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Comparative analysis of phenolic, flavonoid and antioxidant content in root vegetables from organic and conventional production

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Abstract: Vegetables and their products have a low caloric value. Root vegetables are rich in bioactive compounds such flavonoids, polyphenolic acids, carotenoids. It also contains a some percentage of other functional components that can significantly affect human health.In order to achieve a high yield, excessive fertilization with nitrogen fertilizer is performed, which worsens the quality of vegetables. The aim of this paper is to analyze the impact cultivation system on the content of total phenolics and flavonoids, as well as a comparative assessment of their antioxidant activity in selected samples of vegetables. The antioxidant activity was studied using DPPH, and ABTS assays. The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were determined using Folin-Ciocalteuand and Aluminium chloride colorimetric methods, respectively. The highest content of total phenolics was determined for parsley from conventional production (427.38 mg GAE/100 g fresh sample), parsley from organic production the richest source of flavonoids (54.92 mg GAE/100 g fresh sample). Other samples of vegetables from conventional production had a lower flavonoid content compared to organic production. It was confirmed that the antioxidant activity of the analyzed vegetables in the maximum correlation with the content of total phenolics (R2 = 1). Organic samples of vegetables were characterized by a significantly higher content of flavonoids compared to vegetables grown conventionally. Organic production could be a good method to increase the concentration of bioactive compounds with antioxidant properties in vegetables.

Keywords: root plants; farming system; primary antioxidants

Organic agricultural production represents a comprehensive farm management system that combines enviromental protection, biodiversity, conservation of natural resources, prescribed standards and production methods that are suitable for consumers. Organic farming provides relatively good crop yields with minimal impact on environmental factors as opposed to conventional agriculture, which increases yields but causes severe environmental problems. Conventionally produced food has less nutritional value than organically produced food, and it also contains amount of residual chemical agents that are not known to affect human health or are not talked about enough in public (Batelja et al. 2011). According to research by Pokos (2011) vegetables produced organically contains a higher percentage of useful nutrients than vegetables obtained by conventional production. According to the same author, root vegetables, which are the subject of our research, are especially rich in nutrients and phenolic compounds.

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Contain few calories, so they are suitable for everyday consumption. The antioxidant potential of phenolic compounds is due to their redox effect. The phenolic compounds have a key role in absorbing/neutralizing the free radical. Kwiatkowski et al. (2015) in their research shows that the morphological and qualitative characteristics of root vegetables are strongly influenced by agroecological factors, of which fertilization has the most significant impact. Mehri et al. (2021) shows that inadequate fertilization of root vegetable species with nitrogen fertilizer can take to excessive amounts of nitrates and other nitrogen compounds. According to research by a group of authors (Sawicka et al. 2020) the application of mineral fertilizers can affect the nutritional value as well as the content and composition of secondary metabolites in the plant. The obtained results indicate the usefulness of research and enzyme activity as an indicator of soil reaction to nitrogen fertilization and enabling the maintenance of the optimal biological balance of cultivates soil In view of very little data on the influence of mineral fertilizers on the content of secondary metabolites in vegetable roots, we considered it interesting to explore the influence of different production systems on the content and composition of phenolic compounds and antioxidant activity in root vegetables. The aim of this paper is to analyze the impact cultivation system on the content of total phenolics and flavonoids, as well as a comparative assessment of their antioxidant activity in selected samples of vegetable.

MATERIAL AND METHODS

The field experiment was set by using the randomized block in 3 replications. More precisely,the experimentals plots is located were in western Serbia, crop-vegetable region of Kraljevo on the site of Samaila (43°45'44.35"N, 20°32'48.48"E). Field experiment was set up on alluvium-type land in the early spring of 2021. The size of the experimental plot was $3 m^2$ (1.5 m × 2 m). The total area of the experimental field was 54 m^2. The distance between the plants was 0.50 m, and the distance between the rows was 0.20 m. A total of 5 400 plants per/54 m^2. We researched root vegetables: *Petroselinum hortense var.tuberosum* (variety "Berlin semi-long type"), *Daucus carota L.* (variety "Chantenay") and *Beta vulgaris subsp.esculenta* (variety „Detroit"). The basic characteristic of the land on which the experiment was set up were: 6.51 pH (in KCl); 0.25% $CaCO_3$; 3.62% humus; 0.195 % total nitrogen; 6.70 P_2O_5 mg $100g^{-1}$; 11.89 K_2O mg/100g. In conventional grown root vegetable, we used mineral NPK fertilizer formulation 7 : 14 : 21 in the amount of 10 kg/100 m^2. In organiclly grown root vegetable, we used cowshed manure in the amount of 150 kg/100 m^2. Chemical weed control was used only in conventional grown root vegetable. In organiclly grown root vegetable, we didn't use chemicals. Disease control in the conventional grown root vegetable was based on the application of classical fungicides. Root was taken out in fase harvest maturity.

