mail: mlcek@ft.utb.cz