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BIOACTIVE COMPOUNDS AND ANTIOXIDANT CHARACTERISTICS OF VARIOUS TOMATO CULTIVARS FROM SERBIA – CHEMOMETRIC APPROACH

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ABSTRACT. Tomato is one of the most consumed foodstuffs around the world and major dietary source of lycopene. The main objectives of this study were evaluation of antioxidant activity, using DPPH, ABTS, FRAP, TRP and CUPRAC assays, as well as total phenolic, total flavonoid, lycopene and β-carotene content in 30 tomato and cherry tomato cultivars commonly consumed in Serbia. Tomato with the highest total phenol and total flavonoid content was tomato Indigo Rose (17.56 mg GAE g^{-1} DW and 30.30 mg RE g^{-1} DW, respectively), which showed excellent antioxidant characteristics. Total lycopene content was lower in yellow tomato species compared to the red ones, and the highest lycopene content was 0.283 mg g^{-1} DW for tomato Red Pearl Big. Cluster analysis yields dendrogram, separating tomato and cherry tomato cultivars into three statistically significant clusters ($(D_{link} / D_{max})\times100 < 50$).

Keywords: *Tomato, Antioxidant characteristics, Bioactive compounds, PCA, Cluster analysis*

INTRODUCTION

Tomato (*Solanum lycopersicum*) belongs to the family Solanaceae, which includes edible plants such as potatoes, capsicums, and eggplants, but also potentially poisonous plants (jimsonweed and mandrake). All species of this family have toxic alkaloids present in either their leaves or their fruits [1].

Fresh tomatoes are produced worldwide, approximately 180 million tons per year, and it is the third most-produced vegetable. Four times more rice and two times more potatoes are grown around the world [2]. In Serbia,

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tomatoes are grown on about 20000 ha, with the production of 180000 tons of fruit. Tomato is one of the most valuable products since it's consumed fresh as well as processed products. The importance of this vegetable is indicated by the fact that its consumption is recorded in 164 countries. The average tomato consumption in the world is 20.5 kg per capita, and in our country, it is 16 kg per capita.

Tomato is a source of substances with known health benefits, such as vitamins, minerals, and antioxidants [3]. Tomato consumption decreases the risk of certain types of cancer [4] and cardiovascular diseases [5]. Also, beneficial phytochemicals positively affect immune response, atherosclerosis protection and DNA damage. The health benefit of tomato is attributed mainly to carotenoids present in tomato fruits. The primary carotenoid present in tomato is lycopene [6]. It is naturally occurring carotenoid pigment present in tomato, watermelon, grapefruit, guava, and other fruits, giving fruits red color. It's a primary carotenoid in tomato, accounting for more than 80% of total carotenoids in this fruit [7]. The effects of lycopene on various diseases were studied in the past decades, and a positive impact on chronic diseases like cancer and cardiovascular disease was noticed [8]. Lycopene is also proven antioxidant, but the positive health effect could be achieved through different mechanisms that include modulation of intercellular gap junction communication, hormonal and immune systems, and metabolic pathways [8]. A close link between tomato intake and low cancer risk was also established [9]. Besides lycopene, tomatoes also contain α-, β-, γ-, δ-carotene, zeaxanthin and lutein and also neurosporene, phytoene, and phytofluene [10,11]. Carotene and lycopene content is fortified by cooking. Other compounds present in tomatoes are vitamin C, vitamin E, various phenolics, glycoalkaloids (tomatine) and flavonoids [12]. Micro and macro elements are also present, some in quantities higher compared to other products usually consumed.

Having in mind the lack of comprehensive knowledge about the local vegetable varieties, the present research was aimed to evaluate the total phenolic content (TPC), total flavonoid content (TFC), β-carotene content (TCC), lycopene content (TLC) and antioxidant capacities of 30 tomato and cherry tomato varieties consumed in Serbia. As far as we know, this is the first time that some of the varieties were analyzed. Antioxidant activity of tomatoes was determined by applying DPPH, ABTS, TRP, FRAP, and CUPRAC assays. Multivariate statistical analysis was used to achieve the relationship between analyzed tomato species antioxidant properties and various tests used. Chemometric techniques are used to simplify data set to fewer variables, without losing information. Principal component analysis (PCA) reduces multivariate data preserving most of the variance at the same time while cluster analysis (CA) divides the observations into homogeneous and distinct groups.

RESULTS AND DISCUSSION

Phenolic compounds are a widespread group of plant metabolites. They are vital both for the organoleptic properties of foods and for its positive health effects. The most critical outcomes of phenolic compounds are: antioxidant, antibacterial, and antiviral. Other class of natural products commonly analyzed in plant species are flavonoids. They are classified as low-molecular-weight phenolic compounds that are widely distributed in the plant kingdom [13]. Total phenolic and flavonoid content of 30 tomato and cherry tomato varieties were analyzed in this study, and the results were presented in Figures 1 and 2.

