

EVALUATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF ROMANIAN BERRIES

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Received on 30 June 2022

Revised on 22 August 2022

Abstract

The objective of this paper was to evaluate seven different berries most consumed in Romania in order to determine the bioactive compounds: total phenolics content, total flavonoid content, total anthocyanins content, antioxidant activity by DPPH and total antioxidant capacity (water-soluble and lipid-soluble by photochemiluminescence assay, PCL). The analyzed fruits contain significant amounts of phenolic compounds between 18.91 and 383.06 mg gallic acid equivalents per 100 grams of fresh berries. Black currant extract had the highest content of total phenolic compounds, 383.06 mg GAE/100g, while the lowest level was obtained for the gooseberry, 18.91 mg GAE/100g. DPPH values varied between 22.15 and 892.35 μ mol Trolox Equivalent/100g fresh berries. Strong positive correlations between the antioxidant capacity of lipid-soluble compounds measured by PCL and DPPH was found ($r= 0.9153$). The results obtained demonstrated that different Romanian berries studied show a great antioxidant activity with possible benefits on human health.

Keywords: berries, phenolics, flavonoids, anthocyanins, antioxidant activity

Introduction

A diet rich in fruits and vegetables is universally recommended due to its beneficial properties on human health. It is known that fruits and other plant-based foods contain different levels of bioactive compounds, minerals and fiber (Samtiya *et al.*, 2021). Furthermore, fresh fruits contain an abundance of vitamins like vitamin C, vitamin A, vitamin B6, vitamin E, etc. (Abobatta, 2021). The last studies suggested that the total antioxidant capacity of fruits and vegetables is highly correlated with the content of total phenolics (Lee *et al.*, 2015). Regular consumption of fruits and

vegetables prevents the appearance of many diseases such as cardiovascular, chronic, diabetes and cancer (Lockyer *et al.*, 2016).

Berries are generally consumed as fresh fruits. Nowadays, a great number of products are available worldwide, so the production of beverages and confectioneries have grown significant in the last period (Pap *et al.*, 2021). In the last decade, berries have attracted much interest due to their diverse range of bioactive compounds (phenolics such as anthocyanins, flavonols, and flavanols), nutrients and their antioxidant properties (Paredes-Lopez *et al.*, 2010). Phenolic compounds are found in the largest proportion in berries (Sellappan *et al.*, 2002). You *et al.* (2011) have concluded that berries are the main source of phenolics especially anthocyanins. The content of anthocyanins is positively associated with the antioxidant activity of berries (Fernandes *et al.*, 2010).

Berries, especially strawberry, raspberry, blackberry, blueberry, and cranberry are an important dietary sources of bioactive compounds. These fruits contain significant amounts of phenolic compounds such as phenolic acids, flavonoids-flavonols, anthocyanins, tannins, and ascorbic acid and may act as strong antioxidants and, thus, could help in the prevention of numerous diseases, or have protective effects to lower the risk of various cancers (Česonienė *et al.*, 2009). The bioactive compounds are of great interest for nutritionists and food technologists due to the opportunity to use bioactive compounds as functional food ingredients (Skrovankova *et al.*, 2015).

Berries are grown in some regions from Romania. Thus, this paper aimed to characterize some berries: raspberry (*Rubus idaeus*), blackberry (*Rubus fruticosus*), blueberry (*Vaccinium ashei*), black currant (*Ribes nigrum*), red currant (*Ribes rubrum*), white currant (*Ribes rubrum 'White Grape'*), and gooseberry (*Ribes uva-crispa*), grown in Romania as possible sources of phenolics for functional foods application. The different types of berries were analyzed for the content of total anthocyanins, total flavonoids, total polyphenols, Trolox-equivalent (TE) antioxidant activity using DPPH method, and total antioxidant capacity (lipid and water soluble).

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), (+)-rutin, gallic acid, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma Chemical Co. (Switzerland). Folin–Ciocalteu's phenol reagent was purchased from Merck (Germany). All chemicals used were of analytical grade. Standard solutions were prepared with distilled deionized water.

Plant material

The following types of fruits were considered: raspberry (*Rubus idaeus*), blackberry (*Rubus fruticosus*), blueberry (*Vaccinium ashei*), black currant (*Ribes nigrum*), red currant (*Ribes rubrum*), white currant (*Ribes rubrum 'White Grape'*), and gooseberry (*Ribes uva-crispa*). The selected fruits were obtained from markets (Romania). The fruits were homogenized through a mixer.

