

ORIGINAL ARTICLE

Chemometric characterization of peach, nectarine and plum cultivars according to fruit phenolic content and antioxidant activity

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Abstract – Introduction. Deciduous tree fruits like peach (*Prunus persica* (L.) Batsch var. *persica*), nectarine (*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C.K.Schneid), and especially plum (*Prunus domestica* L. ssp. *domestica*) are very common in Serbia. These fruits are known for their nutritional value and therapeutic properties and are valuable sources of antioxidants. **Materials and methods.** The goal of this work was to evaluate fruit tissue antioxidant activity using methanol extracts of 9 peach, 3 nectarine and 7 plum cultivars. The following parameters were measured: total phenolic content (TPC); antioxidant activity as estimated by radical scavenging activity of (2,2-diphenyl-1-picrylhydrazyl, DPPH); cation decolorization activity (2,2-azinobis-3 ethylbenzothiazoline-6-sulphonic acid, ABTS); ferric reducing antioxidant power (FRAP); cupric reducing antioxidant capacity (CUPRAC); and total reducing power (TRP). **Results and discussion.** Total phenolic contents of the plum cultivars were higher than those of peach and nectarine and significant positive correlations were recorded between all antioxidant activity assays and total phenolic contents. Results obtained by principal component analysis (PCA) are in agreement with those obtained by cluster analysis (CA). **Conclusion.** The selected methods revealed antioxidant activities for all plum cultivars significantly higher than in the peach and nectarine cultivars. PCA and CA allow grouping the different fruit species based on TPC, DPPH, ABTS, TRP, FRAP and CUPRAC values.

Keywords: Serbia / peach / plum / nectarine / *Prunus* spp. / antioxidant activity / phenolics

Résumé – Caractérisation chimiométrique de cultivars de pêches, nectarines et prunes en fonction de la teneur en composés phénoliques et de l'activité antioxydante de leurs fruits. Introduction. Les fruits à noyau apparentés aux pêches (*Prunus persica* (L.) Batsch var. *persica*), nectarine (*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C.K.Schneid), et surtout aux prunes (*Prunus domestica* L. ssp. *domestica*) sont très courants en Serbie. Ces fruits sont connus pour leur valeur nutritionnelle et leurs propriétés thérapeutiques, et ils sont une source précieuse d'antioxydants. **Matériels et méthodes.** Le but de ce travail était d'évaluer les activités anti-oxydantes des extraits méthanoliques des fruits de 9 cultivars de pêcher, 3 cultivars de nectarine et 7 cultivars de prunier. Les paramètres suivants ont été mesurés : le contenu phénolique total (PTC) ; l'activité anti-oxydante par piégeage des radicaux (2,2-diphényl-1-picrylhydrazyl ou DPPH) ; l'activité de décoloration cationique (acide 2,2-azinobis 3-éthyl-6-benzothiazoline sulfonique ou ABTS) ; l'activité anti-oxydante ferrique (FRAP) ou cuivrique (CUPRAC) ; et la puissance réductrice totale (TRP). **Résultats et discussion.** Le contenu total en composés phénoliques des cultivars testés de prune était plus élevé que celui des cultivars de pêche et de nectarine. Une corrélation positive significative a été enregistrée entre tous les dosages d'activité anti-oxydante et la teneur totale en composés phénoliques. Les résultats obtenus par analyse en composante principale (PCA) sont en accord avec ceux obtenus par analyse de clusters (CA). **Conclusion.** Les méthodes analytiques choisies ont révélé de fortes activités anti-oxydantes pour tous les cultivars de prune, qui sont significativement plus élevées par rapport aux résultats correspondants pour les cultivars de pêche et de nectarine. La PCA et la CA ont permis le regroupement des différentes espèces fruitières sur la base des valeurs de PTC, DPPH, ABTS, TRP, FRAP et CUPRAC.

Mots clés : Serbie / pêcher / prunier / nectarine / *Prunus* spp. / activité anti-oxydante / composés phénoliques

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1 Introduction

Balanced diets, including the regular consumption of fruits play a major protective role against many diseases such as: different types of cancer, cardiovascular diseases, osteoporosis and atherosclerosis cellular aging [1].

Fruits are valuable sources of nutrients, vitamins, minerals, dietary fiber, nonessential phytochemicals, water, and especially an abundance of antioxidant compounds. Antioxidants are compounds that may inhibit, retard or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions [2]. Phytochemicals that are known for strong antioxidant activity are polyphenols, carotenoids and vitamins (A, C, E), all known to be beneficial for improving human health [3].

Peach and nectarine (*Prunus persica* (L.) Batsch vars. *persica* and *nucipersica*) (family *Rosaceae*) represent one of the most important deciduous fruit crops in the world after apple and pear. They are summer fruit, widely cultivated in the temperate regions of the world, especially in the Italy, Spain, France, Greece, United States and China [4–6]. *Prunus persica* is known for its nutritional value and therapeutic properties. Major constituents of *P. persica* fruit are carbohydrates, organic acids, minerals and dietary fibers [7] which contribute to its nutritional quality [8]. Ripe peach and nectarine fruits have a white or golden yellowish flesh and a sweet taste due to lower acidity. Peaches as well as some other *Rosaceae* family fruits are rich in vitamin A and potassium as well as in organic acids and sugars [9].