The analysis of plant material was done on Fruit Research Institute Cacak. A sample of plant material for chemical analysis was made from 10 plants of each researched variety in three repetitions.

Applied methods

Determination of total phenolic contents (TPC). Total phenolic content was estimated by Folin-Ciocalteu method as described in Jundi et al. (2021). To 0.1 mL of the extract, 1 mL Folin-Ciocalteu reagent (diluted ten times) was added and the mixture was left for 5 minutes and then 1 mL of sodium carbonate was added. The absorbance of the resulting was measured at 765 nm with a double beam UV visible spectrophotometer after incubation for 90 minutes at room temperature. The total phenolic content was estimated from gallic acid (1–100 μg/mL) calibration curve and results were expressed in mg gallic acid equivalents (mg GAE/100 g of fresh extract).

Determination of total flavonoids (TFC). The total flavonoid content was determined as described in Pekal et al. (2014) with minor modifications. The analysis was based on the formation of the yellow color of flavonoid-aluminum complex. Aluminum chloride (2 mL, 2%) was mixed with the same volume of the extract (1 mg/mL). Then absorbance at 415 nm was taken after 1 hour of incubation at room temperature against a blank sample. The total flavonoid content was determined using a standard curve of rutin at (1–40 μg/mL). The results were expressed as mg rutin equivalents per gram fresh sample (mg RE/100 g fresh sample).

Determination of antioxidant activity (DPPH method). Antioxidant activity was determined by the DPPH method which is a modification of the method by Kaur et al. (2017). In summary, 1 mL of extract was added to 2 mL of 0.2 mM DPPH solution. All obtained samples were then incubated for 30 minutes

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at room temperature. The absorbance was measured at 517 nm. Radical removal capacity using free DPPH radicals was estimated by measuring the decrease in absorption at 517 nm. Antiradical activity was calculated from the calibration curve, using gallic acid as standard (10 to 100 mg/L). Results are expressed as mg/100 g gallic acid equivalents.

Determination of antioxidant activity (ABTS method). The free radical-scavenging activity of sample was determined by ABTS. The stock solutions were 7 mM ABTS and 2.45 mM potassium persulfate. The ABTS solution was then prepared by and allowing them to react for 16 hours. This solution was stored in a dark place at room temperature. Crude extracts and fractions (0.3 mL) were allowed to react with 3 mL of ABTS solution and measured at 754 nm. Additionally, 10 mL garlic extract was added 1 mL ABTS solution, mixed thoroughly and subjected to spectrophotometry to measure the absorbance at 734 nm after 30 min stay time. The antioxidant activity using the Trolox standard curve was reported in μmol Trolox /g sample extract.

Statistical analysis

ANOVA Tukey HSD analysis. Statistical analysis was performed using the Statistical Analysis System (SPSS, version 20).

Data were analyzed by analysis of variance (ANOVA) and differences between samples were determined by Tukei's HSD test. Values are expressed as mean ± S.D., $n = 3$.

The mean value within each group with different letters (a-e) showed significant differences with $P < 0.05$. Pearson's correlations between antioxidant activity, total phenols and flavonoid content were considered at $P < 0.05$.

Z-Score analysis. The Z-score analysis used min-max normalization of the vegetable antioxidant potential parameters trans-forming them from their original unit system into a new dimensionless system, where further mathematical calculations with different types of quality parameters are applicable (Filipović et al. 2020.) The maximum value of the normalized score presents the optimum value of all combined analyzed parameters, indicating the optimum antioxidant potential:

$$S_{ki} = \frac{\sum_{i=1}^{6} \frac{x_{ki} - x_{ki\,max}}{x_{ki\,max} - x_{ki\,min}}}{6} \tag{1}$$

Where x_{ki} are total phenols, flavonoids, ABTS, and DPPH based on commercial and organic fertilizer ($k = 1 - 4$). All experimental results were calculated in triplicates.

$$Score_i = \frac{\sum_{k=1}^{4} S_k}{4} \tag{2}$$

max [Score$_i$] → optimum
where S_k is S_1, S_2, S_3, and S_4.