Extraction procedure

An amount of 3.0 g of berries was weighed and brought into 30 mL of 50% aqueous methanol. For the ACW and ACL methods, fresh fruits were homogenized with 30 ml of water and 30 ml of methanol, respectively (Popov and Lewin, 1996). The extracts were vortexed for 3 hours at 10,000 rpm (Heidolph Instruments, Multi Reax). Then, the extracts were centrifuged for 10 min at 10,000 rpm to remove the secondary materials (Corbu *et al.*, 2021). The samples were purchased one day before the analysis and were stored in the refrigerator at 4 °C.

Determination of Total Phenolic Content

Total phenolic content (TPC) was determined by the Folin–Ciocalteu method with minor modifications (Singleton *et al.*, 1999). A total of 500 µL of extract was mixed with 10 µL Folin–Ciocalteu reagent, 90 µL distilled water, and 10 µL of saturated sodium carbonate. The samples were allowed to stand in the dark for 20 min for color development. Absorbance was measured at 765 nm using a Specord 210 UV-VIS (Analytic Jena, Germania) spectrophotometer. A standard curve was prepared by using different concentrations (10–50 µg/mL) of gallic acid in the same conditions with samples ($R^2 = 0.9993$). Total phenolic content was expressed as mg gallic acid equivalent/100g of fruit (mg GAE/100g fresh product).

Determination of Total Flavonoid Content

Total flavonoid content (TFC) was assessed through the $AlCl_3$ method described by Woisky and Salatino (1998). Briefly, 0.1 mL extract/standard solution was mixed with 0.1 mL 10% sodium acetate and 0.12 mL 2.5% $AlCl_3$, the final volume being adjusted to 1 mL with ethanol 70%. The samples were then vortexed and incubated in the dark for 45 min. The absorbance was measured at 510 nm. A standard curve was plotted by using different concentrations (10–60 µg/mL) of rutin ($R^2 = 0.9996$). Total flavonoid content was expressed as mg rutin equivalent/100g of fruit (mg Ru/100g fresh weight).

Determination of Anthocyanins Content

The method consists in extracting anthocyanins in 30 ml buffers with pH = 1.0 and pH = 4.5, followed by reading the absorbances at 520 and 700 nm (Okan *et al.*, 2018). The results are expressed in mg cyanidin-3-glucoside equivalent and are calculated by the formula:

$$\text{cyanidin-3-glucoside (mg)} = A \cdot M \cdot FD \cdot 103 / \epsilon \cdot l,$$

where A = (A₅₂₀ - A₇₀₀) pH₁ - (A₅₂₀ - A₇₀₀) pH_{4.5}; M = 449.2 g/mol (molecular weight of cyanidin-3-glucoside); FD = dilution factor; ϵ = molar extinction coefficient of cyanidin-3-glucoside, 26900; l = length of the measuring tank.

DPPH Radical Scavenging Capacity

DPPH radical scavenging activity was determined based on the reduction in DPPH radical, according to Culetu *et al.* (2016) with slight modifications. The reaction

mixture consisted of 1 mL of methanolic extract and 6 mL of DPPH radical solution, which was incubated for 20 min in the dark. Then, the absorbance was measured at 517 nm. Antioxidant activity was calculated using a calibration curve (0.0156–0.0625 µg/mL) obtained with Trolox ($R^2 = 0.9998$). The results were expressed in µmol TE/100g fresh fruit.

Photochemiluminescence Assay – hydrophilic system (PCL-ACW)

The scavenging activity of fruits was evaluated by a photochemiluminescence (PCL-ACW) method in which superoxide radical anions (O_2^-) are generated from luminol. The extracts were dissolved in water. The reactions were carried out using kits for the determination of antioxidant capacity of water-soluble substances (Analytik Jena, Jena, Germany), mixing 1500 µL of water (reagent 1), 1000 µL of buffer solution (reagent 2), 25 µL of luminol (reagent 3), and 10 µL of extract. Measurement was performed on a Photochem device with PCL soft software (Analytik Jena). Vitamin C was used to prepare the calibration curve. The results are expressed as µmol of vitamin C equivalents per 100 g of fresh fruit. The extraction was made in triplicate.