Including peaches in the diet can suppress reactive oxygen species (ROS) in human plasma and provide protection against chronic diseases [10]. Peach fruits have laxative properties and are thus appropriate for preventing constipation and for the treatment of duodenum ulcers [7]. Nectarine and peach are closely related, differing mainly in the fact that nectarine lacks fruit surface pubescence. Nectarines tend to be somewhat smaller than peaches and some have a firmer texture and sweeter flavor. Both nectarines and peaches are low in calories and contain no saturated fats. They are a source of some of B-complex vitamins including niacin, pantothenic acid, thiamin, and pyridoxine. In addition, they contain an appropriate ratio of minerals and electrolytes such as potassium, iron, zinc, copper and phosphorus.

Plum fruits (*Prunus domestica* L. ssp. *domestica*) have low calorie content and a low glycemic index score but relatively high nutritive value. They contain carbohydrates, first of all sucrose, glucose and fructose, organic acids, e.g. citric and maleic acids, fibers (pectins), tannins, aromatic substances, chlorophyll, carotenoids, anthocyanins and enzymes. These substances determine nutritive value and taste of plums [12]. Plum is a fruit rich in phenolic compounds, characterized by relatively high antioxidant activity, higher than for example orange, apple or strawberries [13]. The taste of plum depends of the relation of sugars and organic acids. Plums are also rich in many minerals and vitamins (C, A, B1, B2). Ascorbic acid is the best known antioxidant and an important molecule in plant tissues that protects plants against oxidative damage caused by the oxidative metabolites of photosynthesis and aerobic processes [14].

Plum has high potassium content and an advantageous sodium/potassium ratio [15] that slows down the absorption of carbohydrates, enhances the sensation of satiety, reduces blood serum triglycerides and homocysteine concentrations as well as the levels of total cholesterol and its LDL fraction, and increases lipid breakdown in the human organism. The consumption of plum remains low despite reports that this tasty fruit with intensive aroma is an important source of compounds with benefits for human health. This might be due to the lack of maturity of the marketed fruit [16].

Peach, nectarine and plum tissues contain ample amounts of polyphenols, carotenes and anthocyanins [17], flavonols such as quercetin 3-rutinoside, hydroxycinnamates such as chlorogenic acid and neochlorogenic acid, and flavan 3-ols such as catechin and epicatechin [18, 19]. The main anthocyanins reported in peach and plum were cyanidin 3-glucoside and cyanidin 3-rutinoside [18, 19], cyanidin 3-acetyl glucoside, cyanidin 3-galactoside [19], peonidin-3-glucoside and peonidin derivatives [18]. Similar phenolic profiles were detected for both nectarine and peach, and no differences were found between white-flesh and yellow-flesh peach cultivars [20]. Phenolic compounds [7, 21] do not have essential importance to the peach and nectarine plant itself, but can affect the quality of fruit sensorial-organoleptic attributes (flavor, aroma, and color), as well as nutritional quality.

The antioxidant content in examined fruits varies greatly across cultivar type (peach, nectarine or plum) [22], growing practices, geographic location and environmental factors (water and light availability, soil composition, stresses, etc.) [3, 5]. Furthermore, as happens for other fruits that are often picked unripe for commercial handling purposes, peach antioxidant content may be affected by the stage of fruit ripening at harvest, storage techniques [12] and time elapsed between harvest and consumption [5]. Although phenolic compounds have bioactivities which could have a positive impact on health [23], they could provoke undesirable effects such as astringency and bitterness [1, 7, 24, 25]. Carotenoids from peach and nectarine [26] especially β -carotene, lutein and β -cryptoxanthin [2] have a role as pigments but also have a protective role against oxidative stress in plant cells [5].

Published data about the contents of phenolic compounds and total antioxidant activity of peach and nectarine methanol extracts are insufficient. Taking into account that these natural antioxidants are multifunctional, the antioxidant capacities of samples cannot be completely described with one single method. Also, since the methods are different from each other in terms of assay principles reaction conditions, and expression of results, one single method is not enough to show all the antioxidant proprieties of examined fruits.

The objective of this study was to compare different peach, nectarine, and plum cultivars to determine fruit phenolic content and antioxidant capacity. This was done by applying five widely used spectrophotometric methods: DPPH, ABTS, FRAP, CUPRAC, TRP and estimating the correlation of antioxidant capacities with total phenolic content. To the authors' knowledge, clustering of different peach and nectarine species based on their antioxidant activity and total phenolic content was done for the very first time.

2 Materials and methods

2.1 Plant materials

Peach, nectarine and plum fruits of various cultivars were collected from orchards in Serbia. Five to ten fruits at the firm ripe stage were chosen from each cultivar of peach ('Maycrest', 'Cardinal', 'Cresthaven', 'Redhaven', 'Colins', 'J.H. Hale', 'Maja', 'Golden', 'Vinogradarska'), nectarine ('Caldesi', 'Fantasia', 'Crimson gold') and plum ('Ruska dzanarika', 'Cacanska lepotica', 'Cacanska rodna', 'Cacanski secer', 'Cacanska najbolja', 'Stanley', 'Moravka'). Fruits were collected during harvest season between June and September 2014 in a rural unpolluted area of Soko Banja (peach and nectarine) and Blace (plum) in South Eastern Serbia.

Fruit tissue (without peel) of frozen peach, nectarine and plum (10 g) was homogenized in a blender. Four consecutive extractions were performed with 15 mL methanol and 15 min in an ultrasonic bath. These extracts were filtered, and diluted with methanol to a final volume of 25 mL.

2.2 Chemicals and instruments

Chemicals and reagents were purchased from Merck (Darmstadt, Germany). Spectrophotometric assays were performed on a double-beam UV–VIS spectrophotometer Perkin Elmer lambda 15 (MA, USA). Each sample was analyzed in triplicate.