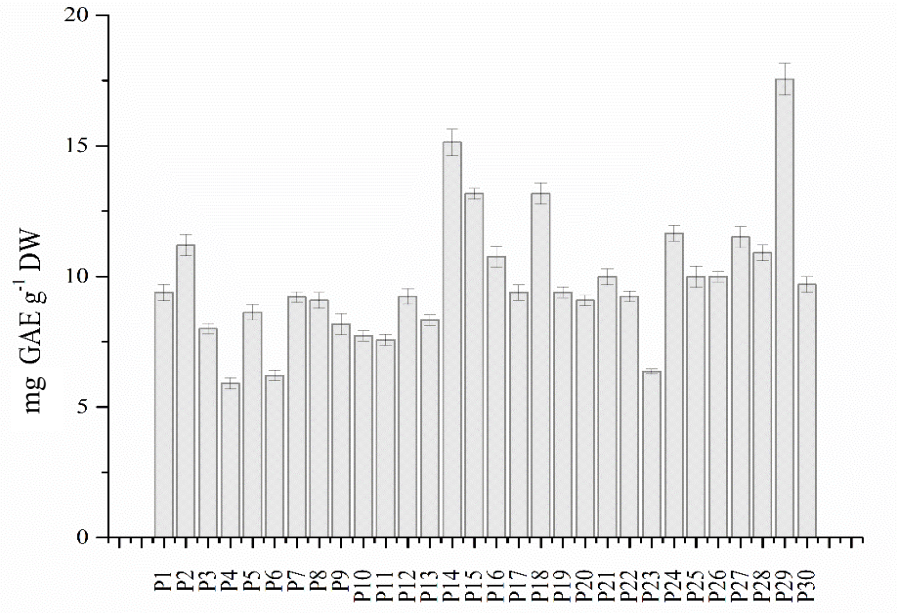

Figure 1. Total phenolic content in analyzed tomato and cherry tomato species

The highest total phenolic content was recorded for sample P29 – Indigo Rose tomato (17.6 mg GAE g^{-1} DW) - tomato cultivar first cultivated the 1960s, crossing tomatoes with wild species from Chile and the Galapagos Islands. Qi [14] analyzed 29 tomato species, and TPC varied significantly from 3.05 to 7.12 mg GAE/g DW. Kahkonen et al. [15] reported that the total phenolic content of tomatoes is up to 2 mg of gallic acid equivalent per 1 g DW, which is slightly lower than our results. The level of phenol compounds in tomatoes can be influenced by various features, including genotype, availability of nitrogen in the root zone, biotic, and abiotic stress-related events [16].

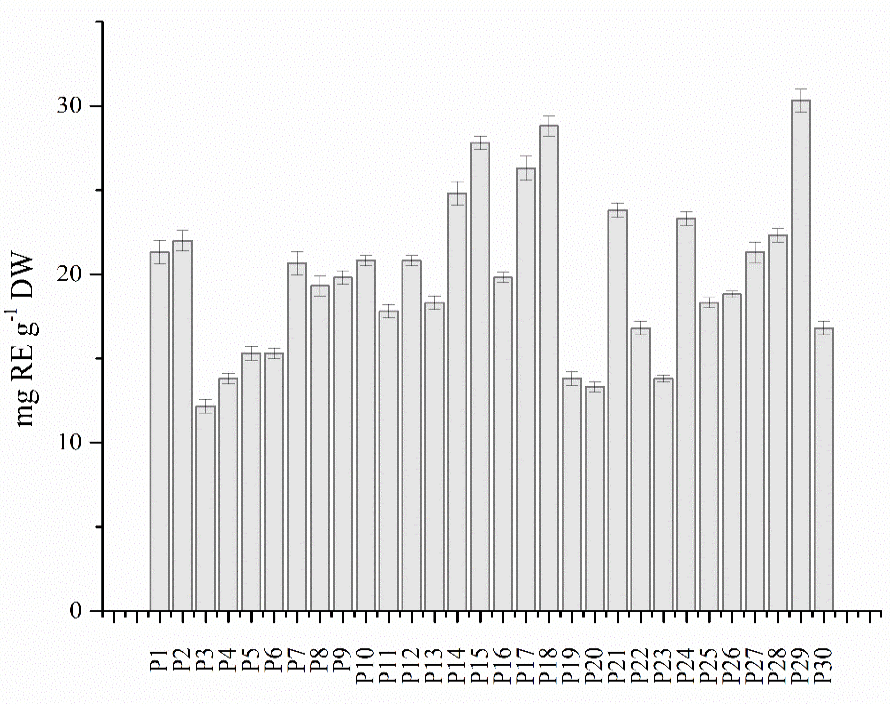

Figure 2. Total flavonoid content in analyzed tomato and cherry tomato species

Barros et al. [17] analyzed phenolic compounds in tomato varieties consumed in Portugal. Cis p-coumaric acid derivative was the most represented compound in yellow and round tomato varieties, while 4-O-caffeoylquinic acid was the most represented in long and heart varieties. The most abundant flavonoid was quercetin pentosylrutinoside in these tomato varieties. According to our study, Indigo Rose tomato also possesses the highest flavonoid content among other analyzed species. The total flavonoid content of Indigo Rose tomato was 30.3 mg RE g^{-1} DW. This tomato is rich in anthocyanins, a class of flavonoids, including delphinidin, petunidin, and malvidin [18].