Photochemiluminescence Assay – lipophilic system (PCL-ACL)

The scavenging activity of fruits was evaluated by a photochemiluminescence method in which superoxide radical anions (O_2^-) are generated from luminol. The extracts were dissolved in methanol. The reactions were carried out using kits for the determination of antioxidant capacity of lipid-soluble substances (Analytik Jena, Jena, Germany), mixing 2300 µL of methanol (reagent 1), 200 µL of buffer solution (reagent 2), 25 µL of luminol (reagent 3), and 10 µL of extract. Measurement was performed on a Photochem device with PCL soft software (Analytik Jena). Trolox was used to prepare the calibration curve. The results are expressed as µmol of TE per 100 g of fresh fruit. The extraction was made in triplicate.

Statistical analysis

Analyses were performed in triplicate and expressed as mean \pm standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey test to assess differences between means (Minitab software). Differences were considered to be significant at $p < 0.05$.

Results and discussion

Total phenolics, flavonoids and anthocyanins

The analyzed berries contain phenolic compounds levels between 18.91 and 383.06 mg GAE/100g fresh weight (FW). Black currant extract resulted in the highest level of total polyphenols (383.06 mg GAE/100gFW), followed by blueberries (174.41 mg GAE/100gFW) and blackberries (157.08 mg GAE/100gFW). The lowest content was obtained for gooseberry (18.91 mg GAE/100gFW). These levels were comparable with results described in the literature for other extracts of berries (Benvenuti *et al.*, 2004; You *et al.*, 2011; Giovanelli *et al.*, 2012; Okatan *et al.*, 2017).

The concentration of polyphenols in each type of berry is significantly different ($p < 0.05$) except raspberry and white currant with $p > 0.05$.

Table 1. Phenolic content and flavonoid content in the analyzed Romanian berries.

Type of berries	Total phenolics (mg GAE/100g FW)	Total flavonoids (mg rutin/ 100g FW)	Total anthocyanins (mg CGE/ 100g FW)	DPPH-RSA ($\mu\text{mol TE}/$ 100g FW)
Raspberry	99.55 \pm 0.48 ^d	10.44 \pm 0.36 ^e	14.86 \pm 0.52 ^c	214.09 \pm 1.36 ^f
Blackberry	157.08 \pm 1.75 ^c	24.72 \pm 0.81 ^c	44.7 \pm 1.58 ^b	465.10 \pm 1.21 ^d
Blueberry	174.41 \pm 1.24 ^b	40.88 \pm 0.12 ^a	56.76 \pm 0.76 ^a	696.83 \pm 2.19 ^c
Black currant	383.06 \pm 0.13 ^a	29.55 \pm 0.76 ^b	53.84 \pm 0.25 ^a	892.35 \pm 1.96 ^a
Red currant	62.77 \pm 0.59 ^c	11.95 \pm 0.20 ^{d,e}	45.95 \pm 1.20 ^b	122.62 \pm 0.69 ^b
White currant	99.73 \pm 0.16 ^d	13.27 \pm 0.45 ^d	0.026 \pm 0.004 ^d	219.29 \pm 1.32 ^c
Gooseberry	18.91 \pm 0.68 ^f	3.03 \pm 0.09 ^f	0.022 \pm 0.0007 ^d	22.15 \pm 0.32 ^g

The values are expressed as means \pm standard deviations ($n = 2$). Values followed by different letters in the same column are significantly different ($p < 0.05$).

The flavonoid content of tested berries was between 3.03 and 40.88 mg rutin/100gFW (Table 1). The results showed that blueberries have the highest flavonoid content (40.88 mg rutin/100gFW), while gooseberries have the lowest flavonoid content (3.03 mg GAE/100gFW). The flavonoid content represents 10.49% of the total polyphenols for raspberries, 15.74% for blackberries, 23.44% for blueberries, 7.71% for black currant, 19.04% for red currant, 13.31% for white currant, and 16.02% for gooseberries. The flavonoid content of the analyzed berries varied significantly ($p < 0.05$), except for red currant-white currant and red currant-raspberry which presented similar concentrations ($p > 0.05$).