2.3 Antioxidant activity

2.3.1 Total phenolic content (TPC)

Total phenolic content and the five estimates of antioxidant activity all involved the procedures described by Dimitrijevic *et al.* [27]. For total phenolic content, 0.05 mL of the extract was mixed with 0.5 mL of Folin-Ciocalteu reagent, 2 mL sodium carbonate solution and 4 mL water. The reaction was carried out in the dark for 30 min and absorbance was measured at 750 nm. Gallic acid was used to calculate the standard curve and the results were expressed as mg of gallic acid equivalents (GAE) per g of fresh weight (mg GAE g⁻¹ fw).

2.3.2 DPPH radical scavenging capacity

For the quantitative assays of the methanol extracts for DPPH radical scavenging capacity, 1.5 mL of DPPH radical methanol solution at the concentration of 100 mmol L⁻¹, 0.1 mL extract at the concentration of 20 mg mL⁻¹ fruit tissue, and methanol to a total volume of 4 mL, were placed in a test tube. The mixture was shaken and after 60 min in the dark, the absorbance was measured at 515 nm. The percentage of scavenging activity was calculated as:

$$A\% = ((A_c - A_s)/A_c) \times 100$$

where A_c is the absorbance of control (without sample), and A_s is the absorbance of sample.

2.3.3 ABTS radical scavenging activity

The ABTS radical was produced by the reaction of ABTS stock solution with potassium persulfate and the mixture was allowed to stand in the dark at 25 °C for 12–16 h before use. The solution was then diluted by mixing 7 mL ABTS⁺ solution with 120 mL methanol to obtain an absorbance of 0.70 ± 0.02 units at 734 nm. An aliquot of each extract, at the concentration of 20 mg mL⁻¹, was mixed with 1.8 mL of diluted ABTS⁺ solution at the concentration of 7 mmol L⁻¹ and diluted with methanol to a total volume of 4 mL. After 6 min at 25 °C, the reduction in absorbance was measured at 734 nm. The percentage of scavenging activity was calculated applying Equation 1.

2.3.4 Ferric-reducing antioxidant power (FRAP) assay

One mL of FRAP reagent was mixed with 0.05 mL of sample, at the concentration of 20 mg mL⁻¹, and diluted with water to make up a volume of 4 mL. After 5 min incubation at 37 °C, the absorbance was recorded at 595 nm. FRAP values expressed as mmol of Fe²⁺ equivalents per g fresh weight (mmol Fe g⁻¹ fw) were obtained by comparing the absorption change in the test mixture with doses obtained from the Fe(II) standard calibration curve.

2.3.5 Total reducing power (TRP) assay

Reaction mixtures were prepared by mixing 0.01 mL of extract, 1 mL of 1% solution K₃[Fe(CN)₆], phosphate buffer (pH 6.6) and water. The mixtures were incubated at 50 °C for 30 min and then 1 mL 10% solution of trichloroacetic acid and 0.6 mL FeCl₃ were added. Results were expressed as mg ascorbic acid equivalents per g of fresh weight (mg AAE g⁻¹ fw).

2.3.6 Cupric reducing antioxidant capacity (CUPRAC) assay

This assay involved the addition of 0.05 mL of extract, 1 mL of phosphate buffer (pH 7.0), neocuproine concentration of 7.5×10^{-3} mol L⁻¹, copper (II) - chloride at the concentration of 0.01 mol L⁻¹, and diluted with water to a total volume of 4.1 mL. The mixture was left for 30 min at 25 °C and after that, absorbance was measured at 450 nm. Trolox was used as a standard and results were expressed as mg Trolox equivalents per g of fresh weight (mg TE g⁻¹ fw).

2.4 Statistical Analysis

Data analysis involved several procedures. The elimination of outliers was carried out by Grubb's test. All data were reported as the mean \pm standard deviation of three replicates ($n = 3$). Correlation analysis examined the interrelationships between the study samples. Cluster analyses (CA) was carried out with the total phenolic content and antioxidant activity data

Table I. Total phenolic contents (TPC) and antioxidant activity criteria of several *Prunus* fruit cultivars grown in Serbia (DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity; CUPRAC: cupric reducing antioxidant capacity; TRP: total reducing power; FRAP: ferric reducing antioxidant power). Values are means \pm SD ($n = 3$).