Tomato contains carotenoids – a group of tetraterpenes fat-soluble pigments. They include β-carotene, β-cryptoxanthin, lutein, and lycopene. Lycopene, one of the most potent antioxidants among dietary carotenoids, is a highly unsaturated hydrocarbon containing 11 conjugated and two unconjugated double bonds, and it is an acyclic isomer of β-carotene. Lycopene and β-carotene content were determined using a spectrophotometer, and the results were presented in Figures 3 and 4.

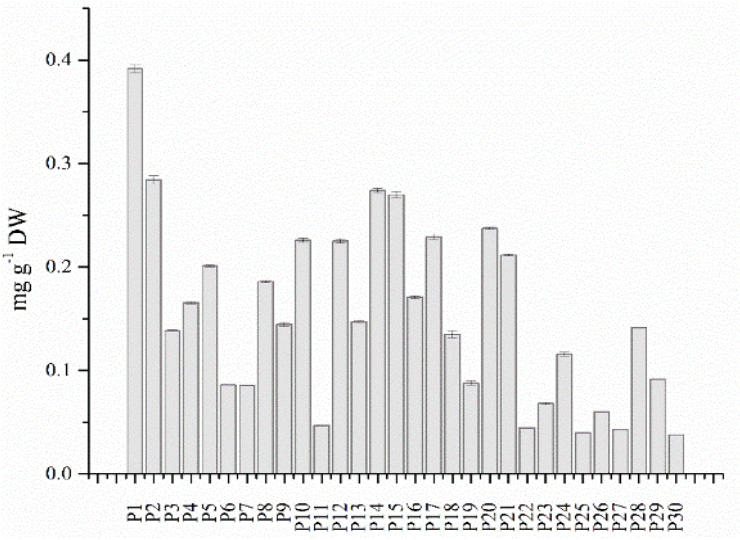

Figure 3. β-carotene content in analyzed tomato and cherry tomato species

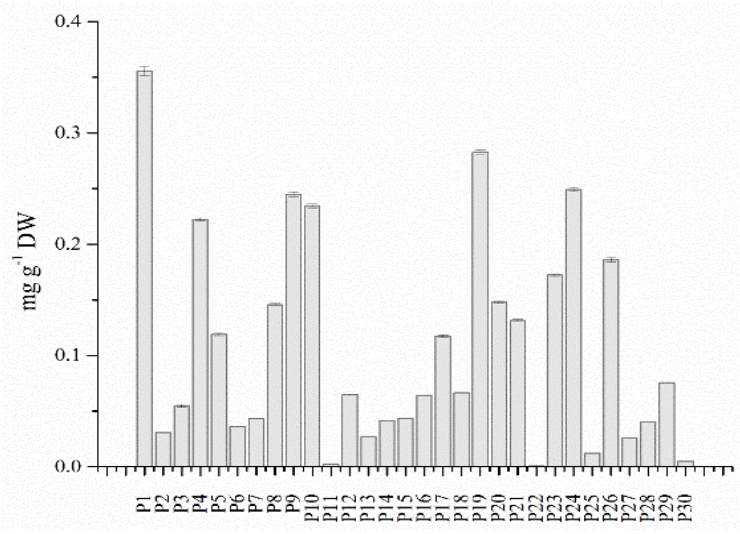

Figure 4. Lycopene content in analyzed tomato and cherry tomato species

Tomato with the highest β-carotene content was P2 - Cherry Datterino Yellow (0.284 mg g^{-1} DW), whereas tomato with the top lycopene content was P19 - Tomato Red Pearl Big (0.283 mg g^{-1} DW). Spectrophotometric assay on β-carotene and lycopene is simple, fast, and inexpensive. Baranska et al. [19]

analyzed β-carotene and lycopene content in tomato using FT-Raman, ATR-IR, and NIR spectroscopy, and results obtained in their study was 2.62−629.00 (lycopene) and 0.23−2.83 mg/100 g (β-carotene). Lycopene content obtained by Baranska et al. [19] was in accordance with results from this study, while β-carotene in our study was slightly higher. Another survey on those pigments in tomatoes was conducted by Burns et al. [20]. Their results recorded that the content of total carotenoids in tomatoes averaged 0.908 mg g^{-1} dry mass, where lycopene represented 0.522 mg g^{-1}. Since carotenoids and lycopene possess antioxidative effects, promote blood flow, and inhibit LDL cholesterol oxidation [18], their analysis is of great importance.

Five different assays were employed to access the antioxidant activity of tomato extracts – DPPH, ABTS, FRAP, TRP, and CUPRAC. Since tests differ in the reaction mechanism, more than one assay should be used to obtain comprehensive information on antioxidant activity. Various standards

Table 1. Antioxidant activity of tomato extracts obtained using ABTS, DPPH, FRAP, TRP, and CUPRAC assays

