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INFLUENCE OF TITANIUM TREATMENT ON ANTIOXIDANTS CONTENT AND ANTIOXIDANT ACTIVITY OF STRAWBERRIES

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Abstract. The spraying with titanium is one of the agronomic practices used to stimulate the flowering, bearing of fruit and production of strawberry plants. The aim of the study was to assess the influence of titanium treatment (Ti^{4+}-ascorbate) on L-ascorbic acid, total polyphenol, antioxidant activity, and phenolics profile of six strawberry fruit cultivars. The experiment was carried out on a commercial plantation where basic fertilization and protection of plants followed the recommendations for the species. The chemical composition of strawberries, as a response to titanium treatment, was differential and cultivar-dependent. A significant increase of total polyphenol content was noted only in 'Elkat' berries. The treatment resulted in the increase of L-ascorbic acid in all the cultivars, except for 'Kent'. However, the influence of Ti on the antioxidant activity of strawberries against ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6sulfonic acid) and DPPH (2-diphenyl-1-picrylhydrazyl) radical was differential. Only in treated 'Elsanta' fruit a significant enhancement of scavenging both radicals was observed. Moreover, the treatment caused a significant increase of total anthocyanin content in 'Kent', 'Selva' and 'Senga' fruit, and a significant decrease of these compounds in 'Dukat' berries. Regarding total proanthocyanidins, the only significant change was a decline observed in treated 'Elsanta' berries. The applied treatment exerted no considerable effect on ellagic acid and p-coumaric acid content in any of cultivars tested.

Key words: *Fragaria* × *ananassa*, cultivar, titanium, antioxidant activity, polyphenols

INTRODUCTION

Strawberries (*Fragaria* × *ananassa Duch.)* like other fruits are the source of beneficial compounds accounting for nutrition and health maintenance of humans which has been widely reviewed by others [Meyers et al. 2003, Hannum 2004]. Chemical composition and biological activity of strawberries varies depending on cultivar (genetic fac-

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Corresponding author – Adres do korespondencji: Dr inż. Katarzyna Skupień, Laboratory of Processing and Storage of Plant Materials of Agricultural University of Szczecin, Słowackiego 17, 71-434 Szczecin, Poland, e-mail: kskupien@agro.ar.szczecin.pl tors), climate, and weather conditions, as well as agronomic practices involved. The usage of titanium [Laszlovszky-Zmarlicka and Żurawicz 2003] apart from phytohormones [Paroussi et al. 2002, Żmuda et al. 2001, Masny et al. 2004] and algae extracts [Masny et al. 2004] is one of the agronomic practices used to stimulate fruition of strawberry plants and improve the quality of fruits. Many investigators [reviewed by Dumon and Ernst 1988] demonstrated the promotion of growth by titanium, whether applied as a fertilizer to the soil, or as a spray to the leaves. Pais [1983] carried out numerous field experiments and found that titanium-chelate-treated apple trees yielded the fruits with higher soluble solids, sugar and acid content. The author observed that Ti-treated grapes showed higher sugar content and lower acid content compared to the control. The saplings of Ti-treated sour cherry and apple showed higher first class yields and lower faulty product. Similarly, gooseberries, black currents, peaches and apricots displayed higher yield under Ti-treatment. Reverte et al. [2000] found the application of Ti^{4+}-ascorbate significantly improved red paprika yield and fruit quality as well as photostability of ground peppers during storage. The positive effects of Ti-treatment were found on rape plant development (an increase of chlorophyll content and photosynthesis intensity), the yield and mass of a thousand seeds of winter wheat, and the yield and sugar content in sugar beets [Grenda 2003]. Beneficial influence of Ti^{4+}-ascorbate spraying in combination with Ca^{2+} and Mg^{2+} was observed on plum trees [Alcaraz--Lopez et al. 2003]; and peach tree performance and fruit size [Alcaraz-Lopez et al. 2004 a]. At harvest, fruits from the Ti-treated trees showed improved resistance to compression and penetration, and a decrease in weight-loss during post-harvest storage. The authors obtained similar results both for plum tree development and fruit quality when Ca^{2+} spraying was combined with Ti^{4+}-ascorbate and/or marine algae extract [Alcaraz--Lopez et al. 2004 b]. Serrano et al. [2004] found that peach and nectarine trees sprayed with a formulation containing Ca^{2+}, Mg^{2+} and Ti^{4+} produced fruits with higher weight and pulp firmness. Whereas, no effect was observed for either colour, total soluble solids, titratable acidity or the time required to ripen on the tree.