Prunus species	Cultivars	DPPH (%) ^y	ABTS (%) ^y	TPC ^y (mg GAE g ⁻¹)	CUPRAC ^y (mg TE g ⁻¹)	TRP ^y (mg AAE g ⁻¹)	FRAP ^y (mmol Fe g ⁻¹)
Peach	Maycrest	13.49 \pm 1.09 ^{cd}	16.67 \pm 2.94 ^{cde}	1.28 \pm 0.02 ^g	0.28 \pm 0.02 ^p	15.52 \pm 3.23 ^{gh}	0.48 \pm 0.02 ⁱ
	Cardinal	17.30 \pm 1.77 ^{bcd}	24.16 \pm 3.02 ^{cd}	2.03 \pm 0.03 ^g	0.32 \pm 0.02 ^q	18.41 \pm 2.54 ^{fgh}	0.80 \pm 0.03 ^g
	Cresthaven	16.98 \pm 1.51 ^{cd}	31.50 \pm 3.75 ^c	1.78 \pm 0.02 ^g	0.32 \pm 0.02 ^q	19.69 \pm 3.01 ^{fg}	0.67 \pm 0.02 ^h
	Redhaven	14.13 \pm 1.16 ^{cd}	12.69 \pm 2.96 ^e	0.69 \pm 0.01 ^{gh}	0.24 \pm 0.01 ^r	13.30 \pm 3.51 ^{gh}	0.31 \pm 0.02 ⁱ
	Collins	17.94 \pm 2.16 ^{abcd}	30.58 \pm 3.13 ^c	1.78 \pm 0.08 ^g	0.32 \pm 0.02 ^q	15.24 \pm 3.51 ^{gh}	0.67 \pm 0.03 ^h
	J. H. Hale	16.67 \pm 1.75 ^{cd}	44.80 \pm 4.71 ^c	4.00 \pm 0.04 ^e	0.45 \pm 0.02 ^g	24.55 \pm 2.68 ^{ef}	1.06 \pm 0.04 ^f
	Maja	15.24 \pm 1.68 ^{cd}	13.76 \pm 1.85 ^{de}	0.55 \pm 0.01 ^h	0.24 \pm 0.02 ^r	12.31 \pm 1.45 ^h	0.28 \pm 0.02 ⁱ
	Golden	17.46 \pm 1.84 ^{abcd}	26.30 \pm 3.75 ^c	1.69 \pm 0.02 ^g	0.31 \pm 0.02 ^q	17.22 \pm 1.68 ^{fgh}	0.69 \pm 0.02 ^h
Nectarine	Vinogradarska	17.94 \pm 2.08 ^{abcd}	44.19 \pm 4.09 ^c	3.17 \pm 0.02 ^{fg}	0.40 \pm 0.02 ^h	23.62 \pm 1.92 ^f	1.15 \pm 0.03 ^f
	Caldesi	18.10 \pm 1.98 ^{abcd}	22.78 \pm 3.39 ^{cde}	1.83 \pm 0.15 ^g	0.33 \pm 0.01 ^q	17.58 \pm 1.87 ^{fgh}	0.73 \pm 0.03 ^h
	Fantasia	13.02 \pm 1.01 ^{cd}	11.93 \pm 2.06 ^e	1.08 \pm 0.18 ^{gh}	0.25 \pm 0.01 ^{kr}	15.07 \pm 1.82 ^{gh}	0.39 \pm 0.02 ⁱ
Plum	Crimson gold	15.40 \pm 2.28 ^{cd}	12.84 \pm 2.74 ^{de}	0.93 \pm 0.51 ^{gh}	0.26 \pm 0.01 ^{pk}	14.36 \pm 1.38 ^{gh}	0.49 \pm 0.02 ⁱ
	Ruska dzanarika	32.86 \pm 3.75 ^{ab}	72.32 \pm 4.63 ^{abc}	6.42 \pm 1.29 ^c	0.63 \pm 0.03 ^d	34.25 \pm 2.51 ^d	2.74 \pm 0.02 ^d
	Cacanska lepotica	32.86 \pm 3.28 ^{ab}	82.26 \pm 4.47 ^{ab}	5.94 \pm 1.07 ^d	0.61 \pm 0.01 ^e	32.43 \pm 2.84 ^{de}	2.40 \pm 0.02 ^e
	Cacanska rodna	40.03 \pm 3.51 ^{ab}	83.94 \pm 5.33 ^{ab}	4.43 \pm 0.93 ^e	0.52 \pm 0.03 ^f	29.01 \pm 2.93 ^{de}	1.50 \pm 0.01 ^f
	Stanley	38.25 \pm 2.75 ^{ab}	78.59 \pm 4.97 ^{ab}	8.33 \pm 1.48 ^c	0.83 \pm 0.05 ^c	40.64 \pm 3.04 ^c	3.56 \pm 0.03 ^c
	Moravka	40.79 \pm 3.26 ^a	86.39 \pm 5.09 ^a	3.91 \pm 0.92 ^{ef}	0.44 \pm 0.02 ^g	28.65 \pm 3.61 ^e	1.47 \pm 0.02 ^f
	Cacanska najbolja	32.70 \pm 1.88 ^{abc}	66.82 \pm 4.34 ^{bc}	9.41 \pm 1.28 ^b	0.88 \pm 0.04 ^b	53.39 \pm 6.92 ^b	3.89 \pm 0.04 ^b
	Cacanska secer	33.49 \pm 2.98 ^{ab}	53.06 \pm 4.11 ^c	13.53 \pm 1.85 ^a	1.11 \pm 0.06 ^a	58.30 \pm 7.68 ^a	6.02 \pm 0.09 ^a

^y Values with different letters within columns are statistically different at $P < 0.05$ by a statistical test on the means (LSD).

to identify various groups. The CA produced a dendrogram by means of the Ward's method of hierarchical clustering, based on the Euclidean distance between pairs of fruit samples.

Principal component analysis (PCA) was performed as it is among the best-known multivariate analysis methods for determining relationships among variables [28]. All computations were done using the Statistica 8 software (StatSoft, Tulsa).

3 Results and discussion

3.1 Fruit phenolic contents

Polyphenols play an important role in antioxidant activity. The results of total phenol content, determined by Folin-Ciocalteu's method, are reported in *table 1*. The polyphenol content of fruit is influenced by numerous factors such as genotype, rootstock, climatic conditions, agronomic practices, harvesting time, and postharvest conditions. Also, the results of total phenolic content depend on sample preparation technique, the assay standards (gallic acid, tannic acid), and the methods used to identify and quantify potential antioxidants (spectrophotometric determination or HPLC-DAD).

The total content of phenolic substances ranged from 0.55 to 4.01 mg GAE g⁻¹ fw for peach and 0.93 to 1.83 mg GAE g⁻¹ fw for nectarine. The lowest total phenol content was determined in the early peach cv. 'Maja' and

highest in the late cv. 'J.H. Hale'. Similarly, the late-season cv. 'Vinogradarska' had significantly higher phenolic content (3.17 mg GAE g⁻¹ fw) compared to other cultivars and it appears that late season peaches tend to exhibit higher total phenolic content.