Sample	ABTS (mgTE1g^{-1}DW)	DPPH (mg TE 1 g^{-1} DW)	FRAP (mg Fe 1 g^{-1} DW)	TRP (mg AAE 1 g^{-1} DW)	CUPRAC (mg TE 1 g^{-1} DW)
P1	7.7±0.2	6.5±0.7	3.4±0.3	0.586±0.008	13.5±0.5
P2	7.7±0.1	10.0±0.8	4.3±0.6	0.699±0.007	14.0±0.7
P3	5.6±0.3	6.9±0.6	2.7±0.2	1.83±0.06	8.5±0.5
P4	7.3±0.2	7.8±0.7	2.7±0.3	1.99±0.07	6.2±0.5
P5	7.7±0.4	8.3±0.5	3.7±0.2	1.84±0.07	11.6±0.9
P6	6.6±0.3	7.0±0.6	2.5±0.3	1.2±0.1	8.6±0.6
P7	9.3±0.6	10.5±0.7	4.0±0.1	2.1±0.2	16±1
P8	7.8±0.6	8.8±0.7	4.0±0.3	2.16±0.09	13.2±0.5
P9	7.8±0.3	9.5±0.6	4.0±0.2	1.95±0.07	13.3±0.9
P10	7.8±0.4	10.0±0.9	5.2±0.3	2.2±0.1	17.3±0.9
P11	7.6±0.4	8.7±0.6	3.6±0.2	1.96±0.08	12.6±0.8
P12	7.8±0.3	9.1±0.6	3.8±0.2	2.3±0.2	12.1±0.6
P13	7.0±0.2	7.7±0.6	2.8±0.1	1.70±0.09	9.3±0.5
P14	7.8±0.3	10.3±0.7	4.2±0.3	3.3±0.3	18±1
P15	7.8±0.6	10.7±0.5	5.0±0.3	2.5±0.1	17.6±0.8
P16	7.8±0.3	10.9±0.6	5.1±0.2	2.5±0.1	17.3±0.9
P17	7.8±0.5	11.1±0.9	5.4±0.4	2.9±0.2	17.5±0.7
P18	7.8±0.2	11.1±0.7	5.6±0.3	2.5±0.01	21±2
P19	7.8±0.6	8.9±0.8	3.6±0.2	2.3±0.1	13.8±0.8
P20	7.8±0.3	9.9±0.5	4.2±0.5	2.57±0.09	14.6±0.5
P21	7.8±0.7	10.2±0.9	5.8±0.3	2.6±0.1	16.9±0.9
P22	7.8±0.6	8.3±0.6	3.8±0.2	3.6±0.2	15.9±0.8
P23	3.9±0.1	6.5±0.3	2.9±0.2	1.86±0.06	11.4±0.4
P24	7.2±0.5	9.0±0.8	5.9±0.3	2.5±0.1	17.7±0.8
P25	4.6±0.4	6.5±0.4	2.7±0.2	1.93±0.08	12.5±0.4
P26	5.0±0.4	7.6±0.3	3.4±0.3	2.8±0.1	14.1±0.6
P27	6.8±0.2	6.7±0.2	2.5±0.3	2.3±0.2	12.6±0.8
P28	5.3±0.3	7.4±0.4	2.3±0.1	2.6±0.1	13.2±0.6
P29	7.8±0.5	8.9±0.7	4.3±0.2	2.4±0.2	20±2
P30	7.3±0.4	7.3±0.6	2.8±0.2	2.11±0.09	11.6±0.5

used and results expressed either on fresh or dry weight makes results from the literature hardly comparable. Results obtained using five different assays were shown in Table 1.

DPPH and ABTS assays are based on the radical scavenging of antioxidants from the extracts and determines scavenging capacity stable radical species by antioxidants compounds present in the extracts. DPPH values (mg TE g^{-1} DW) ranged from 6.5 for P1 (Cherry Datterino Green) and P23 (tomato Green Zebra) to 11.1 for P18 (Cherry Russian Red). Cherry Datterino Yellow (10.0 mg TE g^{-1} DW) showed higher antioxidant activity than Cherry Datterino Green (6.5 mg TE g^{-1} DW), indicating that compounds present in yellow tomato significantly affect antioxidant activity. Klunklin and Savage [21] examined the antioxidant characteristics of tomatoes grown under water stress conditions. The mean DPPH values showed a significant difference between the well-watered (0.25 mg TE g^{-1} DW) and water stress treatments (0.40 mg TE g^{-1} DW) for all cultivars. Those values are lower than the values obtained in our study. The same authors also reported antioxidant activity using the ABTS assay [21]. The mean ABTS assay results in their research for the four cultivars was 0.45 mg TE g^{-1} DW for the well-watered fruits, and this was significantly raised to a mean of 0.83 mg TE g^{-1} DW for the drought-stressed tomatoes. In our study, ABTS values were higher (3.9 mg TE g^{-1} DW for P23 to 7.9 mg TE g^{-1} DW for P19), which is in agreement with the results obtained by Kerkhofs et al. [22]. The antioxidant activity of tomato Black Truffle and Cherry Yellow Pear lipophilic and hydrophilic extracts was analyzed by Zanfini et al. [23]. Hydrophilic extract of Cherry Yellow Pear showed higher antioxidant activity than tomato Black Truffle, which is in accordance with our results.

Assays like FRAP, TRP, and CUPRAC are used to evaluate the ability of antioxidants from the sample to donate electrons. Those methods are SET (single electron transfer) based, and change in color (the solution absorbance change) is linked to the antioxidant concentration [24].