RESULTS AND DISCUSSION

Phenolic compounds are a large group of secondary plant metabolites that contribute to the organoleptic and nutritional properties of fruits and vegetables. Composition and content of secondary metabolites and antioxidants plant capacity depends on genetic factors, production conditions (temperature, light, availability of water and nutrients), agricultural practice (harvest date, etc.) and conditions after harvest (Chen et al. 2022; Mattoo et al. 2022; Pazos et al. 2022). In vegetable samples from conventional production, the total phenolic content was (mg GAE/100 g fresh sample) from 39.28 to 427.38 while in organically grown vegetable was from 23.57 to 292.67 mg GAE/100 g fresh sample) (Table 1). Flavonide content was higher in organically grown vegetables and amounts from 11.13 to 54.92 (mg GAE/100 g fresh sample) while in conventional mode of cultivation of vegetables from 8.56 to 32.63 (mg GAE/100 g fresh sample). Among the tested samples, commercial parsley is the richest source of total phenolic content (427.38 mg GAE /100 g fresh sample), while organic parsley is the richest source of total flavonoids (54.92 mg GAE/100 g fresh sample). However, a very limited number of studies have investigated the effect of cultivation systems on antioxidant content in plants. Some studies have reported that the profile of phenolic acids and flavonoids may differ between organic and conventionally grown fruits (Iswaldi et al. 2013*).* Research results of Oloyede et al. (2012) showed that the content of total phenolic in fruit vegetables significantly decreases with increasing levels of mineral NPK fertilizatio, which confirms our results. The experimental results and application of organic production influenced the reduction of total phenolic and antioxidant activity by DPPH method, ie the increase in the share of total flavonoids and antioxidant activity of ABTS method. When it comes to the antioxidant activity of the DPPH method on the example

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Table 1. Total phenolic, flavonoides content and their antioxidative activity in samples of different root vegetables

Vegetable	Total phenolic	Total flavonoids	ABTS	DPPH
	(mg GAE/100 g fresh sample)		(mmol TE/100 g)	
Parsley (con.)	427.38 ± 21.58[d]	32.63 ± 1.38[d]	1.12 ± 0.05[c]	0.63 ± 0.01[a]
Parsley (org.)	292.67 ± 11.9[c]	54.92 ± 2.77[b]	1.52 ± 0.07[d]	0.57 ±0.07[a]
Carrot (con.)	39.28 ± 4.04[a, b]	8.56 ± 0.37[a]	0.39 ± 0.08[a, b]	0.56 ± 0.01[a]
Carrot (org.)	23.57 ± 2.34[a]	11.13 ± 0.69[a]	0.52 ± 0.02[b]	0.31 ± 0.005[b]
Beet (con.)	70.00 ± 4.08[b]	19.17 ± 2.02[c]	0.35 ± 0.02[a]	0.40 ± 0.01[c]
Beet (org.)	57.38 ± 4.71[a, b]	52.14 ± 1.52[a, b]	0.49 ± 0.01[a, b]	0.41 ± 0.01[c]

[a–d]Different letters in table indicate the statistically significant difference between values, at a significance level of $P < 0.05$

of parsley and beets, the results of the experiments were not statistically significant ($P < 0.05$). Similar results are in carrots, when it comes to the total content of phenols and flavonoids. In further research, the authors plan and recommend a more complex analysis of polyphenolic compounds (including, primarily, anthocyanins), which were not the subject of analysis in this paper. This claim is supported by the results obtained from the analysis of berries where the antioxidant activity is proportional to the content of anthocyanins. Current findings are supported the studies of Elhanafi et al. (2019) of pot cultivation of potted carrot plants fertilized with Ca $(NO_3)_2$ which showed a decrease in the phenolic compound content in storage roots when compared to fertilized with $(NH_4)_2SO_4$, NH_4NO_3, $CO(NH_2)_2$ and the control without nitrogen fertilization.

Antioxidants are substances that, although present in small amounts, protect cells from oxidative action of free radicals and prevent the induction of oxidative stress in the human body (Buljeta 2015*)*. In the organic cultivation system, the highest antioxidant capacity was found in parsley (1.52 TE /100 g), while the lowest antioxidant capacity was found in the conventional beet growing system (0.35 TE/100 g) (Figure 1). Such results coincide with the results of (Eryigit et al. 2014*)*, who determined a higher antioxidant capacity in the organic cultivation system, compared to the stated antioxidant capacity in the conventional cultivation system. Very significantly higher antioxidant capacity between the tested vegetable species was in the organic cultivation system, compared to the determined antioxidant capacity from the conventional cultivation system.

Correlations between antioxidant activity and total phenolic content. In order to determine the correlation between the concentration of total phenolic and the antioxidant effect (obtained according to the applied methods), a correlation was calculated. Values of concentrations of total phenols and flavonoids in relation to the associated proportion of reduced radical determined by DPPH and ABST test (Figure 2). By comparing the correlation coefficients (R-values), it is possible to suggest that phenolic and flavonoid groups are highly responsible for the antioxidant activity of the selected plant extracts.

