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THE EFFECT OF THE CULTURE SUBSTRATE ON THE CONTENT OF BIOACTIVE COMPOUNDS IN SOME RASPBERRY GENOTYPES

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Abstract

Raspberries are shrubs that belong to the genus Rubus idaeus L., Rosaceae's family. The raspberry culture is one of the most widespread among fruit bushes. Raspberries include a large number of varieties with different ripening periods, with summer and autumn fruiting of the remontant ones. The fruits of the genus Rubus are among those rich in bioactive compounds (anthocyanins, dietary fiber, vitamins, minerals and carbohydrates), so beneficial for human and animal health (Vega et al., 2021) The objective of the paper was to evaluate the bioactive compounds with antioxidant properties from raspberry fruits obtained from plants grown on different culture substrates, such as: manure, garden soil, forest compost, semi-fermented compost and spent mushroom substrate (SMS), applied to the soil. The studied plantation was established in the spring of 2020, and the presented results refer to the fruits harvested in 2022. The experiments were set up in the field within SCDP Băneasa - the Moara Domnească Afumati Experimental Farm.

***Key words**: raspberry cultivation, nutritive substrates, bioactive compounds.*

INTRODUCTION

Red raspberry (*Rubus idaeus* L.), a herbaceous plant in the Rosaceae family, is a shrub described as a nanophanerophyte. Raspberry, is an important commercial product in fresh or processed form due to its nutritional, medicinal and cosmetic uses (Gülcin et al., 2011; Papaioanou et al., 2018).

Raspberries contain high concentrations of important nutrients, bioactive compounds and phytochemicals. Raspberries are also an excellent source of vitamin C. It is well known that vitamin C has health and wellness attributes that make the fruit very popular among consumers (Papaioanou et al., 2018). Raspberries contribute to the nutritional value of a diet. They contain phytochemical components with documented biological activity, many of which were originally investigated for their *in vitro antioxidant properties* .

The chemical constitution of raspberry leaves, which are a by-product of raspberry processing, has not been studied as thoroughly as its fruit. In particular, the healing properties of raspberry leaves, known since ancient times, are prescribed for the treatment of a wide variety of diseases, for example, by including them in herbal preparations used to relax the uterus during childbirth. Other applications of raspberry leaves include their use as an additive to beverages, nutritional supplements, functional herbal preparations, teas and chocolate to enhance their nutritional and flavor-forming properties (De Santis et al., 2022; Wu et al., 2022). *Rubus* fruits have been shown to be an increasingly important source of bioactive substances due to their antioxidant, anti-inflammatory, chemopreventive and antibacterial properties, positive effects on blood lipids and atherosclerosis mentioned above, as well as their advantageous composition. Therefore, due to their biological effect, they can potentially be applied as a health promoting factor (Schulz and Chim, 2019).

The objective of the work was the evaluation of the bioactive compounds with antioxidant properties from raspberry fruits (*Rubus idaeus* L.) in correlation with their culture substrate.

MATERIALS AND METHODS

The study was carried out at the Moara Domnească Experimental Base, located NE of Bucharest (in Câmpia Vlăsiei, a subunit of the Romanian Plain), in Ilfov County, just about 17 km from Bucharest. The farm belongs to the Research and Development Station for Pomiculture (RDSFG) Băneasa.

The experimental plot was established in early spring 2020 by planting three varieties of raspberry (*Rubus idaeus* L.): "Citria", "Przehyba" and "Tulameen", on different nutrient substrates (peat moss, semi-fermented compost, forest compost, and mixture of the 4 substrates in equal amounts), at distances of 3.0 m between rows and 0.5 m apart in each row.

The environment in which the plant grows and develops is one of the most important factors in agriculture. Substrates must be able to provide adequate water, nutrients and oxygen for the plant, as well as support for the whole plant. (Kang et al., 2004; Miller and Jones, 1995)

The better the substrate, the healthier and more vigorous the plant

Biochemical investigations were carried out at the Faculty of Biotechnologies of USAMV-Bucharest, using spectrophotometric determination methods.

Determination of total ascorbic acid content in fruit juices was performed by spectrophotometric method using potassium permanganate ($KMnO_4$) as chromogenic reagent (Zanini et al., 2018; Elgailani et al., 2017). The concentration of ascorbic acid in the samples was expressed in mg/L.

Determination of total anthocyanins content

Total anthocyanins content (TA) was carried out using the pH differential spectrophotometric method (Giusti & Wrolstad, 2000).

Determination of total phenolics

For determination of TPC, a method with Folin - Ciocalteu reagent (Sigma-Aldrich) (Singleton, 1999)

Determination of antioxidant activity

It was determined by two methods: the DPPH (2,2-diphenyl-1-picrylhydrazyl) method and the phosphomolybdate method for total antioxidant capacity.

Statistical analysis

All measurements were carried out in three replicates (n = 3) and results were presented as means ± standard deviations (SD). Anova test and Duncan test were performed with SPSS software.

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RESULTS AND DISCUSSIONS

The biological material consisted of three varieties of raspberry (*Rubus idaeus* L.): "Citria", "Przehyba" and "Tulameen", from the Băneasa Pomiculture Research and Development Station - Bucharest. A brief characterization of the three raspberry cultivars and the general appearance of the fruit are presented in Table 1.