FRAP is a fast, reproducible, and non-specific method measuring the reduction of Fe^{3+}-TPTZ to Fe^{2+}-TPTZ by antioxidants at low pH [34]. FRAP values for analyzed tomato species varied from 2.3 mg Fe per 1 g DW for P28 (tomato Black Krim) to 5.9 mg Fe per 1 g DW for P24 (tomato Black Truffle). tomato Omar's Lebenese, Cherry Russian Red, tomato Fantom, tomato San Marzano, and Cherry Brioso showed similar FRAP values like tomato Black Truffle. According to Bernie et al. [25] results from tomato San Marzano showed low FRAP values (0.31 mmol Fe/100 g FW) compared to other tomatoes analyzed in that study, which is opposite to our results, where tomato San Marzano was one of the tomatoes with the highest FRAP values. Tomato Black Truffle showed high amounts of phenolic compounds, flavonoids, and

lycopene. Phenolics are the most abundant antioxidants in the human diet and can donate hydrogen, chelate metal ions, and inhibit enzymatic reactions. However, the main antioxidant in tomatoes might be lycopene, carotenoid class pigment. The presence of phenolic compounds and lycopene makes tomato food of stupendous nutritional characteristics, allowing its consideration as a functional food with positive health effects [26].

In total, reducing power assay (TRP) antioxidants react with potassium ferricyanide to form potassium ferrocyanide, which latter reacts with $FeCl_3$, yielding a blue colored complex, with a maximum absorbance at 700 nm. Searching literature, authors could not find results for this assay regarding all analyzed varieties. Results showed that the antioxidant capacity of analyzed tomatoes ranged from a minimum of 0.586 mg AAE g^{-1} DW in the Cherry Datterino Green variety to a maximum of 3.6 mg AAE g^{-1} DW in the tomato Brandywine Yellow variety. Reducing the power of Brandywine Yellow variety was analyzed by Sidhu et al. [27], and their results for TRP of this tomato were slightly lower (2.0).

As far as we know, CUPRAC assay for determining antioxidant activity has not been used until now on fresh tomato and cherry tomato cultivars. CUPRAC assay is simple and reproducible, and strongly correlated to other assays for antioxidant activity determination [28]. The highest antioxidant capacity using CUPRAC assay was obtained from P18 - Cherry Russian Red (21 mg TE g^{-1} DW). Other cherry tomatoes also showed high antioxidant activity compared to regular tomato fruits Cherry Yellow Pear (18 mg TE g^{-1} DW), Cherry Bell (17.6 mg TE g^{-1} DW), and Cherry Brioso (17.3 mg TE g^{-1} DW). Only tomato Fantom and tomato Indigo Rose (17.5 mg TE g^{-1} DW and 20 mg TE g^{-1} DW, respectively) showed antioxidant activity similar to cherry tomatoes.

Statistical analysis

Statistical analysis was performed to evaluate the relationship between assays used in this study and tomato varieties.

Cooperative effects between antioxidants and plant-food extracts have been previously reported in different chemical systems. The relationship between total phenolic, flavonoid, lycopene, and carotenoid content and antioxidant activity were evaluated using correlation analysis. Correlation coefficients were presented in Table 3. The most significant positive correlations were found between FRAP/DPPH (0.85) and FRAP/CUPRAC (0.81) assays ($p < 0.05$). Interestingly, no correlation was found between lycopene content and any assay used in this study. CUPRAC assay is strongly correlated to total flavonoid (0.77, $p < 0.05$) and total phenolic content (0.72, $p < 0.05$), indicating phenolics and flavonoids are responsible for antioxidant activity of tomato extracts.

Table 2. Correlation matrices of antioxidant activity, total phenolic, flavonoid, lycopene and carotenoid content

	TPC	TFC	TLC	TCC	DPPH	ABTS	FRAP	TRP	CUPRAC
TPC	1.00	**0.78**	-0.23	0.13	**0.37**	0.22	**0.39**	**0.36**	**0.72**
TFC		1.00	-0.13	0.29	**0.54**	0.33	**0.60**	0.25	**0.77**
TLC			1.00	0.34	-0.04	0.05	0.20	-0.20	0.00
TCC				1.00	**0.38**	**0.38**	**0.38**	-0.25	0.19
DPPH					1.00	**0.69**	**0.85**	**0.38**	**0.72**
ABTS						1.00	**0.55**	0.09	**0.41**
FRAP							1.00	0.34	**0.81**
TRP								1.00	**0.50**
CUPRAC									1.00

Cluster analysis was performed to group tomato varieties based on analyzed characteristics. The dendrogram was constructed by Euclidean distance dissimilarities with Ward's Method of Linkage. The dendrogram of analyzed tomato cultivars was presented in Figure 5.