However, nectarine followed a different trend: the highest total phenolic content was determined in the early cv. 'Caldesi' (1.83 mg GAE g⁻¹ fw) and the lowest in late cultivar 'Fantasia' 1.08 mg GAE g⁻¹ fw.

Total phenolic contents in plum ranged from 3.91 mg GAE g⁻¹ fw in 'Moravka' to 13.53 in 'Cacanski secer'. Other high phenol cultivars were 'Stanley' (8.33) and 'Cacanska najbolja' (9.41). These values are almost five times higher than published results [29] for 'Stanley' (1.74) and almost three times more for 'Cacanska najbolja' (3.19).

Considering that all plum cultivars were grown under identical conditions and in the same locality, it is possible to conclude that our results are strongly influenced by varietal variability, which is quite typical of plum. We observed higher contents of phenolics and stronger antioxidant activities in regional plum cvs ('Cacanska najbolja', 'Cacanski secer') than in more commercial ones (e.g. 'Stanley').

In general, the phenolic contents found in plum were significantly higher than those reported for peach and nectarine. It is very difficult to compare our data on total phenolic content with published data. Moreover, phenolic compounds are not

uniformly distributed within the fruit tissue, and most of them are concentrated in the epidermal and sub epidermal layers of the fruit, so it is very important how fruits material was prepared - with or without peel tissues. Phenolic distribution is an important aspect of the overall phenolic composition and antioxidant capacity because peach skin is usually not eaten and therefore it does not contribute to the human diet. In general, the values we obtained are comparable to data reported in the literature expressed as mg GAE g⁻¹ fw: 0.74 [30]; 0.70 [31]; 0.33 [32]; 0.28 [33]; 0.29–0.55 [5]. In fruit samples from Croatia [34], phenolic content of the peach cv. ‘Redhaven’ was 0.41 mg GAE g⁻¹ fw, very similar to what we determined (0.69) for the same cultivar. Since higher values of phenolic content were determined in peach samples from Italy and Korea (3.95–7.28 mg GAE g⁻¹ fw [3], 4.03 mg GAE g⁻¹ fw [35], respectively), climatic conditions might be responsible for the differences observed.

Our results for total phenolic content of different plum cultivars were comparable with the recent reports from other origins (in mg GAE g⁻¹ fw): 3.48–4.95 [36]; 1.25–3.72 [18]; 1.60–3.00 [37]; 2.37 [38]; 1.29–6.25 [39]; 2.82–9.22 [40, 41]).

3.2 Fruit antioxidant activities

To determine free radical scavenging activity of peach, nectarine and plum extracts, we used two types of radicals, DPPH and ABTS. Antioxidants interacting with these radicals transfer an electron, thus neutralizing an unpaired electron. Electron transfer-based DPPH and ABTS assays generally set a fixed time for the redox reaction and measure thermodynamic conversion (oxidation) during that period.

DPPH radical activity can be expressed in many different ways, making the comparison of obtained data with previously reported data very difficult [42]. The majority of authors evaluated DPPH activity *via* evaluation of EC₅₀ (the sample concentration necessary to reduce the initial DPPH activity to 50%), or percentage inhibition (%). DPPH has been widely used for free radical-scavenging assessments due to its simplicity and convenience. In the present study, peaches’ and nectarines’ extracts were found to be very similar in their ability as DPPH radical scavengers (*table I*). The results of examined peach extracts were in the range 13.50–17.94% inhibition of DPPH radical. The highest values came from the peach cultivars ‘Vinogradarska’ and ‘Colins’ (17.94%). The cv. ‘Redhaven’ inhibited 14.13% of DPPH radical. The highest DPPH free radical scavenging capacity amongst nectarine cultivars came from the early cv. ‘Caldesi’ (18.01%); the lowest came from the late cv. ‘Fantasia’ (13.02%).

These results differ slightly from those reported earlier [34], which is probably the consequence of different cultivars being studied. However, they are very similar to some other results [42] for similar cultivars.

Antioxidant capacity of plum extracts, as evaluated by the DPPH radical scavenging assay, was in the range of double that found for peach and nectarine, ranging from 32.70% (‘Cacanska najbolja’) to 40.79% (‘Moravka’) (*table I*). The high scavenging property of plum extracts may be due to hydroxyl

groups existing in the phenolic compounds’ chemical structure that can provide the necessary component as a radical scavenger.

ABTS method is also a common method for determination of antioxidant activity of extracts and is based on the decolorization of the ABTS⁺ cation radical. Various peach cultivars showed statistically significant differences in antioxidant activity estimated by this method (*table I*). The cultivar ‘Vinogradarska’ possessed a strong scavenging capacity for the ABTS⁺ radical (44.20%) and was very similar to that for ‘J.H. Hale’ (44.80%). These late peach cultivars showed stronger activity against ABTS radical than early varieties (12.69% and 13.76%, ‘Redhaven’ and ‘Maja’). These results could be explained by the fact that ‘J.H. Hale’ and ‘Vinogradarska’ have significantly higher phenolic content (4.01 and 3.17 mg GAE g⁻¹ fw) compared to other cultivars.

The nectarine cv. ‘Fantasia’ exhibited ABTS-estimated antioxidant activity (11.93%) only half that of the cv. ‘Caldesi’ (22.78%). Amongst the peach and nectarine cultivars, ‘Vinogradarska’ showed the highest antioxidant capacity considering both methods.