Table 1. General description of the raspberry genotypes studied

Genotype	General description	The appearance of the fruit
"Citria"	**The "Citria" raspberry (origin Romania)** is a variety with yellow fruits. It forms a bush with a tall stem, with few branches and medium sprouting capacity. The fruits are medium-sized, short-shaped, conical, with small seeds, yellow in color and excellent taste. The planting period is spring and autumn, and the harvesting period in June. The fruits can be consumed fresh or processed. It is an extremely early variety, adaptable to different environmental conditions, resistant to frost and raspberry diseases and is very productive.	
"Przehyba"	**"Przehyba" (origin Poland)** is an early variety, created in recent years and appeared on the market in 2016. It is available from autumn 2017 for testing, and from 2018 for professional crops. It keeps very well, has good firmness and withstands transport in suitable packaging. The Przehyba raspberry belongs to seasonal varieties with an early ripening period. The fruits are elongated, cylindrical, of a bright red, of sizes that come out of any pattern, up to 5 cm in length, very sweet and tasty. It is a dessert variety.	
"Tulameen"	**The "Tulameen" raspberry variety (origin Canada**), is a variety that easily adapts to environmental conditions, being widespread in many climate zones sweet noted for its excellent flavor. For optimal development, it needs a rich, deep soil that retains water quite well but not with excess moisture and sunny exposure. The fruit is very large, weighing between 5-7 g, intense red, shiny, conical, elongated. Fruit characters of Tulamen, apparent derived from black raspberries,include late ripenng and relatively firm textured fruit.	

The soil at Moara Domnească is a reddish prevosol. Several soil profile analyses were carried out in the in-house agrochemical laboratory to determine the soil's physico-chemical properties.

The following soil characteristics were determined (by particle size analysis to determine the clay, dust and sand content of the soil): a high percentage of clay ranging from 40.55% in the upper horizon 0-40 cm to 41.63% at depths of 41-53 cm and 47.39% at depths greater than 54 cm (Table 2).

Table 2. The granulometric composition of the soil (Experimental Base Moara Domnească, 2019)

Horizon	Depth (cm)	Clay (%)	Coarse sand (%)	Fine sand (%)	Dust (%)	Texture
Ao	0-40	40.55	0.36	34.33	24.75	Clay loam
Ao/Bt	41-53	41.63	0.52	21.54	56.28	Clay loam
Bt	54-200	47.39	0.37	27.59	30.34	Clay loam
C	Over 200	36.18	0.42	32.04	32.04	Clay loam

The clay texture results in low nutrient mobility and poor soil water permeability.
Soil humus content is good in the first 40 cm of the profile, where most of the roots of young trees, reaching a value of 3.26%, then drops sharply to 1% in the Bt horizon profile (Table 3).

Table 3. Physical and chemical properties of the profile soil (Experimental Base Moara Domnească, 2019)

Properties \ Horizons	Ao	Ao/Bt	Bt	C
Humus (%)	3.26	1.87	1.0	1.0
Soluble Ca (mg/100 g soil)	55	32	32	30
Hydrolitic acidity (meq)	2.8	2.04	1.72	0.18
Exchangeable Bases (meq)	22.6	23.62	26.28	-
Total cation exchange capacity (meq)	28.65	28.04	30.01	-
Degree of saturation in bases (%)	78.94	84.28	87.53	-
pH	6.4	6.6	6.8	8.3
Total N (%)	0.144	0.102	0.075	0.07
Soluble P (mg / 100 g soil)	50	40	40	30

The pH is slightly acidic at the soil surface (6.4), reaching alkaline in the C horizon (8.3). Other indicators such as nitrogen index (NI), hydrolytic acidity, humus, organic carbon were determined during 2020
The climate at Moara Domnească is temperate continental
The plants were grown on different nutrient substrates (peat moss, semi-fermented compost, forest compost, and a mixture of the 4 substrates in equal amounts) (Table 4).

Table 4. Variants of nutrient substrates in which blackberry varieties are planted

Nr. crt.	Variants of nutrient substrates
1	Control
2	Bramble
3	Mushroom Compost
4	Forest Compost
5	Semifermented Compost
6	Mixture Compost 25 % of the 4 substrate variants

Biochemical determinations were performed by analyzing fresh raspberry fruit from "Citria", "Przehyba" and "Tulameen", Fruits were obtained from plants grown on different culture substrates: peat moss, compost from mushroom growing substrates, forest compost, semi-fermented compost and compost substrates, forest compost and semi-fermented compost). The plants produced fruit on all substrates used for their culture.

Determination of total ascorbic acid content

The determination of the total content of ascorbic acid in fruit juices was carried out by the spectrophotometric method using potassium permanganate ($KMnO_4$) as a chromogenic reagent (Zanini et al., 2018; Elgailani et al., 2017). The decrease in absorbance was measured when a potassium permanganate solution was reacted with ascorbic acid solution in acid medium.
The results for the total ascorbic acid content (g/L) of blackberry fruit are summarised in Table 5 and Figure 1.