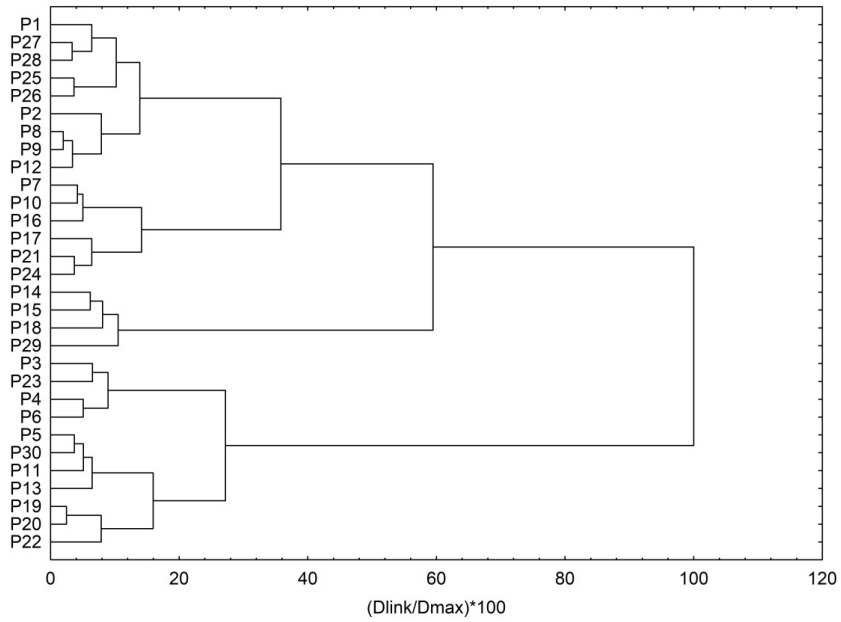

Figure 5. Dendrogram obtained in the cluster analysis of antioxidant activity and total phenolic, total flavonoid, lycopene and carotene content of tomato cultivars

Cluster analysis grouped analyzed tomato cultivars in three statistically significant clusters ($(D_{link} / D_{max})\times100 < 50$). These clusters were separated due to differences in antioxidant activity and analyzed phytochemicals among cultivars. The first cluster consists of 15 analyzed tomato cultivars – Cherry Datterino Green, tomato Black Plum, tomato Black Krim, tomato Azoychka, tomato Chreokee Purple, Cherry Datterino Yellow, Cherry Genio, Cherry Anna-Aasa, tomato Couer de Boeuf (in the first subcluster) and Cherry Lipso, Cherry Brioso, tomato San Marzano, tomato Fantom, tomato Omar's Lebanese, tomato Black Truffle (in the second subcluster). These varieties showed higher antioxidant activity, total phenolic, flavonoid, lycopene, and carotene content compared to other analyzed tomatoes. The smallest Euclidean distance was recorded between Cherry Anna-Aasa and Cherry Genio (1.2), indicating those two varieties are the most similar according to analyzed characteristics. Maximum Euclidean distance was found between tomato Indigo Rose and tomato Rio Grande (24.3). Euclidean distance between analyzed tomatoes was small (1.2-24.3), so it can be concluded that 30 tomato varieties analyzed in this study did not differ significantly in terms of analyzed characteristics. Cluster analysis did not separate cherry tomatoes from tomatoes but gave us the ability to quickly interpret an extensive data set.

A principal component analysis (PCA) was performed to obtain an overview of the similarities between samples and investigate the relationship between the assays used for evaluating the antioxidant activity. PCA produced scores and loading plots (Fig. 6a and 6b). Scores plot is a visualization of the differences among analyzed samples, where each tomato was plotted on a graph in which the first two principal components make up x and y axes, while the loading plot explains the contribution of each variable to the total variance. The number of significant principal components was chosen according to criteria set by Kaiser [29], where eigenvalues should be higher than 1. Only PC1 (3.23) and PC2 (1.07) had eigenvalues higher than 1. PC1 explained 64.68% of the total variance, and PC2 explained 19.41%, totalizing 84.09%. Variables grouped together were strongly positively correlated (TPC, TFC, CUPRAC, DPPH, and FRAP). Major contributors to the PC1 were DPPH (-0.93) and FRAP (-0.92). It seems that they are similar to analyzed characteristics, and some observations could be made:

- Cherry Datterino Green and Cherry Datterino Yellow are grouped together, indicating there is no significant difference in analyzed parameters in Green and Yellow Datterino variety;
- Variety with the highest negative contribution to PC1 was Cherry Russian Red (-2.98), variety with the highest antioxidant activity according to DPPH and CUPRAC assays, and high antioxidant activity according to FRAP assay, indicating PCA analysis can be used as a powerful tool in the study of an extensive data set;

- Tomatoes with negative loading on PC2 (tomato Azoychka, tomato Green Zebra, tomato Black Plum, tomato Cherokke Purple, Cherry Yellow Pear, tomato Brandywine Yellow, tomato Black Truffle, tomato Indigo Rose) are tomatoes with no red skin/meat color, and they have similar antioxidant characteristics;
- Tomatoes grouped in the upper and lower right quadrants possess lower values for analyzed characteristics, and they are on the reverse side of the methods used in this study.