Marinova *et al.* (2005) analyzed 42 food products – 20 fruit and 22 vegetable species including blackberries, raspberries and blueberries. The data clearly outlined that these berries are a rich source of polyphenols: blackberries, 355.3 mg GAE/100gFW, raspberries, 178.6 mg GAE/100gFW, blueberries, 670.90 mg GAE/100gFW. Also, the results indicated high levels of flavonoids, blackberries, 55.5 mg catechin/100gFW, raspberries, 26.6 mg catechin/100gFW, blueberries, 190.30 mg catechin/100gFW. The results are higher than those obtained in this research. Šavikin *et al.* (2009) analyzed the total phenolics content in fresh berries (black currant, black raspberry, wild bilberry) from Serbia. The results showed that the total phenolics content in fresh berries ranged from 380 mg GAE/100g in black currant to 1660 mg GAE/100 g fresh mass in black raspberries. Giovanelli and Buratti (2009) examined four varieties of cultivated blueberries and a wild crop originating from Modena (Italy) in order to determine their phenolic composition. Total phenolic content ranged between 251 and 310 mg GAE/100gFW in cultivated blueberries, while the wild crop contained 614 mg GAE/100gFW. Our results are lower than

those reported by Giovanelli and Buratti (2009). Okan *et al.* (2018) have found that the content of phenolic compounds and flavonoids in 28 varieties of blueberries from Turkey varied in the range 76.20-215.12 mg GAE/100g FW and 30.44-91.69 mg quercetin/100g FW, respectively. Stanciu *et al.* (2019) analyzed some berries (blueberry, raspberry, blackberry and red currant) and showed that the highest total phenolic content was registered for blueberries (543.50 mg GAE/100g FW), followed by red currants (432.30 mg GAE/100gFW), blackberries (425.00 mg GAE/100gFW), and raspberries (344.50 mg GAE/100gFW).

Anthocyanin pigments reversibly change color with a change in pH; the colored oxonium form exists at pH 1.0, and the colorless hemiketal form predominates at pH 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration (Okan *et al.*, 2018).

Anthocyanins are a class of water-soluble flavonoids, which are mostly found as glycosides (Koponen *et al.*, 2007). It is known that anthocyanins are responsible for the red, blue and purple colors of most berries and fruits. The total anthocyanins content in fresh berries ranged from 0.022 mg CGE/100g in fresh gooseberries to 56.76 mg CGE/100g fresh blueberries (Table 1). Black currant-blueberry, red currant-blackberry and white currant-gooseberry presented similar concentrations of anthocyanins ($p > 0.05$).

According to the results of the present study, anthocyanins comprised 0.11% to 73.20% of total phenolic content. Some authors (Kähkönen *et al.*, 2003; Heinonen, 2007) reported that in blueberries, red raspberries and lingonberries, anthocyanins accounted for 90%, 30% and 22% of their phenolic profiles, respectively.

Plessi *et al.* (2007) concluded that raspberry contain 43 mg cyanidine/100gFW, black currant 262 mg cyanidine/100g FW, and red currant 22 mg cyanidine/100gFW. Rubinskienė and Viškelis (2002) have found that black currant contains an amount of anthocyanins between 233.5 and 450.0 mg CGE/100gFW. As for the anthocyanin amounts among the berry species, raspberries have similar content to red currants, little more than strawberries, but about 2.5-times fewer anthocyanins than blackberries and about six-times fewer than black currants (Benvenuti *et al.*, 2004). In blackberry, blueberry and raspberry, Marhuenda *et al.* (2016) have found a content of 57.2 mg CGE/100g FW, 5.1 mg CGE/100g FW and 57.5 mg CGE/100g FW, respectively.

Lugasi *et al.* (2011) analyzed different varieties of blackberries, reporting an amount of total anthocyanins ranging between 50 and 233 mg anthocyanins/100g FW. Gavriloova *et al.* (2011) analyzed different varieties of blueberries and they have found concentration of anthocyanins, from 41.99 to 83.64 mg /100gFW. McDougall *et al.* (2005) reported different contents of anthocyanins in raspberry, from 14.5 to 78.4 mg anthocyanins/100gFW. Brazilian blueberries had relatively high concentration of total phenolics of 1622–3457 mg GAE/100 g dry weight (DW) and total anthocyanins of 140–318 mg CGE/100 g DW (Pertuzatti *et al.*, 2014).