Whereas, in the literature there is evidence on the effect of titanium on fruit quality of different plant genus; to our knowledge, there is no information on the possible influence of titanium on antioxidants and antioxidant status of strawberries.

The objective of the study was to estimate the influence of titanium treatment on antioxidant activity (scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH radical) and 2,2'-azinobis-(3-ethylbenzothiazoline-6sulfonic acid) (ABTS) radical) and antioxidants content (vitamin C and polyphenols) of six strawberry cultivars ('Dukat', 'Elkat', 'Elsanta', 'Kent', 'Selva' and 'Senga Sengana').

MATERIALS AND METHODS

The six cultivars of strawberries: 'Dukat', 'Elkat', 'Elsanta', 'Kent', 'Selva' and 'Senga Sengana' ('Senga') were grown on a plantation near Szczecin (West Pomerania, Poland). The plantation was established on medium clay of pH 6.9-7.1. The field experiment was carried out in the year 2004. In autumn of 2002, organic manure was applied, and in autumn of 2003, mineral fertilization (Hydrokompleks) was used. The program of disease control was performed according to recommendations for the species [Żurawicz 2001]. The plot of 0.25-0.50 ha for each cultivar, was sprayed with Tytanit (0.8% of Ti^{4+}-ascorbate content) and with an addition of fine quality adjuvant Atpolan 80EC (paraffin oil (11-13) 76%). The sprayings were performed on three occasions, every 7 days starting with inflorescence emergence. The Tytanit solution concentration was 0.02%. Within the conditions of commercial plantation, control plants were cultivated in a similar area for each cultivar and were not sprayed with titanium but received both organic and mineral fertilization exactly at the same time and doses as Ti-treated ones. The fruits of the highest class were harvested at the optimum stage of ripeness (color and shape characteristic for the cultivar and diameter $\geq$ 25 mm according to the Polish standard PN-R-75535: UN/ECE FFV-35) at the beginning of July. The 2 kg bulk samples of treated and non-treated berries for each cultivar were collected mainly from third- and fourth-year bushes.

The total polyphenol, proanthocyanidin content, and antioxidant activity against DPPH radical were determined in strawberries packed in poliethylene bags (ca 300 g) and stored at –20°C for 5-6 days. The content of L-ascorbic acid, phenolics profiles and antioxidant activity against ABTS radical were performed on cooled strawberries (kept for 2-3 days at 4-6°C).

The fertilizers used in the field experiment were of Polish production. The 'Tytanit' was purchased from Intermag and the adjuvant 'Atpolan 80EC' was obtained from Agromix.

The chemicals used for titratable acidity and total polyphenol determination were of analytical grade and purchased from POCh (Gliwice, Poland). The DPPH radical (2,2--diphenyl-1-picrylhydrazyl), ABTS radical (2,2'-azinobis-(3-ethylbenzothiazoline-6--sulfonic acid)), Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) and potassium persulfate were obtained from Sigma-Aldrich (USA). HPLC chemicals were of HPLC purity and obtained from Sigma-Aldrich (USA).