From the graph it can be concluded, and above all, to confirm that the antioxidant activity of the analysed vegetables is in maximum correlation with the content of total phenolic (R2 = 1), (Figure 2).

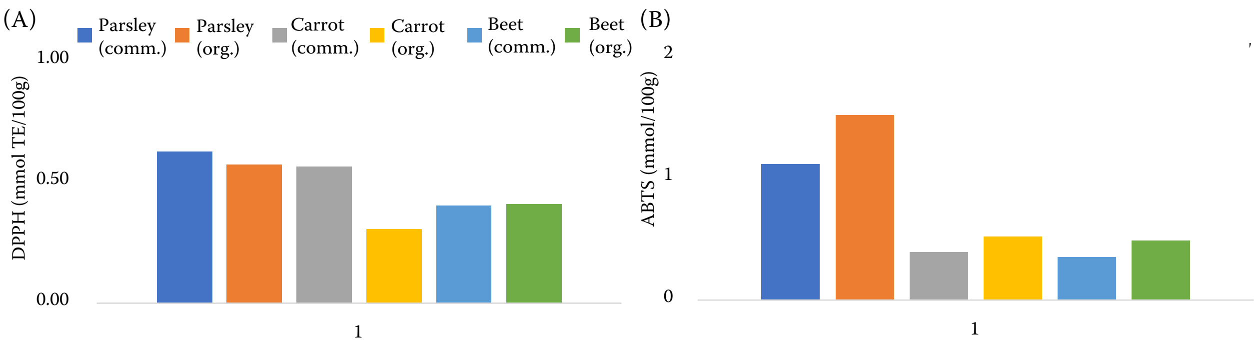

Figure 1. Antioxidant activity in organically and conventionally grown root vegetables
DPPH – determination of antioxidant activity; ABTS – determination of antioxidant activity

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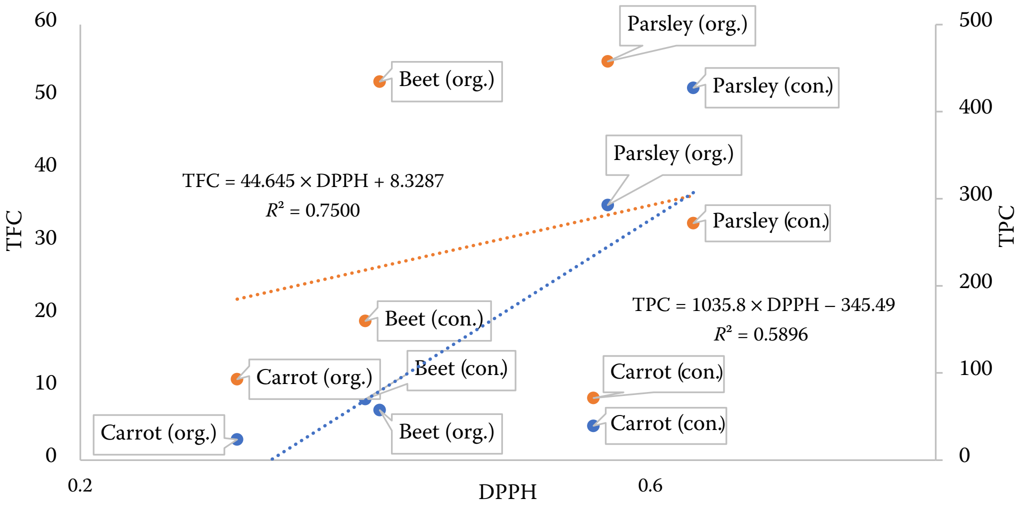

Figure 2. Correlations of antioxidant activity (DPPH) in organically and conventionally grown root vegetables
TFC – total flavonoids content; DPPH – determination of antioxidant activity; TPC – total phenolic content

While a slightly lower coefficient of determination (R2 = 0.75) in the case of dependence of antioxidant activity on flavonoids would be due to their lower antioxidant power and proportion in vegetables (since phenols include flavonoids, anthocyanins, etc.). The presented results coincide with the research of other scientists who point out the connection between the total phenolic content and the antioxidant activity of fruits and vegetables (Diniyah et al. 2020; Ameni et al. 2022). Phenolic and flavonoid molecules are important antioxidant components which are responsible for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals. They also have ideal structural characteristics for free radical scavenging. Different literature reports indicate a linear correlation of total phenolic and flavonoid content with antioxidant capacity, as shown in our experimental results in Figure 3. The results of the Z-score analysis provide information about the segment and the total antioxidant potential of parsley, carrot, and beet (Table 2).