The variation in total phenolic content, total flavonoid content and total anthocyanins content could be due to the varietal differences, cultivar, genotype, variety, growing

location, cultivation techniques, cultivation conditions, growing season, processing, and storage (Skrovankova *et al.*, 2015). Furthermore, the levels of TPC and TFC depends on the extraction solvent. Khalil *et al.* (2018) observed that the best extraction solvent for the total phenolic contents and total flavonoid contents of pomegranate peel extracts is methanol, followed by ethanol and ethyl acetate.

DPPH Radical Scavenging Ability

The radical scavenging activity of fresh berries was measured using the DPPH radical assay (Table 1). Among fresh berries, black currant showed the strongest DPPH radical scavenging activity (892.35 $\mu\text{mol TE}/100\text{g FW}$), followed by blueberries (696.83 $\mu\text{mol TE}/100\text{g FW}$) and blackberries (465.10 $\mu\text{mol TE}/100\text{g FW}$). Raspberry and white currant exhibited similar antioxidant activity, 214.09 $\mu\text{mol TE}/100\text{g FW}$ and 219.29 $\mu\text{mol TE}/100\text{g FW}$, respectively. The results showed a strong positive correlation of TPC with antioxidant activity on DPPH radical, with a correlation coefficient of 0.9344. Moreover, a high correlation was recorded between DPPH and TFC (0.8984), and DPPH and anthocyanins (0.7438). The antioxidant activities of all analysed berries are significantly different ($p < 0.05$).

Giovanelli and Buratti (2009) analyzed antioxidant activity in four varieties of blueberries. The results ranged between 379 and 549 $\mu\text{mol TE}/100\text{g FW}$, this is in agreement with our result. Pertuzatti *et al.* (2014) determined antioxidant activity for 16 varieties of blueberries in different years, through ORAC, FRAP, and ABTS methods. The ORAC values ranged between 600–1028 $\mu\text{mol TE}/\text{g DW}$ for the cultivars analyzed in the 2010/2011 harvest and 533–778 $\mu\text{mol TE}/\text{g DW}$ for the 2011/2012 harvest. In the FRAP assay, the values ranged from 128 to 312 $\mu\text{mol TE}/\text{g DW}$. There was a range of 0.28–16.3 $\mu\text{mol TE}/\text{g DW}$ in the ABTS antioxidant assay for lipophilic extracts. All the methods for determining antioxidant activity of blueberries produced significant differences between cultivars.

Gramza-Michałowska *et al.* (2019) measured antiradical activity using DPPH assay for extracts of raspberry and blackberry. The antioxidant activity of analyzed fruits in the presence of DPPH radical were 294 $\mu\text{mol TE}/100\text{g}$ fresh raspberry, and 351 $\mu\text{mol TE}/100\text{g}$ fresh blackberry, respectively. Okan *et al.* (2018) analysed 28 varieties of blueberries produced in Turkey. To measure the antioxidant capacities of blueberries, Okan *et al.* (2018) used the Ferric Reducing Antioxidant Capacity (FRAP) and the DPPH radical scavenging activity test. The total antioxidant capacity values for blueberries for FRAP were found to be in the range 454.93–3632.96 $\mu\text{mol TE}/100\text{g FW}$, and for DPPH between 1.01 and 4.78 mg/mL . Chen *et al.* (2014) determined antioxidant activity in various colored berries using two methods, namely the FRAP assay and the DPPH assay. The DPPH antioxidant activity values of the extract of wild raspberry, blackcurrant and blueberry were 100.50 $\text{mg ascorbic acid equivalent/kg FW}$, 35.82 $\text{mg ascorbic acid equivalent/kg FW}$ and 65.59 $\text{mg ascorbic acid equivalent/kg FW}$, respectively. The FRAP values ranged between 109.97 and 334.08 $\text{mg ascorbic acid equivalent/kg FW}$.

Antioxidant activity of berries and other fruits was reported in many studies. Its values varied significantly due to the use of different oxidation system and methods (Heinonen and Meyer, 2002).

Antioxidant Capacity by photochemiluminescence assay

Photochemiluminescence assay (PCL) reflects the antioxidant capacity of water-soluble (ACW) and lipid-soluble components (ACL). In this study, ACW and ACL were expressed as μmol ascorbic acid per 100 grams of fresh fruit and $\mu\text{mol TE}$ per 100 grams of fresh fruits, respectively.

Table 2. Antioxidant capacity by photochemiluminescence assay in the analysed Romanian berries.