The total polyphenol content in methanol extracts was estimated according to Singleton and Rossi [1965] with the Folin-Ciocalteu reagent. The data is expressed as mg of gallic acid equivalents (GAE) per 100 g of fruit tissue. The effect of strawberry fruit extract on DPPH radical was determined according to the method of Yen and Chen [1995]. A 10 g of strawberry sample was homogenized in 200 mL of distilled water. The slurry was filtered and 5 mL of filtrate was diluted in 25 mL of distilled water. A 1 mL aliquot of strawberry extract was added to 3 mL of methanol ($0.79\ kg \cdot L^{-1}$) and 1 mL of $DPPH^{\cdot}$ ($0.012\ g\ DPPH^{\cdot}\ 100\ mL^{-1}$ of methanol). The mixture was shaken and left at room temperature for 10 min; the absorbance was measured spectrophotometrically at 517 nm. The percent inhibition of DPPH radical was evaluated according to the formula: Percent inhibition = $[100 - (A_t/A_r) \times 100]$ where A_t and A_r were the absorbances of test and reference solutions, respectively [Rossi et al. 2003]. The effect of strawberry extract on ABTS radical was measured according to Re et al. [1999]. The $ABTS^{\cdot}$ stock solution (7 mM concentration) and 2.45 mM potassium persulfate were left at room temperature for 16 h to produce ABTS radical cation ($ABTS^{\cdot +}$). A 5 g of fruit sample was homogenized in 50 mL of methanol ($0.79\ kg\ L^{-1}$) and filtered. 1 mL of filtrate was diluted 10 times in methanol. ABTS radical solution was diluted with distilled water to an absorbance of 0.70 (±0.02) at 734 nm. A 5 mL of $ABTS^{\cdot +}$ solution was added to 0.5 mL of strawberry extract; the absorbance was monitored at 734 nm after 6 min. A concentration-response curve for the absorbance at 734 nm after 6 min for ABTS radical as a function of different Trolox concentrations was prepared. The decrease in absorption at 734 nm, 6 min after addition of the compound was used for calculating the TEAC (Trolox equivalent antioxidant capacity). The TEAC of fruit extract represents the concentration of a Trolox solution that has the same antioxidant capacity as the fruit extract [Berg et al. 1999]. The HPLC apparatus (Waters Associates, Milford, USA) consisting of the Hitachi L-7455 diode array detector and quaternary pump L-7100 equipped with a D-7000 HSM Multisolvent Delivery System (Merck--Hitachi) was employed. Cooled fruits were homogenized with DIAX 900 (Heidolph Germany) homogenizer. The L-ascorbic acid was estimated using a LiChroCART® 125-3 Purospher® RP-18 column (5 µm; Merck). The elution was carried out using 0.1M phosphoric acid, the flow rate was 1 mL min^{-1}. The absorbance was monitored at 254 nm. The L-ascorbic acid was identified by comparison with the standard. The calibration curve was prepared by plotting different concentrations of standard versus area measurements in HPLC. To estimate phenolics profile for each cultivar, 5 g sample of berry purée was sonicated with acidified methanol (1 mL HCl in 1 L of methanol) for 15 min and then extracted with acidified methanol, and gradually filtered through the Schott inlet (G2). The methanol extracts were combined and made up to 50 mL. The extracts were centrifuged at 8000 g for 5 min and analyzed with the HPLC system. Furthermore, the fruit samples were hydrolyzed. A 1 g of homogenate and 2 mL 2 N HCl were placed in a vial, sealed with teflon plug and hydrolyzed at 100°C for 60 min. After cooling, 5 mL of methanol and 2 mL of 2 N NaOH were added. Mixtures were centrifuged at 8000 g, and 20 µL of supernatant was injected into HPLC system. The separation was performed on LiChro CART 125-3 Purospher RP-18 (5 µm) MerckLabs column. The mobile phase consisted of solvent A (4.5% formic acid) and solvent B (80% acetonitril and 20% of solvent A). The program began with isocratic elution with 95% A and 5% B (0-1 min). Then a linear gradient was used until 16 min lowering A to 20% (increasing B to 80%); from 17 min to 24 min 100% B; and finally from 25 min to 35 min 100% A. The flow rate was 1 mL min^{-1}. The runs were monitored at the following wavelengths: catechins and p-coumaric acid at 280 nm, ellagic acid at 360 nm and anthocyanins at 520 nm. Scanning was performed between 200 and 600 nm. The retention times and spectra were compared to those of pure standards within 200 and 600 nm. The analyses of proanthocyanidins were carried out by direct thiolysis of freeze-dried strawberry powder prior to HPLC analysis as described by Guyot et al. [2001]. The all chemical analyses were carried out in duplicate.