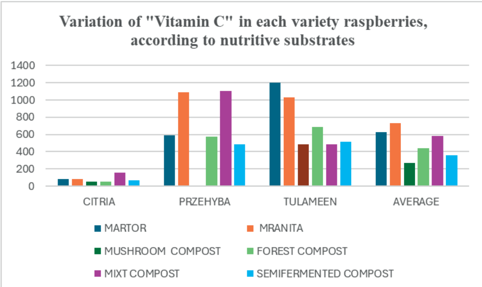

Figure 1. Variation in total ascorbic acid content (g/L) of three varieties of raspberry as a function of nutrient substrates

To raspberry fruits from the "Citria" variety obtained from the plants grown on different substrates, it was found that, compared to the control substrate (84.90 ± 1.02 mg/L), the highest amounts of vitamin C were recorded in the fruits of the plants grown on the substrate

(157.35 ± 1.20 mg/L) represented by the compost obtained from the mixture of 25% each of the other substrates taken into the study (Mraniţă; Mushrooms Compost; Forest compost; Semi-fermented compost).In the case of fruits from plants grown on the other substrates, the vitamin C content varied between 50.28 ± 0.47 mg/L and 79.40 ± 0.47 mg/L (Figure 1).

Table 5. Total ascorbic acid content (mg/L) of raspberry fruit

Descriptive Statistics

Dependent Variable: Total Ascorbic Acid (mg/l)

Substrat	Variety	Mean	Std. Deviation	N
CONTROL	CITRIA	84.90	1.02	3
	PRZEHYBA	592.13	0,61	3
	TULAMEEN	1204.29	1.72	3
	AVERAGE	627.11	1.12	9
BRAMBLE	CITRIA	79.4	0,47	3
	PRZEHYBA	1087	1.00	3
	TULAMEEN	1027.86	1.77	3
	AVERAGE	731.42	1.08	9
MUSHROOM COMPOST	CITRIA	50.28	0,47	3
	PRZEHYBA	-	-	3
	TULAMEEN	484.67	1.01	3
	AVERAGE	267.48	0.74	9
FOREST COMPOST	CITRIA	56.1	0,48	3
	PRZEHYBA	574.00	1.0	3
	TULAMEEN	688.33	1.04	3
	AVERAGE	439.48	0.84	9
MIXTURE COMPOST	CITRIA	157.35	1.20	3
	PRZEHYBA	1106	1.00	3
	TULAMEEN	485.17	1.04	3
	AVERAGE	582.84	1.08	9
SEMIFERMENTED COMPOST	CITRIA	67.72	0.50	3
	PRZEHYBA	485.67	0.76	3
	TULAMEEN	514.33	0.76	3
	AVERAGE	355.91	1.01	9

In the raspberry fruits of the "Przehyba" variety, the highest values of vitamin C compared to the control (592.13 ± 0.61 mg/L) were obtained in the fruits from the plants grown on the substrates (mixed compost) (1106 ± 1.0 mg/L) and (bramble) (1087 ± 1.0 mg/L) (Figure 1).

On the substrate variant (compost from mushroom culture) no fruit was obtained until the time of harvest, as a result the experiments could not be performed. On the substrate variant forest compost, the ascorbic acid content (574.00 ± 1.0 mg/L) was close to that obtained from the fruits of plants grown on the control variant (592.13 ± 0.61 mg/L). The lowest values of vitamin C, 485.67 ± 0.76 mg/L, were recorded in the fruits of plants grown on semi-fermented compost substrate (Table 5, Figure 1).

In the raspberry fruits from the "Tulameen" variety, the highest values regarding the total content of ascorbic acid (vitamin C) were obtained in the case of the fruits from plants grown on bramble substrate (1027.86 ± 1.77 mg/L) and from the control variant (1204.29 ± 1.72 mg/L), followed by the variant (forest compost substrate) with 688.33 ± 1.04. In the fruits obtained from the variants (484.67 ± 1.01 mg/L) (514.33 ± 0.76 mg/L) and (485.17 ± 1.04 mg/L), the values were relatively close (Table 5, Figure 1).

From the data regarding the total content of ascorbic acid (vitamin C) in the raspberry fruits obtained from the plants grown on the different culture substrates, it can be noted that the mixed compost substrate (V5) was conducive to the accumulation of vitamin C in large quantities in the case fruits from the "Citria" and "Przehyba" varieties. In the "Tulameen" raspberry variety, vitamin C recorded significant levels in the control and substrate variant V1 (bramble). The V1 variant was conducive to the accumulation of vitamin C also in the case of the "Przehyba" variety.

Among the 3 analyzed varieties, the highest levels of vitamin C are found in the "Tulameen" and "Przehyba" varieties.

Determination of total monomeric anthocyan

The intense red color of the raspberry itself is related to its composition, including the content of anthocyanins, the concentration of which is influenced by several factors, such as the variety, the stage of ripening and the climatic and soil characteristics of the cultivation areas, among others Anthocyanins, and by extension raspberry color, are important elements for growers because they improve consumers' perception of quality.

The results regarding the total content of anthocyanins (mg equivalent of cyanidin-3-glucoside/L) from raspberry fruits resulting from plants grown on different substrates are summarized in Table 6 and Figure 2.