Type of berries	PCL-ACW (μmol ascorbic acid/100g FW)	PCL-ACL ($\mu\text{mol TE}/100\text{g FW}$)
Raspberry	472.84 \pm 1.62 ^c	698.36 \pm 0.93 ^d
Blackberry	990.92 \pm 0.79 ^c	1169.51 \pm 1.03 ^c
Blueberry	1288.79 \pm 0.74 ^b	1844.20 \pm 2.67 ^b
Black currant	3359.80 \pm 0.69 ^a	3898.80 \pm 0.64 ^a
Red currant	376.91 \pm 1.03 ^f	527.76 \pm 2.11 ^c
White currant	962.72 \pm 0.52 ^d	1183.01 \pm 3.70 ^f
Gooseberry	293.40 \pm 1.29 ^g	413.53 \pm 1.22 ^g

The values are expressed as means \pm standard deviations (n =2). Values followed by different letters in the same column are significantly different (p <0.05).

The water-soluble antioxidant capacity data of the seven berries were between 293.40-3359.80 μmol ascorbic acid/100 g FW. Black currant presented the highest value of ACW (3359.80 μmol ascorbic acid/100 g FW) followed by blueberry (1288.79 μmol ascorbic acid/100 g FW) and blackberry (990.92 μmol ascorbic acid/100 g FW) while gooseberry have the lowest value (293.40 μmol ascorbic acid/100 g FW).

The values of lipid soluble antioxidant capacity of the analysed berries measured by the photochemiluminescence assay varied in the range 413.53-3898.80 $\mu\text{mol TE}/100\text{g FW}$. Black currant (3898.80 $\mu\text{mol TE}/100\text{gFW}$) presented the highest ACL, while gooseberry (413.53 $\mu\text{mol TE}/100\text{g FW}$) had the lowest value of ACL. It is observed that black currant had the highest values of ACW and ACL, while gooseberry had the lowest. The values of PCL of analysed berries are significantly different (p<0.05). Strong positive correlations between the antioxidant capacity of lipid-soluble compounds measured by photochemiluminescence assay (PCL-ACL) and the DPPH method used for determining antioxidant activity was found (r=0.9153). Also, TPC and ACL are significantly correlated (r=0.9797). This may partly be due to the methanol-based measuring or extraction buffer used for these analyses (Balogha *et al.*, 2010).

Balogha *et al.* (2010) analysed antioxidant capacity (ACW and ACL) in 13 cultivars of four species including red currant, raspberry, and black currant. The results showed that the ACW values of red currant varieties ranged between 1562-2533 μmol ascorbic acid/100g DW, 2397-3067 μmol ascorbic acid/100g DW for raspberry, and 3590-7543 μmol ascorbic acid/100g DW for blackcurrant. The ACL values for

red currant ranged between 1279.75-7409.50 $\mu\text{mol TE}/100\text{g DW}$, 1230-10743.25 $\mu\text{mol TE}/100\text{g}$ for raspberry DW, and 16868-32843.75 $\mu\text{mol TE}/100\text{g DW}$ for black currant. Stanciu *et al.* (2019) examined the antioxidant capacity (ACL) of the lipophilic antioxidants (tocopherols, tocotrienols and carotenoids) from the alcoholic extracts of berries by photochemiluminescence method. The highest antioxidant capacity of lipid-soluble phenolic compounds was found in black raspberries (938.25 $\mu\text{mol TE}/100\text{g FW}$) followed by red currants (643.50 $\mu\text{mol TE}/100\text{g FW}$), blackberries (72.74 $\mu\text{mol TE}/100\text{g FW}$), and blueberries (54.00 $\mu\text{mol TE}/100\text{g FW}$). Gramza-Michałowska *et al.* (2019) investigated the antioxidant capacity of some berries including raspberry and blackberry. The ACW values were 97 $\mu\text{mol TE}/100\text{g FW}$ for raspberry, and 128 $\mu\text{mol TE}/100\text{g FW}$ for blackberry, respectively while the ACL presented a value of 253 $\mu\text{mol TE}/100\text{gFW}$ for raspberry and 261 $\mu\text{mol TE}/100\text{g FW}$ for blackberry.

Conclusions

In this paper, the phenolic content, flavonoid content, anthocyanins content and the water-soluble and lipid-soluble antioxidant capacity of seven Romanian berries were measured. Results confirmed that berries were a good source of many biological functional substances having considerable amounts of total phenolic content. In all methods performed, the highest content of biochemical compounds and antioxidant capacity was for the blackberry except total flavonoid and total antocyanins where the highest content was represented by blueberry.