Statistical analysis was done by means of Statistica software package version 7.1 (Statsoft, Poland). The Student's test for comparison the pairs of the means was used. The differences were tested at significance level $p = 0.05$.

RESULTS

The chemical composition of strawberries as a response to titanium treatment was differential and cultivar-dependent. Regarding total polyphenol content, only treated 'Elkat' berries showed significantly higher amount of phenolics (297.6 mg gallic acid·100 g^{-1}) compared to non-treated fruit (266.8 mg gallic acid·100 g^{-1}; Table 1). Neither increased values observed for treated 'Dukat' and 'Senga' berries, nor decreased amounts noted for 'Elsanta', 'Kent' and 'Selva' fruit were statistically significant.

All of the cultivars, except for 'Kent', displayed higher ascorbic acid content under Ti-treatment (Table 1). Significantly higher amount of ascorbic acid was found for treated, 'Dukat', 'Elkat' and 'Selva' berries whereas significantly less for 'Kent' (33.8 mg·100 g^{-1} for treated fruit compared to 58.6 mg·100 g^{-1} for non-treated berries).

Table 1. Total polyphenol, ascorbic acid content, and antioxidant activity of Ti-treated (a) and non-treated (b) strawberry fruit

Tabela 1. Zawartość polifenoli ogółem, kwasu askorbinowego i aktywność przeciwutleniająca owoców truskawki nawożonych Ti (a) i nienawożonych (b)

Characteristics Cechy	Cultivar – Odmiana											
	Dukat		Elkat		Elsanta		Kent		Selva		Senga	
	a	b	a	b	a	b	a	b	a	b	a	b
Total polyphenol mg gallic acid $100\ g^{-1}$ Polifenole ogółem mg kwasu galusowego $100\ g^{-1}$	316.6 ±2.3	306.5 ±3.1	297.6 ±0.9	266.8 ±0.9	368.5 ±1.3	377.7 ±	284.4 ±1.0	324.0 ±2.4	220.2 ±1.7	278.2 ±2.6	290.8 ±1.7	283.2 ±2.1
P	*0.065*		*0.001**		*0.051*		*0.022**		*0.001**		*0.058*	
L-ascorbic acid mg $100\ g^{-1}$ Kwas askorbinowy mg $100\ g^{-1}$	49.5 ±0.9	40.2 ±0,3	64.5 ±0,6	55.9 ±1,3	64.0 ±2,8	59.7 ±1,4	33.8 ±0,9	58.6 ±0,6	52.6 ±1,3	46.9 ±1,3	47.7 ±0,4	45.3 ±0,4
P	*0.005**		*0.013**		*0.194*		*0.001**		*0.046**		*0.030*	
Antioxidant activity against ABTS radical mg TEAC $100\ mL^{-1}$ Aktywność przeciwutleniająca wobec rodnika ABTS mg TEAC $100\ mL^{-1}$	3.79 ±0.01	3.47 ±0.14	4.34 ±0.14	2.70 ±0.08	5.09 ±0.13	4.51 ±0.07	3.36 ±0.10	4.15 ±0.21	3.63 ±0.10	3.45 ±0.35	4.13 ±0.24	2.61 ±0.07
P	*0.086*		*0.005**		*0.030**		*0.041**		*0.560*		*0.013**	
Antioxidant activity against DPPH radical percent inhibition Aktywność przeciwutleniająca wobec rodnika DPPH procent inhibicji	63.0 ±1.1	69.8 ±1.0	64.9 ±1.3	68.8 ±1.3	51.8 ±1.1	36.5 ±3.7	67.4 ±0.8	58.1 ±2.7	62.0 ±1.0	64.4 ±1.1	64.9 ±1.6	71.9 ±1.3
P	*0.024**		*0.092*		*0.030**		*0.043**		*0.152*		*0.039**	

Values are the mean of two determinations ±SD and are expressed per fresh weight.
P indicates the level of significance. The values < 0.05 are considered significant (*).
Wyniki są średnią z dwóch powtórzeń ± odchylenie standardowe i odnoszą się do świeżej masy.
P oznacza poziom istotności. Wartości $< 0{,}05$ są statystycznie istotne (*).