Table 6. Total monomeric antocyan content (mg/L) of raspberry fruit

Descriptive Statistics				
Dependent Variable: Total Ascorbic Acid (mg/l)				
Substrat	**Soi**	**Mean**	**Std. Deviation**	**N**
CONTROL	CITRIA	1.4	0.1	3
	PRZEHYBA	50.6	0.65	3
	TULAMEEN	62.05	0.21	3
	AVERAGE	38.02	0.32	9
BRAMBLE	CITRIA	5.49	0.02	3
	PRZEHYBA	20.19	0.3	3
	TULAMEEN	76.33	0.6	3
	AVERAGE	34.00	0.31	9
MUSHROOM COMPOST	CITRIA	1.82	0.02	3
	PRZEHYBA			3
	TULAMEEN	66.5	0.49	3
	AVERAGE	34.16	0.26	9
FOREST COMPOST	CITRIA	6.33	0.24	3
	PRZEHYBA	6.7	0.99	3
	TULAMEEN	99.08	0.26	3
	AVERAGE	58.37	0.50	9
MIXTURE COMPOST	CITRIA	5.99	0.01	3
	PRZEHYBA	37	0.23	3
	TULAMEEN	82.92	0.21	3
	AVERAGE	41.97	0.15	9
SEMIFERMENTED COMPOST	CITRIA	4.99	0.01	3
	PRZEHYBA	40.96	0.11	3
	TULAMEEN	59.62	0.52	3
	AVERAGE	35.19	0.21	9

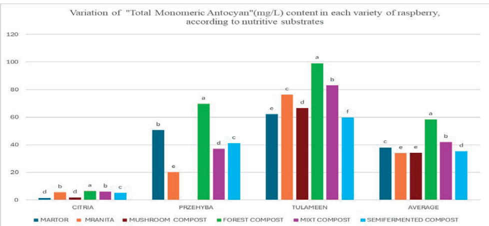

Figure 2. Variation of Total monomeric antocyan (mg/L) content of three varieties of raspberry as a function of nutrient substrates

The raspberry variety "Citria" is a variety with yellow fruits, as a result the total content of anthocyanins in mg cyanidin-3-glucoside equivalents / L was lower than in the raspberry varieties with red fruits.

In the results of this work, in the fruits of "Citria" obtained from plants grown on different substrates, high values of anthocyanins were noted, relatively close, in the case of the fruits obtained from the substrate variants of: forest compost (6.33 ± 0.24 mg/L), semi-fermented compost (5.99 ± 0.01 mg/L), bramble substrate (5.49 ± 0.02 mg/L) and respectively on the mixed compost substrate (4.99 ± 0.01 mg /IT). The lowest anthocyanin values were recorded in the control (1.40 ± 0.1 mg/L) and the substrate variant represented by compost from mushroom culture (1.82 ± 0.02 mg/L) (Table 6 and Figure 2).

In the raspberry variety "Przehyba" with bright red fruits, the highest amount of anthocyanins, 69.7 ± 0.99 mg ECy 3-Glu/L, was recorded in the case of the fruits of the plants grown on the forest compost variant (V3), followed by the control variant with 50.6 ± 0.65 mg ECy 3-Glu/L and respectively by V5 (mixed compost) with 40.96 ± 0.11 mg ECy 3-Glu/L and V4 (semi-fermented compost) with 37.0 ± 0.23 mg ECy 3-Glu/L. The lowest values of anthocyanins were recorded in the fruits of the plants grown on the V1 variant of the bramble substrate (20.19 ± 0.30 mg ECy 3-Glu/L) (Table 6 and Figure 2).

In the **raspberry variety "Przehyba"** with bright red fruits, the highest amount of anthocyanins, 69.7 ± 0.99 mg ECy 3-Glu/L, was recorded in the case of the fruits of the plants grown on the forest compost variant (V3), followed by the control variant with 50.6 ± 0.65 mg ECy 3-Glu/L and respectively by V5 (mixed compost) with 40.96 ± 0.11 mg ECy 3-Glu/L and V4 (semi-fermented compost) with 37.0 ± 0.23 mg ECy 3-Glu/L. The lowest values of anthocyanins were recorded in the fruits of the plants grown on the V1 variant of the bramble substrate (20.19 ± 0.30 mg ECy 3-Glu/L) (Table 6 and Figure 2).

In the **"Tulameen" raspberry variety** with intense red fruits, the results revealed that the highest content of anthocyanins was in the fruits from plants grown on the forest compost substrate (V3), with 99.08 ± 0.26 mg ECy 3-Glu/L, followed by the data obtained from the fruits of plants grown on V4 - semi-fermented compost - with 82.92 ± 0.21 mg ECy 3-Glu/L and V1 - blackberry with 76.33 ± 0.60 mg ECy 3-Glu/L. On the variants of substrates V2 (compost from mushrooms), control substrate (C) and V5 (mixed substrate) the results were relatively close. The lowest values of the anthocyanin content were recorded for the substrate variant V5 - compost mixture (Table 6 and Figure 2).

Following the results obtained regarding the content of anthocyanins in the fruits of plants grown on different substrates, it is noted that the most favorable substrate for the accumulation of anthocyanins in the fruits was represented, for all three raspberry varieties, by the compost substrate of forest (V3). Among the 3 raspberry varieties analyzed, the "Tulameen" and "Przehyba" varieties have the highest levels of anthocyanins.

Anthocyanins are a group of natural phenolic compounds responsible for the color of many plants, flowers and fruits. These compounds are of great importance due to their proven pharmacological activities. Forest fruit extracts (*Rubus* spp.) contain large amounts of anthocyanins, and their consumption either in food or in some therapeutic applications is common. The anthocyanin composition of the fruits of different species of Rubus species are quite distinct. Raspberry (*R. idaeus*) contains a distinct spectrum of anthocyanins (Dugo et al., 2001). The major components are cyanidin and cyanidin-3-sophoroside, with smaller amounts of other anthocyanins, including cyanidin-3-(2G-glucosylrutinoside), cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin and its glycosides. More recently, Chen et al. (2007) established an ultrasound-assisted extraction of anthocyanins from *R. idaeus*. The anthocyanin composition of the extracts was identified by high-performance liquid chromatography-mass spectrometry. The content of anthocyanins, found in large quantities in red raspberries, is influenced by several factors, such as: the variety, the ripening stage and the climatic and pedological characteristics of the cultivation areas, among others (Stamenkovic et al., 2019).