As was the case for the DPPH assay, ABTS-estimated antioxidant activity for the plums was significantly higher for peach and nectarine (*table I*). The antioxidant activity in plum is known to be dependent on the cultivar [18, 34, 43]. We found that ‘Moravka’ had the highest ABTS-estimated activity (88.39%) followed by ‘Cacanska rodna’ (83.94%) and ‘Cacanska leptotica’ (82.63%). The lowest activity was shown for ‘Cacanski secer’ (55.06%), but it is still double that of the highest result for nectarine.

In this study FRAP, CUPRAC and TRP assays were also used to estimate the reductive capacity of the examined extracts (*table I*). Previously reported data for the FRAP [30, 33, 45, 46], CUPRAC and TRP [7] assays of peach and plum extracts were considered insufficient and partly contradictory. This is due not only to different contents and proportions of particular phenolic compounds in different cultivars of peach, nectarine and plum, but above all to different methods and the various methodological approaches that were used. The comparison of literature data is thus very complicated and even impossible in some cases because of the different standard substances used.

Nectarine tended to have lower values obtained by FRAP assay (0.39–0.73 mmol Fe g⁻¹ fw) compared to peach (0.28–1.15) but a wide variation in the total antioxidant capacity and phenolic content of peach was observed among cultivars. The peach cultivar showing the highest antioxidant capacity by this measure was ‘Vinogradarska’ (1.15) followed by ‘J.H. Hale’ (1.06). In contrast, ‘Maja’ (with the lowest phenolic content) showed low Fe²⁺ reduction activity (0.28 mmol Fe g⁻¹ fw). Amongst the nectarine cultivars, ‘Fantasia’ showed low antioxidant activity (0.39), while ‘Caldesi’ (0.73) had both the highest antioxidant capacity and highest phenolic content.

The highest antioxidant activity was found among the plum cultivars. However, these levels overlapped those found among the other examined fruits. Among the seven plum cultivars, ‘Cacanski secer’ showed the highest antioxidant activity (6.02 mmol Fe g⁻¹ fw), whereas ‘Moravka’ had the lowest

(1.47). Values of antioxidant activity obtained by FRAP assay for ‘Cacanska najbolja’ and ‘Stanley’ were very similar (3.89 and 3.56, respectively).

The CUPRAC method for determining antioxidant activity had not been done until now on peach and nectarine. Nectarine cultivars (‘Fantasia’, ‘Caldesi’) expressed lower values (0.25–0.33 mg TE g⁻¹ fw) compared to values observed for peach (0.24 in ‘Maja’ and ‘Redhaven’, 0.45 for ‘J.H. Hale’). Early varieties of nectarine had lower CUPRAC values than late ones, which is different from peach. The highest CUPRAC values in peach were found in ‘J.H. Hale’ (0.45) and in the late cv. ‘Vinogradarska’ (0.40). All plum cultivars had higher CUPRAC values than the peach and nectarine cultivars examined. Amongst the plum cultivars, ‘Cacanski secer’ had the highest value, followed by ‘Cacanska najbolja’ and ‘Stanley’ (1.11, 0.88 and 0.83 mg TE g⁻¹ fw, respectively). The lowest antioxidant activity value obtained by this method was found in ‘Moravka’ (0.44).

Reducing power of the tissue extracts as estimated by the conversion of the Fe³⁺/ferricyanide complex to the ferrous form should serve as a significant indicator of its potential antioxidant activity. The best of our knowledge, there are no data about total reducing power of peach and nectarine extracts measured by this method. Total reducing power of nectarine ranged from 14.36–17.58 mg AAE g⁻¹ fw (‘Crimson gold’, ‘Caldesi’) and for peach (12.32–24.55 for ‘Maja’ and ‘J.H. Hale’). Plum cv. ‘Cacanski secer’ showed the greatest reducing power amongst all examined fruits (58.30). Also, the plum cultivar ‘Cacanska najbolja’ exhibited a high value (53.39). The lowest reducing power values were found in cvs ‘Moravka’ and ‘Cacanska rodna’ (29.01 and 28.65 mg AAE g⁻¹ fw, respectively), which is still greater than the highest values found in peach and nectarine fruits. The antioxidant activity of plum is known to be relatively high compared to other fruit species [46].

The antioxidant (reducing) ability of a sample is associated with the presence of a reductant species that breaks the free radical chain by donating a hydrogen atom or preventing peroxide formation by electron scavenging (ES). Methods used for estimating antioxidant activity are extremely diverse with regard to mechanism and applied reagents and standards. ES-based methods include ABTS and DPPH while TRP, TPC, FRAP and CUPRAC belong to the methods using various chromogenic reagents with different standard redox potentials. The final result of these antioxidant methods (disregarding the mechanism) is mostly similar regarding their capability for quenching or reducing active species. However, their kinetics, eventuality of side reactions, and dependence on reaction conditions may differ. Phenolic and some non-phenolic substances exert different antioxidant activities and the high phenolic contents in fruits may not always lead to higher antioxidant action. Thus, it is of essential importance to estimate antioxidant action using at least one ES and one redox based assay.

Because the antioxidant activity measured by an individual assay reflects only the chemical reactivity under the specific conditions applied in that assay, as pointed out by many authors [48–52], it is inappropriate and misleading to generalize the data obtained by a single method as indicators of antioxidant activity. There are no standardized assays for determining

Table II. Correlations between phenolic content and several antioxidant activity criteria of the tested *Prunus* species.