The favourable influence of titanium on vitamin C content in 5 out of 6 cultivars tested was concurrent with higher ABTS radical scavenging activity found in these cultivars (Table 1). The most positive effect on ABTS radical antioxidant activity was observed for ‘Elkat’, ‘Senga’ and ‘Elsanta’ berries (a significant increase by 60, 58 and 13%, respectively). For Ti-treated ‘Kent’ fruits significantly lower ascorbic acid content was followed by significantly weaker ABTS radical scavenging ability (lowering by 19% compared to the control).

For majority of cultivars the activity of fruit extracts against DPPH radical was not parallel to that of ABTS radical (Table 1). Ti-treatment stimulated antioxidant activity against both radicals only in ‘Elsanta’ berries. The other cultivars displayed diverse abilities of scavenging DPPH radicals. The treated berries of ‘Elsanta’ and ‘Kent’ showed a significant increase of DPPH radical percent inhibition (by 42 and 16%, respectively), while other cultivars a decline (by 4-10%). Significant lowering was observed in 'Dukat' and 'Senga' berries.

However, the most differential response to titanium treatment was reflected in phenolics profiles of the strawberries. The alterations were unequivocal neither for individual compounds, nor for the cultivars (Table 2). (+)-catechin content was significantly enhanced in treated fruit of 'Elsanta' and 'Selva' (respectively 2.3-fold and 1.3-fold more than control fruit). On the other hand, 'Elkat', 'Kent' and 'Senga' cvs showed significantly less amount of (+)-catechin under Ti-treatment.

Table 2. Phenolics content in Ti-treated (a) and non-treated (b) strawberry fruit
Tabela 2. Zawartość polifenoli w owocach truskawki nawożonych Ti (a) i nienawożonych (b)

Phenolic compounds mg 100 g^{-1} fresh weight Związki polifenolowe mg 100 g^{-1} świeżej masy	Cultivar – Odmiana											
	Dukat		Elkat		Elsanta		Kent		Selva		Senga	
	a	b	a	b	a	b	a	b	a	b	a	b
(+)-catechin (+)-katechina	10.57 ±0.10	10.73 ±0.00	8.82 ±0.27	17.42 ±0.59	3.15 ±0.18	1.37 ±0.07	3.10 ±0.21	9.87 ±0.24	10.28 ±0.01	8.02 ±0.11	9.44 ±0.44	12.17 ±0.69
P	*0.150*		*0.003**		*0.006**		*0.001**		*0.001**		*0.042**	
Anthocyanins – Antocyjany												
Cyanidin-3-glucoside Cyjanidyno-3-glukozyd	1.40 ±0.17	1.15 ±0.08	0.62 ±0.01	1.38 ±0.10	0.84 ±0.13	0.78 ±0.08	2.28 ±0.33	1.37 ±0.06	1.66 ±0.08	1.65 ±0.13	1.67 ±0.27	1.32 ±0.25
P	*0.203*		*0.009**		*0.635*		*0.060*		*0.935*		*0.313*	
Pelargonidin-3-glucoside Pelaragonidyno-3-glukozyd	47.68 ±3.47	52.05 ±3.06	22.58 ±2.45	28.46 ±0.76	22.63 ±3.36	21.15 ±2.47	50.67 ±2.45	33.22 ±1.47	45.03 ±1.65	32.24 ±2.50	45.14 ±1.78	37.94 ±1.64
P	*0.033**		*0.083*		*0.666*		*0.013**		*0.026**		*0.052*	
Total – Ogółem	49.08 ±3.64	53.20 ±3.14	23.20 ±2.46	29.84 ±0.86	23.47 ±3.49	21.93 ±2.56	52.95 ±2.77	34.59 ±1.53	46.69 ±1.57	33.89 ±2.63	46.81 ±1.51	39.26 ±1.89
P	*0.038**		*0.069*		*0.665*		*0.015**		*0.027**		*0.048**	
Proanthocyanidins after thiolysis Proantocyjanidyny po tiolizie	73.26 ±2.76	65.36 ±2.90	81.69 ±3.69	75.27 ±1.17	74.07 ±3.78	122.53 ±5.6	53.59 ±3.71	66.75 ±2.21	60.60 ±2.32	67.65 ±2.09	64.50 ±2.12	62.26 ±1.00
P	*0.108*		*0.144*		*0.010**		*0.050*		*0.086*		*0.310*	
Phenolic acids after hydrolysis – Kwasy fenolowe po hydrolizie												
Ellagic acid Kwas elagowy	10.95 ±2.09	12.26 ±2.32	8.32 ±0.92	11.97 ±1.54	6.44 ±0.79	9.56 ±0.79	11.94 ±2.79	10.89 ±1.22	10.56 ±2.19	10.83 ±2.11	10.61 ±1.97	11.64 ±2.11
P	*0.613*		*0.103*		*0.060*		*0.674*		*0.912*		*0.663*	
p-coumaric acid Kwas p-kumarowy	33.67 ±3.65	36.04 ±2.81	39.83 ±4.07	32.64 ±2.32	35.95 ±0.27	32.23 ±1.74	33.70 ±3.42	32.19 ±2.69	32.89 ±3.00	34.34 ±0.52	38.96 ±1.70	42.03 ±2.96
P	*0.263*		*0.162*		*0.096*		*0.672*		*0.570*		*0.331*	