Determination of total phenol content

The total phenolic content, expressed as gallic acid equivalent, **in "Citria" with yellow fruits,** originating from plants grown on different substrates, varied from 355.86 ± 1.78 mg EAG/L, with the highest value (recorded in the sample control), at 175.38 ± 1.26 mg EAG/L (value recorded in samples V5- compost mixture). Among the samples from plants grown on different substrates, the highest values were recorded in sample V4 – semi-fermented compost (197.32 ± 1.13 mg EAG/L). The other samples had close values (Table 5 and Figure 2). **In the red fruits of raspberry cv. "Przehyba"**, the highest values (125.67 ± 1.13 mg EAG/L) were recorded in sample V3 (forest compost), and the lowest (85.78 ± 0.80 mg EAG/L) in sample V1 (bramble). With the exception of the compost substrate variant from mushroom culture for which no fruits were obtained until the analysis was carried out, the control sample (M) and the other samples (V4 and V5) recorded close values (Table 7 and Figure 3 . **In the case of red raspberry fruits cv. "Tulameen"**, the most significant values were noted in samples V4 (256.43 ± 0.89 mg EAG/L) and V3 (220.41 ± 1.13 mg EAG/L), and the lowest in the control sample (137.41 ± 0.88 mg EAG/L) (Table 7 and Figure 3).

As noted from the obtained results, the concentration of polyphenols can vary depending on the variety and the specific nutritional needs of each variety, provided by the culture substrate. Probably, the raspberry studied had different climatic and agrotechnical conditions during growth, which it could have a significant impact on the amount of analyzed compounds. Contaminants from fertilizer use and the lower nutritional quality of the fruit could affect the low concentration of these compounds.

Table 7. Total Poliphenoli content (mg EAG/L) of raspberry fruit

Descriptive Statistics

Dependent Variable: Total Ascorbic Acid (mg/l)

Substrat	Variety	Mean	Std. Deviation	N
CONTROL	CITRIA	355.86	1.78	3
	PRZEHYBA	110.51	1.02	3
	TULAMEEN	137.41	0.88	3
	AVERAGE	201.26	1.23	9
BRAMBLE	CITRIA	188.08	0.47	3
	PRZEHYBA	85.78	0.80	3
	TULAMEEN	176.74	1.23	3
	AVERAGE	150.20	0.83	9
MUSHROOM COMPOST	CITRIA	187.72	1.48	3
	PRZEHYBA			3
	TULAMEEN	169.33	1.41	3
	AVERAGE	178.53	1.45	9
FOREST COMPOST	CITRIA	181.22	0.83	3
	PRZEHYBA	125.67	1.13	3
	TULAMEEN	220.41	0.89	3
	AVERAGE	175.77	0.95	9
MIXTURE COMPOST	CITRIA	197.32	1.13	3
	PRZEHYBA	112.23	1.24	3
	TULAMEEN	256.43	0.89	3
	AVERAGE	188.66	1.09	9
SEMIFERMENTED COMPOST	CITRIA	175.38	1.26	3
	PRZEHYBA	115.74	0.81	3
	TULAMEEN	166.49	1.24	3
	AVERAGE	152.54	1.10	9

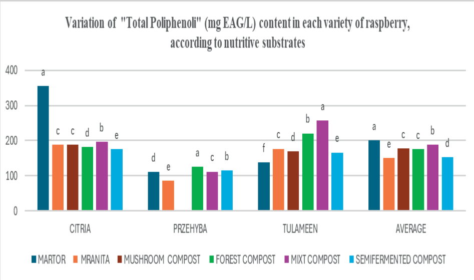

Figure 3. Variation of total poliphenoli content (mg EAG/L) of three varieties of raspberry as a function of nutrient substrates

Polyphenols can act in several ways: as reducing agents, as compound that blocks free radicals, chelates metal ions that catalyze oxidation reactions (thus preventing reactions caused by a single active oxygen atom) and inhibits the activity of oxidative enzymes such as lipoxygenases. Polyphenols have an ideal chemical structure to scavenge free radicals and form chelates with metals, making them more effective than antioxidant vitamins (Ruskovska et al., 2020; Kruk and et al., 2022).

Determination of Total oxidant capacity (µg/ml)

The total antioxidant capacity of the samples was evaluated by the phosphomolybdate method, according to the procedure described by Prieto (Prieto et al., 1999). The results were expressed in µg equivalent of ascorbic acid/ml.

In the phosphomolybdenum test, which is a quantitative method for evaluating antioxidant capacity, all analyzed samples presented different degrees of activity, as shown in Table 7 and Figure 2. **In the "Citria" cultivar**, all the samples obtained from different substrates showed an antioxidant activity ranging between 306.92 ± 1.77 µg/ml of ascorbic acid (for the V1 variant - bramble substrate) and 202.79 ± 0.78 µg/ml of ascorbic acid (at V2 – mushroom compost). A significant total antioxidant activity of 284.01 ± 1.54 µg/ml of ascorbic acid was also recorded in fruit samples from plants grown on semi-fermented compost (V4).