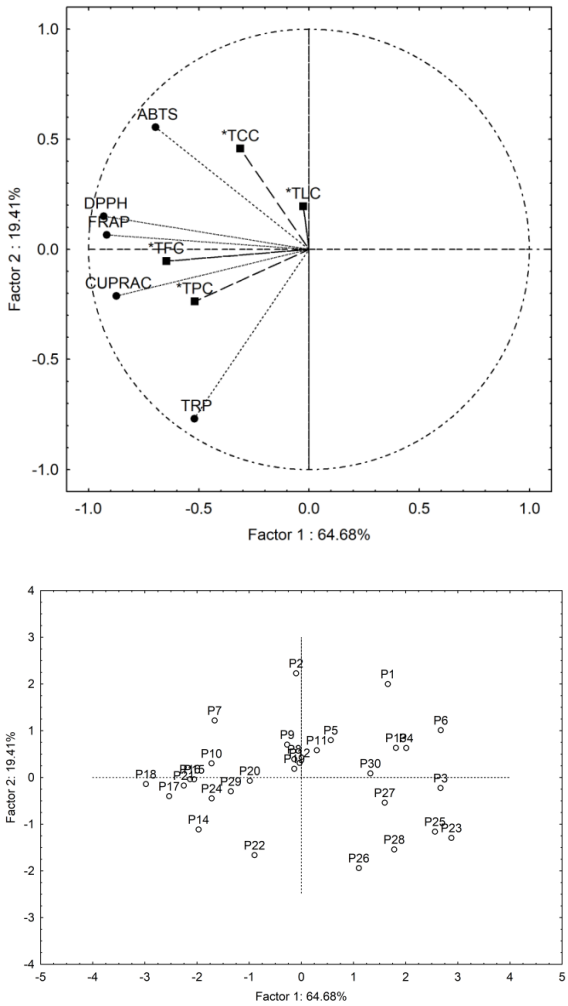

Figure 6. Plots obtained by principal component analysis (PCA); a) loading plot; b) scores plot

EXPERIMENTAL

Plant material and extract preparation

Thirty tomato fruits were obtained from local growers (Nis, Serbia) in July 2018 (Table 3). All samples received similar water and fertilizer treatments and were harvested at the same time. Grounded fresh fruits were lyophilized and kept in the freezer up to analysis. Lyophilized samples were extracted three times (3 x 15 minutes) using methanol in an ultrasonic bath at 25 ºC. Obtained extracts were filtered, evaporated to dryness and dissolved to a final volume of 25mL.

Table 3. List of analysed tomato and cherry tomato

Tomato	Label	Tomato	Label
Cherry Datterino Green	P1	San Marzano	P16
Cherry Datterino Yellow	P2	Fantom	P17
Cherry Tastery	P3	Cherry Russian Red	P18
Rio Grande	P4	Red Pearl Big	P19
Grapola	P5	Teton de Venus	P20
Pink Rock	P6	Omar's Lebenese	P21
Cherry Lipso	P7	Brendywine Yellow	P22
Cherry Genio	P8	Green Zebra	P23
Cherry Anna-Aasa	P9	Black Truffle	P24
Cherry Brioso	P10	Azoychka	P25
Cherry Goldwin	P11	Cherokke Purple	P26
Couer de Bouef	P12	Black Plum	P27
Cherry Koktelpalci	P13	Black Krim	P28
Cherry Yellow Pear	P14	Indigo Rose	P29
Cherry Bell	P15	Ananas	P30

Chemicals and instruments

2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), 2, 2-Diphenyl-1-picrylhydrazyl, Trolox(6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), iron(III) chloride hexahydrate, Folin–Ciocalteu reagent, gallic acid, ascorbic acid, and methanol were obtained from Sigma, St. Louis, Missouri, USA. Acetone, Na_2CO_3, HCl, neocuproine, copper(II) chloride dihydrate, 2, 4, 2-tri(2-pyridyl)-s-triazine(TPTZ), phosphate buffer (NaH_2PO_4–Na_2HPO_4), ammonium acetate buffer, $K_3[Fe(CN)_6]$, CCl_3COOH, $K_2S_2O_8$, and $FeSO_4 \times 7H_2O$ were purchased from Merck, Darmstadt, Germany.

Spectrophotometric measurements were performed using a double beam ultraviolet-visible spectrophotometer (Perkin Elmer Lambda 15, Massachusetts, USA).

Total phenolic content (TPC)

The total phenolic content was measured by applying the Folin-Ciocalteu reagent described by Dimitrijevic et al. [30]. A volume of the extract (25 µL) was mixed with 0.5 mL of Folin-Ciocalteu reagent, 2 mL sodium carbonate solution, and 4 mL water. Reaction mixtures were left to stand in the dark for 30 min, and the absorbance was measured at 750 nm. Gallic acid solution was used for calibration curve construction and the results were expressed via mg gallic acid equivalents (GAE) per g of dry weight (mg GAE g^{-1} DW).

Total flavonoid content (TFC)

The total flavonoid content of analyzed samples was determined by a method described by Mitic et al. [31]. Extract aliquot (50 µL) was mixed with 0.15 mL of a $NaNO_2$ solution. After 5 minutes, 0.75 mL of $AlCl_3$ solution was added, and the solution was kept 5 min at room temperature. Then, 1 mL of NaOH solution was added to the mixture, and water was added to a final volume of 5 mL. The absorbance was measured at 510 nm. Rutin solution was used for calibration curve construction and results were expressed as mg rutin equivalents (RE) per g of dry weight (mg RE g^{-1} DW).