	Correlations					
	DPPH	ABTS	TPC	CUPRAC	TRP	FRAP
DPPH	1.00					
ABTS	0.95	1.00				
TPC	0.73	0.67	1.00			
CUPRAC	0.74	0.68	1.00	1.00		
TRP	0.76	0.70	0.99	0.99	1.00	
FRAP	0.71	0.62	0.99	0.99	0.97	1.00

antioxidant capacity of any sample, and their “importance” or usefulness depends exclusively on the mechanism of action of antioxidants expected to be found in the particular sample. This is why several antioxidant assays are usually employed in each study, so that the overall antioxidant capacity is evaluated more accurately and all antioxidants compounds are taken into account.

3.3 Correlations between all measured criteria

The relationship between antioxidant activity and total phenolic content was tested using correlation analysis. Correlation coefficients are presented in *table II*. Significant positive correlation values were recorded between all antioxidant activity assays and total phenolic content. The most significant positive correlations were found between TPC/CUPRAC ($r = 1$, $P < 0.05$) and TPC/TRP and TPC/FRAP ($r = 0.99$, $P < 0.05$). This indicates that phenolic compounds were the most active compounds measured by the CUPRAC, FRAP and TRP assays. These results are in agreement with Badarinath *et al.* [53], who reported that CUPRAC findings correlated well with the results of ABTS/TEAC and TPC assays, as well as Yildiz [54], who showed a positive strong correlation between TPC and FRAP antioxidant capacity.

Among antioxidant activity assays, the strongest correlations were found between CUPRAC/FRAP ($r = 0.99$, $P < 0.05$), CUPRAC/TRP ($r = 0.99$, $P < 0.05$), and ABTS/ DPPH ($r = 0.95$, $P < 0.05$), all in agreement with Mitic *et al.* [55].

Clustering of different peach and plum cultivars based on their antioxidant activity and total phenolic content is presented in *figure 1*. Cluster analysis grouped the analyzed fruits in two clusters. These clusters were separated due to differences in antioxidant activity and total phenolic content amongst cultivars. Cluster 1 includes peach and nectarine accessions, while plum accessions belong to cluster 2. The tendency to form natural sample groupings arising from common analytical characteristics is clearly highlighted with such a data analysis procedure. Cluster 1 can be divided into three sub-clusters. Sub-cluster 1 contains five peach cultivars, sub-cluster 2 contains five, and sub-cluster 3 only two cultivars. Peach in sub-cluster 3 (cvs ‘J.H. Hale’ and ‘Vinogradarska’) are late-maturing varieties, characterized by higher ABTS radical scavenging activity. Cluster 2 containing the plum cultivars is characterized by higher antioxidant activity and exhibits two sub-clusters. The smallest Euclidean distance in this cluster was recorded for cvs ‘Cacanska rodna’/‘Moravka’ and

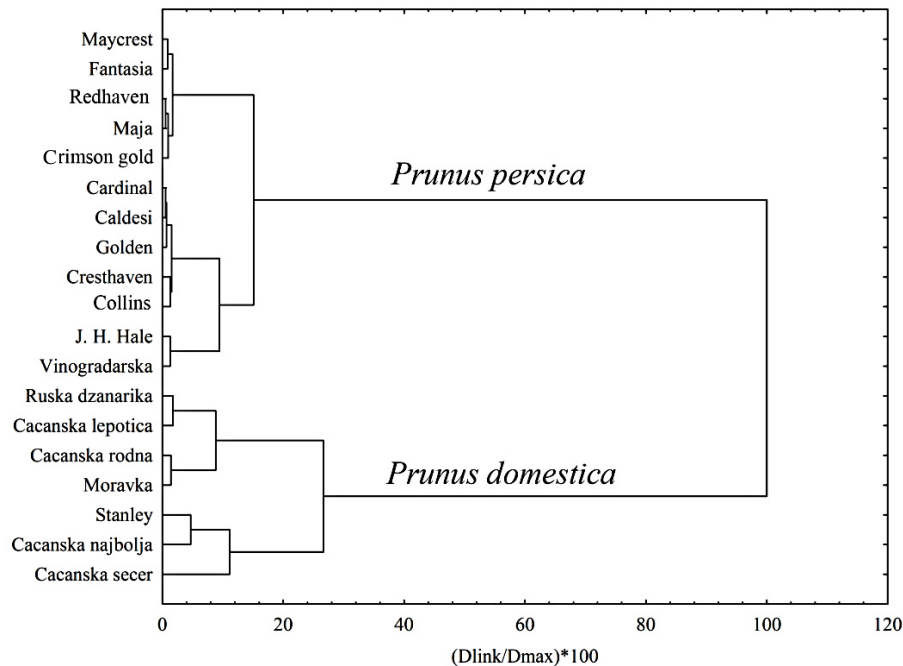


Figure 1. Two-dimensional dendrogram obtained in the cluster analysis of antioxidant activity and total phenol content of peach, nectarine and plum cultivars.

‘Ruska dzanarika’/‘Cacanska leptotica’, indicating their similarity.