In the "Przehyba" cultivar, the highest antioxidant activity was recorded in fruit samples from plants grown on forest compost substrate (V3) (206.76 ± 0.63 µg/ml of ascorbic acid), on semi-fermented compost substrate (V4) (143.47 ± 0.17 µg/ml of ascorbic acid) and on mixed compost substrate (V5) (138.53 ± 0.42 µg/ml). The lowest antioxidant activity was presented by the fruit samples from the plants grown on bramble substrate (V1) (108.77 ± 0.16 µg/ml). On the substrate variant V2 - the fruits did not develop.

In the "Tulameen " variety, in the raspberry samples from different substrates, a significant total antioxidant capacity is possessed by the fruit samples from the forest compost substrate (V3) (211.35 ± 0.63 µg/ml) and respectively those from the substrate of sea buckthorn (V1) (201.48 ± 1.49 µg/ml). The sample from the mushroom compost substrate (V2) presented the lowest antioxidant activity, equivalent to 143.16±0.77 µg/ml of ascorbic acid (Table 8, Figure 4).

Table 8. Total oxidant capacity(µg/ml) content of raspberry fruit

Descriptive Statistics				
Dependent Variable: Total Ascorbic Acid (mg/l)				
Substrat	**Variety**	**Mean**	**Std. Deviation**	**N**
CONTROL	CITRIA	275.92	1.05	3
	PRZEHYBA	141.23	0.25	3
	TULAMEEN	232.37	1.21	3
	AVERAGE	216.51	0.84	9
BRAMBLE	CITRIA	306.92	1.77	3
	PRZEHYBA	108.77	0.16	3
	TULAMEEN	201.48	1.49	3
	AVERAGE	205.72	1.14	9
MUSHROOM COMPOST	CITRIA	202.79	0.78	3
	PRZEHYBA			3
	TULAMEEN	143.16	0.77	3
	AVERAGE	172.98	0.78	9
FOREST COMPOST	CITRIA	227.31	1.18	3
	PRZEHYBA	206.76	0.63	3
	TULAMEEN	211.35	0.63	3
	AVERAGE	215.14	0.81	9
MIXTURE COMPOST	CITRIA	284.01	1.54	3
	PRZEHYBA	143.47	0.17	3
	TULAMEEN	170.51	0.13	3
	AVERAGE	199.33	0.61	9
SEMIFERMENTED COMPOST	CITRIA	230.49	1.61	3
	PRZEHYBA	138.53	0.42	3
	TULAMEEN	161.19	1.06	3
	AVERAGE	176.74	1.03	9

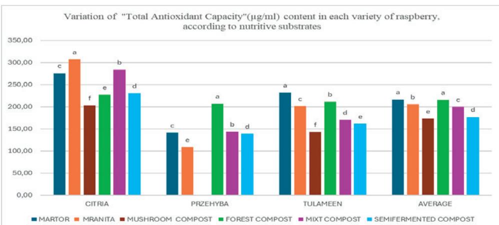

Figure 4. Variation of Total oxidant capacity (µg/ml) content of three varieties of raspberry as a function of nutrient substrates

Determination of antioxidant activity using DPPH radical scavenging activity . Fruit samples were evaluated for antioxidant activity using the DPPH method and were expressed as percentage DPPH · inhibition (Table 9)

Table 9. Total Antioxidant activity of the samples expressed as DPPH (RSA %) content of raspberry fruit

variants	Substrate type/variety	DPPH RSA%*		
		Citria	Przehyba	Tulameen
m	Blank substrate	**87.14±0.38**	**94.37±0.24**	**88.22±0.48**
V1	garden soil	61.38±0.48	59.92 ±0.79	82.01±0.33
V2	Compost from mushrooms	78.58±0.09	when	**90.79±0.45**
V3	Forest compost	77.26±0.98	59.93±0.79	80.61±0.66
V4	Semi-fermented compost	**88.30±0.25**	**79.93±0.73**	83.50±0.86
V5	Compost mix	**88.40±0.09**	**77.48±0.20**	**88.11±0.57**

Regarding the antioxidant activity of the plant raspberry fruits, significant differences were found between the control samples of the three cultivations and the tested samples. **In "Citria",** a strong antioxidant activity - with approximately the same values, was found both in samples V4 - semi-fermented compost (88.30 %) and V5 - compost mixture (88.40%), as well as in the control sample (87.14%) (Table 9). **In "Przehyba** ", a high antioxidant activity was registered especially in the control sample (94.37%) and similar to "Citria", in samples V4 - semi-fermented compost (79.93%) and V5 - mixed compost (77.48%). **The fruits of the "Tulameen" variety** also recorded antioxidant activities of over 80%, among which the sample grown on mushroom compost substrate (V2) (90.79%) stands out.

The strong antioxidant activities of these raspberry samples may be a possible result of the high level of phenolic compounds and flavonoids that have been shown to have antioxidant properties (Zheng et al., 2009). These results were consistent with previous studies that reported that phenolic compounds in various plant extracts are the major constituents with the free radical scavenging property of donating a hydrogen atom from their phenolic phenolic hydroxyl (Wang et al., 2011).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical that is frequently used in the measurement of antioxidant activities due to the following strengths: direct inhibition measurement, simplicity, and rapid analysis (Mammadov et al., 2011).