β-carotene (TCC) and lycopene (TLC) content

β-carotene and lycopene were determined using the method described by Barros et al. [32]. Lyophilized samples were thawed in the dark and 10 mL of acetone: hexane mixture (4:6, v/v) was added. The solution was filtered, and then the filtrate was read in comparison to a blank (acetone: hexane solution) at different wavelengths (453, 505, and 663 nm). Contents of lycopene and β-carotene contents were calculated according to the equations:

$$Lycopene\ (mg\ 100\ mL^{-1}\ of\ extract) = -0.0458 \times A_{663} + 0.372 \times A_{505} - 0.0806 \times A_{453} \quad (1)$$

$$\beta\text{-}Carotene\ (mg\ 100\ mL^{-1}\ of\ extract) = 0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453} \quad (2)$$

Antioxidant activity

DPPH radical scavenging capacity: Antioxidant activity on DPPH radicals was performed according to Dimitrijevic et al. [30]. Extract aliquot (25 µL), 1.5 mL of 100 mmol L^{-1} DPPH methanol solution, and methanol to a final volume of 4 mL was shaken, allowed to stand for 60 minutes in the dark and the absorbance was measured at 515 nm. Trolox was used for calibration curve construction. Results were expressed as mg of Trolox equivalents (TE) per g dry weight (mg TE g^{-1}DW).

ABTS radical scavenging capacity: The ABTS radical was produced by the reaction of the stock solution with potassium persulfate [30]. Extract aliquot (25 µL) was mixed with 1.8 mL of ABTS solution and diluted with methanol to a total volume of 4 mL. After 6 minutes at room temperature, absorbance was measured at 734 nm. Trolox was used for calibration curve construction and results were expressed as mg of Trolox equivalents (TE) per g dry weight (mg TE g^{-1} DR).

Total reducing power (TRP) assay: Total reducing power of tomato extracts was determined by the method of Oyaizu [33]. Extract aliquot (10 µL) was mixed with 1 mL $K_3[Fe(CN)_6]$, 1 mL pH 6.6 phosphate buffer, and water. The reaction mixtures were incubated for 30 minutes at 50°C. After that, 1 mL CCl_3COOH and 0.6 mL $FeCl_3$ were added. Ascorbic acid solution was used for calibration curve construction. Absorbance was measured at 700 nm, and results were expressed as mg ascorbic acid equivalents per g of dry extract weight (mg AAE g^{-1}DW).

Ferric-reducing antioxidant power (FRAP) assay: FRAP assay was performed using the method previously described by Benzie and Strain [34]. Extract aliquot (50 µL) was mixed with 1 mL FRAP reagent and diluted with water to a final volume of 4 mL, and after 5 minutes at 37 ºC absorbance was recorded at 595 nm. Ferrous sulfate was used for calibration curve construction. FRAP values of analyzed extracts were expressed as mg of Fe(II) equivalents per g dry weight (mg Fe g^{-1}DW).

Cupric reducing antioxidant capacity (CUPRAC) assay: Cupric ion reducing antioxidant capacity assay was performed using the method of Apak et al. [35]. Extract aliquot (50 µL) was mixed with 1 mL phosphate buffer (pH 7), 1 mL neocuproine, 1 mL $CuCl_2$, and diluted with water to a final volume of 4 mL.

After 30 minutes at room temperature, the absorbance was measured at 450 nm. Trolox was used for calibration curve construction and results were expressed as mg Trolox equivalents per g of dry weight (mg TE g^{-1} DW).

Statistical analysis

Statistical analysis was carried out in Statistica 8.0 software (StatSoft, Tulsa, Oklahoma, USA). A probability level of $p < 0.05$ was considered statistically significant. Correlation between metal content was established using regression analysis at a 95% significance level ($p \leq 0.05$). Cluster analysis was used for sample grouping based on antioxidant activity. In contrast, PCA reckons the correlation structure of the variables creating hypothetical new variables (principal components - PC) that account for as much as possible of the variance (or correlation) in a multidimensional data set [36].

CONCLUSION

Tomato is one of the most consumed foodstuffs both around the world and Serbia. This vegetable is rich in many phytochemicals such as phenolics, flavonoids, vitamins, and minerals. This study aimed to evaluate antioxidant activity, using DPPH, ABTS, FRAP, TRP and CUPRAC assays, as well as total phenolic, total flavonoid, lycopene and β-carotene content in 30 tomato and cherry tomato varieties commonly consumed in Serbia. The most abundant total phenolic and total flavonoid content was recorded for tomato Indigo Rose (17.56 mg GAE g^{-1} DW and 30.30 mg RE g^{-1} DW, respectively). Cherry Russian Red showed the best antioxidant characteristics in two out of five used methods, and excellent results in other methods used. This variety had the highest contribution to the PC1, together with DPPH, FRAP and CUPRAC assays. Cluster analysis permitted separation of analyzed tomato and cherry tomato varieties in three statistically significant clusters. The most significant positive correlations were found between FRAP/DPPH (0.85) and FRAP/CUPRAC (0.81) assays ($p < 0.05$). This indicates that multivariate techniques can be used as a powerful tool to evaluate results obtained by a large number of samples.

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