Photochemiluminescence (PCL-ACL) analysis and DPPH assay were fully applicable to the evaluation of the antioxidant capacity of lipophilic fraction of berries, with correlation coefficients of 0.9153. Also, ACL and phenolic content presented a high correlation ($r=0.8718$). The Romanian berries investigated in the present study are a potential source of antioxidants that may be beneficial to human health.

The present studies were performed in order to determine the antioxidant capacity in berries. In the future, an evaluation of the classes / subclasses of bioactive compounds, of the total antioxidant capacity of fruits with local consumption in Romania and the establishment of a database with the obtained results are desired.

Acknowledgments

This research was achieved through Core Program, with the support of the Ministry of Research, Innovation and Digitization (MCID), contract 22N/2019, project PN 19 02 02 04.

References

Abobatta, W.F. 2021. Nutritional and Healthy Benefits of Fruits. *Biomedical Journal of Scientific & Technical Research*, **40**(2), 31979-31983.

- Balogha, E., Hegedus, A., Stefanovits-Banyai, E. 2010. Application of and correlation among antioxidant and antiradical assays for characterizing antioxidant capacity of berries. *Scientia Horticulturae*, **125**, 332-336.
- Benvenuti, S., Pellati, F., Melegari, M., Bertelli, D. 2004. Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of Rubus, Ribes, and Aronia. *Journal of Food Science*, **69**, 164-169.
- Česonienė, L., Jasutiene, I., Šarkinas, A. 2009. Phenolics and anthocyanins in berries of European cranberry and their antimicrobial activity. *Medicina (Kaunas)*, **45**(12), 992-999.
- Chen, L., Xin, X., Yuan, Q., Su, D., Liu, W. 2014. Phytochemical properties and antioxidant capacities of various colored berries. *Journal of the Science of Food and Agriculture*, **94**, 180-188.
- Corbu, V.M., Gheorghe, I., Marinas, I.C., Geană, E.I., Moza, M.I., Csutak, O., Chifiriuc, M.C. 2021. Demonstration of Allium sativum Extract Inhibitory Effect on Biodeteriogenic Microbial Strain Growth, Biofilm Development, and Enzymatic and Organic Acid Production. *Molecules*, **26**, 7195.
- Culetu, A., Fernandez-Gomez, B., Ullate, M., Del Castillo, M.D., Andlauer, W. 2016. Effect of theanine and polyphenols enriched fractions from decaffeinated tea dust on the formation of Maillard reaction products and sensory attributes of breads. *Food Chemistry*, **197**, 14-23.
- Fernandes, I., Faria, A., Azevedo, J., Soares, S., Calhau, C., De Freitas, V., Mateus, N. 2010. Influence of anthocyanins, derivative pigments and other catechol and pyrogallol-type phenolics on breast cancer cell proliferation. *Journal of Agricultural and Food Chemistry*, **58**, 3785-3792.
- Gavrilova, V., Kajdzanoska, M., Gjamovski, V., Stefova, M. 2011. Separation, characterization and quantification of phenolic compounds in blueberries and red and black currants by HPLCDAD-ESI-MSn. *Journal of Agricultural and Food Chemistry*, **59**(8), 4009-4018.
- Giovanelli, G., Buratti, S. 2009. Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. *Food Chemistry*, **112**(4), 903-908.
- Giovanelli, G., Brambilla, A., Rizzolo, A., and Sinelli, N. 2012. Effects of blanching pre-treatment and sugar composition of the osmotic solution on physico-chemical, morphological and antioxidant characteristics of osmodehydrated blueberries (*Vaccinium corymbosum* L.). *Food Research International*, **49**, 263-271.
- Gramza-Michałowska, A., Bueschke, M., Kulczyński, B., Gliszczynska-Świągło, A., Kmiecik, D., Bilaska, A., Purlan, M., Wałęsa, L., Ostrowski, M., Filipczuk, M., Jędrusek-Golińska, A. 2019. Phenolic compounds and multivariate analysis of antiradical properties of red fruits. *Journal of Food Measurement and Characterization*, **13**, 1739-1747.
- Heinonen, I.M., Meyer, A.S. 2002. Antioxidants in fruits, berries and vegetables. *Fruit and vegetable processing (W Jongen, ed.)*, Woodhead Publishing Limited, Cambridge, 23-51.
- Heinonen, M. 2007. Antioxidant activity and antimicrobial effect of berry phenolics – Finnish perspective. *Molecular Nutrition & Food Research*, **51**(6), 684-691.
- Kähkönen, M.P., Heinämäki, J., Ollilainen, V., Heinonen, M. 2003. Berry anthocyanins: isolation, identification and antioxidant activities. *Journal of the Science of Food and Agriculture*, **83**, 1403-1411.