The results of the antioxidant evaluation based on the two models (DPPH and Total Antioxidant Capacity) used in this study , showed that raspberry samples from plants grown on different substrates possess an interesting antioxidant activity that varies depending on the culture substrate and the variety of raspberries that benefited from these substrates.

Table 10 includes the data obtained regarding the biochemical compounds with antioxidant activity in raspberry fruits in correlation with their culture substrates.

Table 10. Biochemical compounds with antioxidant activity in raspberry fruits harvested from plants grown on different substrates

	The variety	VIT. C (mg/L)	ANTHOCYANS (mg/L)	Phenols mg GAE/L	DPPH RSA%	CAT µg/ml Total antioxidant capacity
1	**Citria -witness**	**84.90±1.02**	**1.40±0.1**	**355.86±1.78**	**87.14±0.38**	**275.92±1.05**
2	**V1**	79.40±0.47	5.49±0.02	188.08±0.47	61.38±0.48	306.92±1.77
3	**V2**	50.28±0.47	1.82±0.02	187.72±1.48	78.58±0.09	202.79±0.78
4	**V3**	56.10 ± 0.48	6.33±0.24	181.22±0.83	77.26±0.98	227.31±1.18
5	**V4**	67.72 ± 0.50	5.99 ± 0.01	197.32±1.13	88.30±0.25	284.01±1.54
6	**V5**	157.35 ± 1.20	4.99 ± 0.01	175.38±1.26	88.40±0.09	230.49±1.61
7	**Przehyba - witness**	**592.13±0.61**	**50.6 ± 0.65**	**110.51±1.02**	**84.37±0.76**	**141.23±0.25**
8	**V1**	**1087 ± 1.0**	20.19±0.30	85.78±0.80	59.92 ±0.79	108.77±0.16
9	**V2**					
10	**V3**	574.00 ± 1.0	69.69 ± 0.99	125.67±1.13	59.93±0.79	206.76±0.63
11	**V4**	485.67 ± 0.76	37.00±0.23	112.23±1.24	79.93±0.73	143.47±0.17
12	**V5**	1106 ± 1.0	40.96±0.11	115.74±0.81	77.48±0.20	138.53±0.42
13	**Tulameen - witness**	**1204.29 ± 1.72**	**62.05 ± 0.21**	**137.41±0.88**	**88.22±0.48**	**232.37±1.21**
14	**V1**	1027.86 ± 1.77	76.33±0.60	176.74±1.23	82.01±0.33	201.48±1.49
15	**V2**	484.67 ± 1.01	66.5±0.49	169.33±1.41	90.79±0.45	143.16±0.77
16	**V3**	688.33 ± 1.04	99.08±0.26	220.41±.1.13	80.61±0.66	211.35±0.63
17	**V4**	514.33 ± 0.76	82.92±0.21	256.43±0.89	83.50±0.86	170.51±1.03
18	**V5**	485.17 ± 1.04	59.62±0.52	166.49±1.24	88.11±0.57	161.19±1.06

M: Witness; V1: Bramble; V2: Compost from mushrooms; V3: Forest compost; V4: Semi-fermented compost; V5: Compost mix.
* Data are expressed as mean and standard deviation (±SD) of three replicates (n= 3).

CONCLUSIONS

The results of the study led to the following conclusions:

- From the data regarding **the total content of ascorbic acid (vitamin C)** in the raspberry fruits obtained from the plants grown on the different culture substrates, it was noted that the **mixed compost substrate (V5)** was conducive to the accumulation of vitamin C in a significant amount in the case of the fruits from the "**Citria" and "Przehyba**" varieties.
- **"Tulameen"** raspberry variety , vitamin C recorded significant levels both in **the control variant and in the substrate variant V1 (mraniță**).
- The bramble substrate was conducive to the accumulation of vitamin C also in the case of the "Przehyba" variety.
- Among the 3 raspberry varieties analyzed, the highest levels of vitamin C were found in the "Tulameen" and "Przehyba" varieties (the last one without the V2 substrate variant);
- Following the results obtained regarding the content of anthocyanins in the fruits of plants grown on different substrates, it was noted that the **most favorable substrate for the accumulation of anthocyanins in the fruits was represented, in all three raspberry varieties, by the compost substrate of forest (V3).**
- Among the 3 raspberry varieties analyzed, the "Tulameen" and "Przehyba" varieties had the highest levels of anthocyanins.
- **For cv. "Citria"** with yellow fruits, the most favorable substrate both for the accumulation of phenolic compounds and for the two antioxidant activity evaluation models was the **semi-fermented compost substrate (V4)**; A significant accumulation of phenols, with a high total antioxidant activity, was also recorded in the case of "Citria" raspberry fruits grown on bramble substrate (V1);
- **For cv. "Przehyba"** with red fruits, the most favorable substrate for the three parameters analyzed was also **semi-fermented compost (V4);** Significant results were also obtained in the case of fruits from the substrate represented by forest compost, but only for the supply of phenols and for an increased total antioxidant capacity;
- **For cv. "Tulameen"** with red fruits, for the accumulation of phenolic compounds in high quantities, the favorable substrates were **forest compost (V3)** and semi-fermented compost **(V4)**; For the accumulation of compounds with DPPH antioxidant activity, **the growth substrates V2 (compost from mushrooms)** and **V5 (compost mixture) stood out** , and for the total antioxidant capacity and viability , the best results were obtained in the case of the samples from substrates V1 (bramble) and V3 (forest compost).