Values are the mean of two determinations ±SD.
P indicates the level of significance. The values < 0.05 are considered significant (*).
Wyniki są średnią z dwóch powtórzeń ± odchylenie standardowe.
P oznacza poziom istotności. Wartości < 0,05 są statystycznie istotne (*).

The usage of titanium affected mainly pelargonidin-3-glucoside (and thus total anthocyanin content) over cyanidin-3-glucoside content (Table 2). The positive effect of Ti on pelargonidin-3-glucoside was found in 'Kent' and 'Selva' berries (a significant increase by 17.5 and 12.8 mg·100 g^{-1}) whereas, a significant decrease was observed in 'Dukat' cv. (by 4.4 mg·100 g^{-1}, respectively). On the other hand, only treated 'Elkat' berries showed a significant drop of cyanidin-3-glucoside content. The treatment enhanced significantly total anthocyanin content in 'Kent', 'Selva' and 'Senga' berries and reduced considerably in 'Dukat' cv. For majority of the cultivars tested, Ti-treatment exerted no considerable effect on proanthocyanidins content (Table 2). The only significant response was found for 'Elsanta' berries (a decrease by 40%).

Regarding phenolic acids content, the usage of titanium caused no statistically significant alterations (Table 2). The treated berries of 'Kent' cv. had slightly higher ellagic acid content, while, other cultivars showed the decrease of ellagic acid under the treatment (Table 2). The greatest lowering was found in 'Elkat' and 'Elsanta' berries (by 3.7 and 3.1 mg·100 g^{-1}, respectively). The Ti-treatment caused an increase of p-coumaric acid content especially in 'Elkat' berries (by 7.2 mg·100 g^{-1}) whereas, lowering of the compound was observed in 'Senga', 'Dukat' and 'Selva' fruit (by 3.1, 2.4 and 1.5 mg·100 g^{-1}).

DISCUSSION

In our study, the content of ascorbic acid ranged from 33.8 mg·100 g^{-1}('Kent' treated fruit) to 64.5 mg·100 g^{-1} ('Elkat' treated fruit). The values were similar to the results obtained by Hakala et al. [2003] 32.4-66.9 mg·100 g^{-1} ('Senga' and 'Bounty' cvs, respectively) and lower than reported by Cordenunsi et al. [2005] 47-80 mg·100 g^{-1} (for 'Oso Grande' and 'Campineiro' cvs). In present research, except for 'Kent' cv. (a significant 42% decrease), the treated fruit of other tested cultivars showed higher vitamin C content by 23% ('Dukat') to 5% ('Senga'). Similarly, the stimulating effect of titanium on vitamin C content in parsley (an increase by 15% under 5 ppm of Ti spraying) and paprika (an increment by 114 and 369% under 1ppm and 10 ppm of Ti, respectively) was noted by Pais [1983].