The highest value of S_4 in parsley indicated the best DPPH antioxidant potential, unlike the lowest value of S_4 in beet. Generally, the importance of S was very similar (0.54, 0.51 and 0.47 for parsley, carrot, and beet, respectively) where according to the commercial or organic fertilizer the best antioxidant potential (S_{max} = 0.54 %) was observed for the parsley, ex-

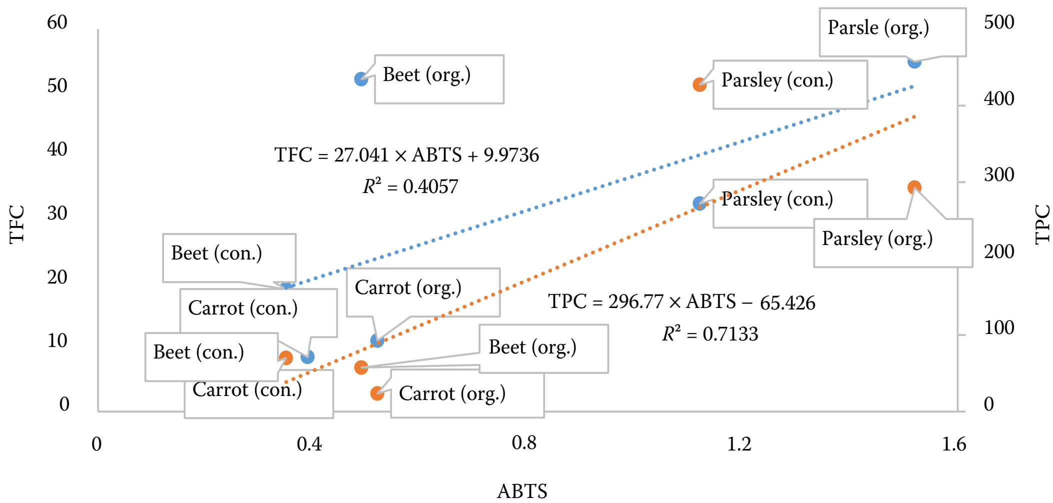

Figure 3. Correlations of antioxidant (ABTS) activity in organically and conventionally grown root vegetables
TFC – total flavonoids content; ABTS – determination of antioxidant activity; TPC – total phenolic content

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Table 2. Score value of the antioxidative parameters for different vegetables based on conventional and organic fertilizer

	Parsley	Carrot	Beet
		S	
S_1 – TPC	0.46	0.50	0.51
S_2 – TFC	0.46	0.47	0.51
S_3 – ABTS	0.48	0.58	0.54
S_4 – DPPH	0.75	0.50	0.33
Saverage (max)	0.54	0.51	0.47

TPC – total phenolic content; TFC – total flavonoids content

S_1 – the segment score of antioxidant activity based on TPC

S_2 – the segment score of antioxidant activity based on TFC

S_3 – the segment score of antioxidant activity based on ABTS

S_4 – the segment score of antioxidant activity based on DPPH

S – the total score of antioxidant activity based on TPC, TFC, ABTS, and DPPH

pressed as maximal score value. Research has shown that TEAC and FRAP tests show differences in total antioxidant capacity depending on the phenolic content in fruits and vegetables; consequently, the higher the content of total phenolic, the stronger the antioxidant capacity. Our results are in good agreement with *(*Ulewicz et al. 2019). Various studies by other authors confirm the link between total phenol content and antioxidant activity of fruits, plants and vegetables (Diniyah et al. 2020; Ameni et al. 2022).

CONCLUSION

Based on the obtained results, the conclusions can be drawn as follows: The production system had a significant effect on the concentration of phenolic compounds and antioxidant capacity. It can be stated that the examined types of vegetables, which are most often consumed in households, could be considered as an extremely good source of natural phenols and flavonoids. The higher content of these compounds was found in organic compared to the conventional system of cultivated vegetable species. *Z*-score analysis indicated optimal segment and total antioxidant potential of parsley, carrot, and beet according to the commercial or organic fertilizer. The best antioxidant potential (S_{max} = 0.54%) was observed for the parsley. In this study, the assessment of antioxidant activity indicates that root vegetables with higher phenolic and flavonoid contents could be a significant source of natural antioxidants. The increased biological value of the tested vegetable species, ie higher antioxidant capacity, obtained in the organic cultivation system that should be one of the basic motives of agricultural producers to decide more for the organic cultivation system.

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