The results obtained may vary depending on the variety and the specific nutritional needs of each variety, provided by the culture substrate. Probably, the raspberry studied had different climatic and agrotechnical conditions during growth, which it could have a significant impact on the amount of analyzed compounds. Contaminants from fertilizer use and the lower nutritional quality of the fruit could affect the low concentration of these compounds.

REFERENCES

Braca, A., Tommasi, ND, Bari, LD, et al. (2001). Antioxidant Principles from *Bauhinia tarapotensis*. *Journal of Natural Products*, 64, 892-895.

Chen F, Sun Y, Zhao G, Liao X, Hu X, Wu J, Wang Z. (2007). Optimization of ultrasound-assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using high-performance liquid chromatography-mass spectrometry. *Ultrasonics Sonochemistry*, 14, 767-778.

De Santis, D.; Carbone, K.; Garzoli, S.; Laghezza Masci, V.; Turchetti, G. (2022). Bioactivity and Chemical Profile of *Rubus idaeus* L. Leaves Steam-Distillation Extract. *Foods,* 11, 1455.

Dugo P., Mondello L., Errante G., Zappia G., Dugo G. (2001). Identification of anthocyanins in berries by narrow- base high performance liquid chromatography with electrospay ionization detection. *Journal of Agricultural and Food Chemistry*, 49, 3987-3992.

Elgailani H. Isam Eldin, Mohamed A.M. Gad-Elkareem; Elnoor A.A. Noh; Omer E.A. Adam; Ahmed M.A. Alghamdi (2017). Comparison of Two Methods for the Determination of Vitamin C (Ascorbic Acid) in Some Fruits. *American Journal of Chemistry*, 2(1): 1-7.

Giusti MM, Wrolstad RE (2000). *Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy*, John Wiley & Sons, Inc., 2000.

Gülcin, I., Topal, F., Cakmakc¸ R., Bilsel, M., Ahmet C. Gören, CA, Erdogan, U. (2011). Pomological

Features, Nutritional Quality, Polyphenol Content Analysis, and Antioxidant Properties of Domesticated and 3 Wild Ecotype Forms of Raspberries (*Rubus idaeus* L.). *Journal of Food Science*, 764.

Kruk, J.; Aboul-Enein, B.H.; Duchnik, E.; Marchlewicz, M. (2022). Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. *J. Physiol. Sci*., 72, 19.

Lee J., Durst R.W., Wrolstad R.E. (2005). Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *Journal of AOAC International*, Vol. 88, No. 5, 1269 – 1278.

Mammadov R., Ili, P. and Ertem Vaizogullar H. (2011). Antioxidant activity and total phenolic content of *Gagea fibrosa* and *Romulea ramiflora*. *Iran J. Chem. Chem. Eng.*, 30: 57-62.

Papaioanou, M., Chronopoulou, GE, Ciobotari, G., Efrose, CR, Duke, Chatzikonstantino MLS, Pappa, E., Ganopoulos, I., Madesis, P., Obeidat, NI, Zeng, T., Labrou, EN (2018). Cosmeceutical Properties of Two Cultivars of Red Raspberry Grown under Different Conditions.*Cosmetics*, 520.

Prieto P., Pineda M., Aguilar M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem*, 269: 337-341.

Ruskovska, T.; Maksimova, V.; Milenkovic, D. (2020). Polyphenols in human nutrition: From the in vitro antioxidant capacity to the beneficial effects on cardiometabolic health and related inter-individual variability - An overview and perspective. *Bro. J. Nutr*., 123, 241–254.

Schulz, M.; Chim, J.F. (2019). Nutritional and bioactive value of *Rubus berries. Food* Biosci., 31, 100438.

Singleton V.L., Orthofer R., Lamuela -Raventos R.M., Lester P. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteu reagent. *Meth. Enzymol*, 299, 152-178.

Stamenkovic, Z.; Pavkov, I.; Radojˇcin, M.; Tepi´c Horecki, A.; Kešelj, K.; Bursac Kovacevic, D.; Putnik, P. (2019). Convective Drying of Fresh and Frozen Raspberries and Change of Their Physical and Nutritive Properties. *Foods*, 8, 251.

Wang W., Yagiz Y., Buran T.-J., Nunes C.-N. and Gu L. (2011). Phytochemical from berries and grapes inhibited the formation of advanced glycation endproducts by scavenging reactive carbonyls, *Food Res. International*, 44: 2666-2673.

Wu, L.; Yang, J.; Wang, C.; Li, N.; Liu, I.; Duan, A.; Wang, T. (2022). Chemical compositions of raspberry leaves influenced by growth season, cultivars and leaf position. *Sci. Hortic*., 304, 111349.

Zanini D.J., Silva M.H., Aguiar-Oliveira E., Mazalli M.R., Kamimura E.S., Maldonado R.R. (2018). Spectrophotometric analysis of vitamin C in different matrices using potassium permanganate. *European International Journal of Science and Technology*, Vol. 7 No. 1, pp 70-84.

Zheng G.M., Xu L.X., Wu P., Xie H.H., Jiang Y.M., Chen F. and Wei X.Y. (2009). Polyphenols from longan seeds and their radical-scavenging activity, *Food Chem*., 116: 433-436.