To our knowledge, in the literature there is a lack of data referring to influence of Ti-usage on phenolic compounds and antioxidant activity in treated plants. However, in the literature there is much evidence on the influence of environmental and climatic conditions as well as agronomic practices on chemical composition of strawberries [Hoppula and Karhu 2006, Anttonen et al. 2006]. On the other hand, recent studies have shown that the phytochemicals, especially phenolics are the major bioactive compounds with human health benefits [Vinson et al. 2001]. The consumption of fruits and vegetables is associated with the prevention of chronic diseases such as heart disease, cancer, diabetes, and Alzheimer's disease [Sun et al. 2002]. In this study, total polyphenol content (GAE) determined for control berries varied from 266.8 ('Elkat') to 377.7 mg·100 g^{-1} ('Elsanta') while, for treated fruit 220.2-368.5 mg·100 g^{-1}. Generally, the values were lower compared to the scope estimated for the same cultivars (non-treated) in the year 2003 317.2 ('Senga') – 443.4 mg·100 g^{-1} ('Kent') [Skupień and Oszmiański 2004]. It may be due to different weather conditions in the two seasons. The adverse reaction to Ti-treatment was found for 'Kent' and 'Selva' berries (21 and 12% significant decrease, respectively). On the contrary, the treatment significantly enhanced total polyphenol content in 'Elkat' berries (by 12%). The influence of Ti-spraying on strawberry antioxidant status was not unequivocal. The clear response was found for 'Elkat' cv. In these berries, a significant increase of total polyphenol and L-ascorbic acid content was concurrent with the significant enhancement of antioxidant capacity towards ABTS radical however, not towards DPPH radical. Except for 'Elsanta', other cultivars' antiradical activity against ABTS˙ was not parallel to DPPH˙ percent inhibition.

As evidenced in the literature, anthocyanins, catechins, proanthocyanidins, and phenolic acids have significant antioxidative, antiviral, antimutagenic and anticancer activity [Maas et al. 1991, Bruyne et al. 1999, Häkkinen and Törrönen 2000, Tomás-Barberán and Espin 2001, Kong et al. 2003, Nakajima et al. 2004, Yilmaz and Toledo 2004]. Moreover, the amount of anthocyanins is important for the attractiveness and maturity assessment of the strawberries [Cordenunsi et al. 2005]. In our study, the sum of pelargonidin and cyanidin, two basic strawberry pigments, ranged in non-treated fruit from 21.93 to 53.20 mg·100 g^{-1}. In treated fruit, the scope was similar 23.20-52.95 mg·100 g^{-1}. Wang and Zheng [2001] found that the total of pelargonidin-3-glucoside and cyanidin-3-glucoside was 309.8 $\mu g \cdot g^{-1}$ in 'Earliglow' and 391.7 $\mu g \cdot g^{-1}$ for 'Kent' cvs, when grown in cool day and night temperatures (18/12°C). Whereas, the temperature cycle 30/22°C enhanced the pigment content to 782.7 and 990.9 $\mu g \cdot g^{-1}$ in 'Earliglow' and 'Kent', respectively. In present research cultivar-dependent responses were noted. The positive effect of Ti-treatment on total anthocyanin content was found for 'Senga' (a significant increase by 19%), 'Selva' (by 38%) and 'Kent' cv. (by 53%); whereas, in 'Dukat' berries a significant decrease was observed (by 8%). Regarding measured anthocyanins, treated 'Elkat' fruit showed a significant decrease of cyanidin-3-glucoside content; while, for 'Kent' and 'Selva' the significant increase of pelargonidin-3-glucoside was observed.

The divergence of (+)-catechin content in control berries was wider (1.37-17.42 mg·100 g^{-1}) than in treated fruit (3.10-10.50 mg·100 g^{-1}). A more narrow variation was estimated for these cultivars in 2003 [Skupień and Oszmiański 2004] 6.10-8.74 mg·100 g^{-1}. The most positive response to the treatment was noticed for 'Elsanta' (an increase by 130%) and 'Selva' berries (28% increase). On the other hand, 'Kent', 'Elkat' and 'Senga' berries showed a significant lowering of (+)-catechin by 69, 49 and 22%, respectively.