Principal component analysis (PCA) was applied to evaluate the data for total phenolic content and antioxidant activity. PCA produced two visual plots: scores and loading (*figure 2*). The scores plot is a visualization of the differences among accessions, where each fruit sample is plotted on a graph in which the first two or three principal components make up the axes. The loading plot explains the contribution of each variable to the total variance, and shows key variables causing variation in the dataset. PC1 explained 86.44% of the total variance and PC2 explained 12.21%, totalizing 98.65% (*figure 2*). Variables grouped together were strongly positively correlated (TPC, TRP, CUPRAC and FRAP). Considering the position of the fruit samples it was possible to separate them into two major groups. The first group is made of seven plum varieties, same as in cluster 2, while peach varieties belong to the second group. Plum accessions are located on the left side of the plot, since they have higher antioxidant activity and total phenolic content compared to peach and nectarine. The plum cv. ‘Cacanski secer’ is located quite a distance from other accessions, indicating that its antioxidant activity and total phenolic content differs significantly from the other plum samples. Using the plots in *figures 2a* and *2b*, it is possible to suggest reasons for the location of accessions on the basis of their antioxidant activity. Location of ‘Cacanska najbolja’ and ‘Cacanski secer’ in the lower left-hand quadrant of *figure 2b* may be explained by their high total phenolic contents and TRP, FRAP and CUPRAC values, which are located in the same quadrant in loading plot. In contrast, peach cultivars had lower values for antioxidant activity analyzed by these as-

says, and they are located at the opposite side of the score plot. Plums in the upper left quadrant have higher ABTS and DPPH radical scavenging activity than the other fruit species, which is confirmed by the position of ABTS and DPPH in the loading plot. Results obtained by PCA analysis are in agreement with those obtained from CA.

4 Conclusion

The various fruits examined in this study are commonly represented in the traditional human diet in Serbia, and the evaluation of their antioxidant properties is valuable for those interested in consumption patterns (consumers’ association, nutrition and health policy makers) as well as for scientists pursuing more comprehensive studies that will encompass more fruit species and broader geographic areas. Having in mind that most consumers recognize fruit mostly at the species level, the results of the present study are valuable for making proper choices of fruit with regard to their antioxidant potential.

Regarding the antioxidant properties of the selected fruit species and varieties, all applied analytical methods (DPPH, ABTS, TRP, FRAP and CUPRAC) are reliable, simple, robust, and they do not require a lot of time for perform, nor complicated and expensive equipment. Although we have found a good correlation among all the methods used here for assessing antioxidant capacity, using more than one antioxidant assay is strongly recommended – a single method will provide basic information about antioxidant properties, but a combination of methods describes the antioxidant properties of the sample in more detail. This is how we can confirm that plum is generally

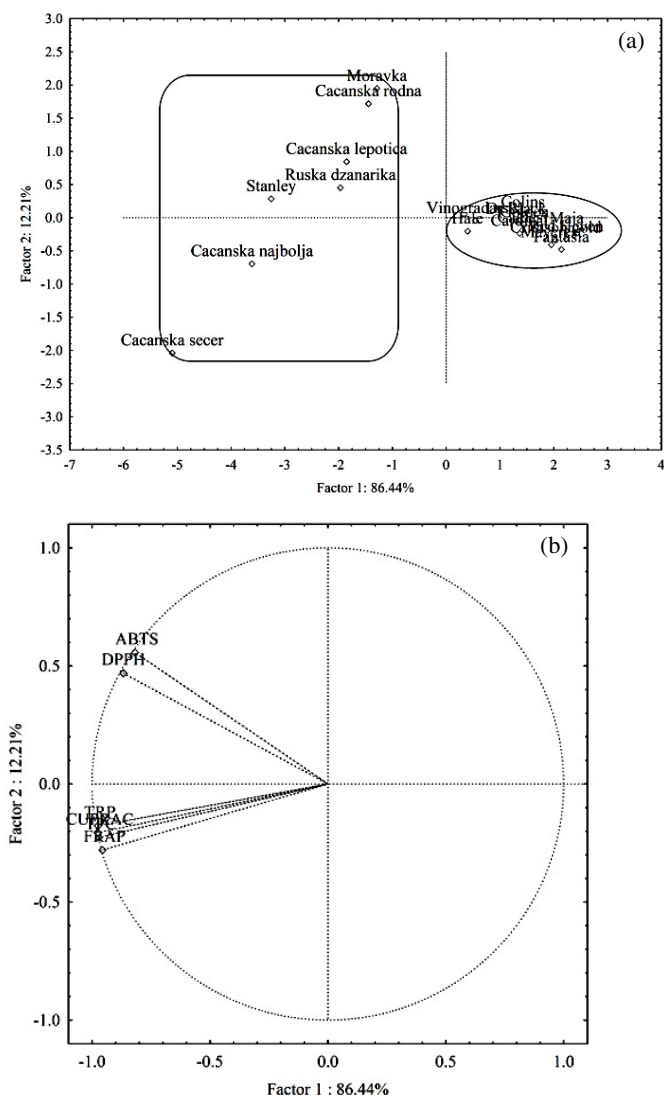


Figure 2. Plot obtained by principal component analysis (PCA); antioxidant activity (measured from DPPH, ABTS, TRP, FRAP and CUPRAC assays) and total phenol content (TPC) are used as variables of peach, nectarine and plum cultivars.

the most potent source of antioxidants, and among the cultivars tested, especially ‘Cacanski secer’. Considering all applied assays, the tested peach fruits expressed less than half the antioxidant properties of the tested plums. Among them ‘J.H. Hale’ and ‘Vinogradarska’ showed the best characteristics. Nectarine is the fruit species with the lowest antioxidant potential, and among the tested cultivars the best was cv. ‘Caldesi’, although without significant difference from the other ones.

Fruit maturation has been recognized as an important stage for estimating antioxidant property (FRAP). In the case of peach, late cultivars had significantly higher values than the early ones, while in case of nectarine the relation was reverse. PCA and CA allowed grouping of different fruit species and varieties based on TPC, DPPH, ABTS, TRP, FRAP and CUPRAC values. Also, it should be emphasized that, to our

knowledge, these are the first data on the antioxidant activity of these fruit species, determined applying the CUPRAC method.

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