The proanthocyanidin content in control berries ranked from 62.26 to 122.53 mg·100 g^{-1} ('Senga' and 'Elsanta', respectively); while, in treated ones from 53.59 to 81.69 mg·100 g^{-1} ('Kent' and 'Elkat', respectively). The data confirms that strawberries are an appreciable source of these compounds. Under Ti-treatment, a slight enhancement of proanthocyanidins amount occurred in 'Dukat', 'Elkat' and 'Senga' berries by 12, 9 and 4%, respectively. However, the only significant response was a 40% drop observed in 'Elsanta' fruit. According to Cheng and Breen [1991], tannin content per individual berry increases along with strawberry development. Foo and Porter [1980] found that condensed tannins account for 63% of the total tannins (condensed and hydrolyzable) isolated from a ripe strawberry.

Regarding our findings, higher amount of ellagic acid was determined in the control fruit (9.56-12.26 mg·100 g^{-1}) than in the treated ones (6.44-11.94 mg·100 g^{-1}). Only Ti-treated 'Kent' berries, showed an increase of ellagic acid content by 10%. Häkkinen and Törrönen [2000] report ellagic acid content in strawberries 34.4-58.6 mg·100 g^{-1}, prior to hydrolysis, and Williner et al. [2003] 28.5-68.4 $mg \cdot kg^{-1}$, prior to hydrolysis.

Whereas, Cordenunsi et al. [2005] 2.4-2.5 mg·100 g^{-1}, without hydrolysis. Comparing our data, obtained prior to hydrolysis, along with the above results, it can be concluded that sample extraction and hydrolysis procedure, apart from genetic and environmental factors, account for the differences observed between the studies.

The amount of p-coumaric acid in control fruit (32.19-42.03 mg·100 g^{-1}) was very close to the values observed for treated berries (32.89-39.83 mg·100 g^{-1}). Häkkinen and Törrönen [2000] obtained a much lower p-coumaric acid level in strawberries 0.7--4.1 mg·100 g^{-1}. In our research, a positive response to Ti-treatment was found for 'Elkat', 'Elsanta' and 'Kent' (the increase by 22, 12 and 5%, respectively).

CONCLUSIONS

Titanium treatment caused disparate and multi-directional alterations in chemical composition of strawberries.

1. The most positive effect of the treatment was observed for 'Selva' fruit which manifested in a significant increase of L-ascorbic acid, pelargonidin-3-glucoside, total anthocyanin, and (+)-catechin.
2. The significant increase of total polyphenol was found only in treated 'Elkat' fruit; whereas, a significant decrease of proanthocyanidins was noted in treated 'Elsanta' berries and cyanidin-3-glucoside content in treated 'Elkat' fruit.
3. The applied treatment enhanced total anthocyanin content in majority of cultivars; however, a significant lowering was determined in treated 'Dukat' berries.
4. Ti-treatment did not affect significantly the content of ellagic acid and p-coumaric acid.
5. The influence of titanium on antioxidant status of strawberries was not unequivocal. The increased activity against ABTS$^{\cdot}$ was found in the treated fruit of 'Elkat', 'Elsanta' and 'Senga' cvs; whereas, against DPPH$^{\cdot}$ in the treated berries of 'Elsanta' and 'Kent'. Apart from 'Elsanta', for other cultivars antioxidant activity against DPPH radical was not parallel to that of ABTS radical.
6. The results indicate that the response of strawberries to titanium treatment is cultivar-dependent and this study may contribute to elucidation of the problem.

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WPŁYW STOSOWANIA TYTANU NA ZAWARTOŚĆ ANTYOKSYDANTÓW I AKTYWNOŚĆ PRZECIWUTLENIAJĄCĄ TRUSKAWEK

Streszczenie. Stosowanie tytanu w formie oprysku jest jednym z zabiegów agrotechnicznych mających na celu stymulowanie kwitnienia, zawiązywania owoców i produktywności roślin truskawki. Celem badań była ocena wpływu tytanu (w formie askorbinianu Ti^{4+})