- Khalil, A.A., Khan, M.R., Shabbir, M.A. 2018. In vitro antioxidant activity and punicalagin content quantification of pomegranate peel obtained as agro-waste after juice extraction. *Pakistan Journal of Agricultural Sciences*, **55**, 197–201.
- Koponen, J.M., Happonen, A.M., Mattila, P.H., Törrönen, A.R. 2007. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. *Journal of Agricultural and Food Chemistry*, **55**, 1612–1619.
- Lee, S.G., Vance, T.M., Nam, T.G., Kim, D.O., Koo, S.I., Chun, O.K. 2015. Contribution of Anthocyanin Composition to Total Antioxidant Capacity of Berries. *Plant Foods for Human Nutrition*, **70**, 427–432.
- Lockyer, S., Spiro, A., Stanner, S. 2016. Dietary fibre and the prevention of chronic disease—should health professionals be doing more to raise awareness? *Nutrition Bulletin*, **41**(3), 214-231.
- Lugasi, A., Hovari, J., G. Kadar, G., Denese, F. 2011. Phenolics in raspberry, blackberry and currant cultivars grown in Hungary. *Acta Alimentaria*, **40**(1), 52–64.
- Marhuenda, J., Alemán, M.D., Gironés-Vilaplana, A., Pérez, A., Caravaca, G., Figueroa, F., Mulero, J., Zafrilla, P. 2016. Phenolic Composition, Antioxidant Activity, and In Vitro Availability of Four Different Berries. *Journal of Chemistry*, **2016**, 1-7.
- Marinova, D., Ribarova, F., Atanassova, M. 2005. Total phenolics and flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy*, **40**(3), 255-260.
- McDougall, G.J., Fyffe, S., Dobson, P., Stewart, D. 2005. Anthocyanins from red wine—their stability under simulated gastrointestinal digestion. *Phytochemistry*, **66**(21), 2540–2548.
- Okan, O.T., Deniz, I., Yayli, N., Güngör Şat, I., Öz, M., Hatipoğlu Serdar, G. 2018. Antioxidant activity, sugar content and phenolic profiling of blueberries cultivars: a comprehensive comparison. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **46**(2), 615.
- Okatan, V., Gündoğdu, M., Güçlü, S.F., Çelikay, A., Özeydin, A.M.Ç., Korkmaz, N., Aşkın, M.A. 2017. Phenolic Profiles of Currant (*Ribes* spp.) Cultivars. *YYU Journal of Agriculture Sciences*, **27**(2), 192-196.
- Pap, N., Fidelis, M., Azevedo, L., Araujo Vieira do Carmo, M., Wang, D., Mocan, A., Penha Rodrigues Pereira, E., Xavier-Santos, D., Sant’Ana, S., Yang, B., Granato, D. 2021. Berry polyphenols and human health: evidence of antioxidant, anti-inflammatory, microbiota modulation, and cell-protecting effects. *Current Opinion in Food Science*, **42**, 167–186.
- Paredes-Lopez, O., Cervantes-Ceja, M.L., Vigna-Perez, M., Hernandez-Perez, T. 2010. Berries: improving human health and healthy aging, and promoting quality life—a review. *Plant Foods for Human Nutrition*, **65**, 299–308.
- Pertuzatti, P.B., Teixeira Barcia, M., Rodrigues, D., Nogueirada Cruz, P., Hermosín-Gutiérrez, I., Smith, R., Teixeira Godoy, H. 2014. Antioxidant activity of hydrophilic and lipophilic extracts of Brazilian blueberries. *Food Chemistry*, **164**, 81-88.
- Plessi, M., Bertelli, D., Albasini, A. 2007. Distribution of metals and phenolic compounds as a criterion to evaluate variety of berries and related jams. *Food Chemistry*, **100**(1), 419-427.
- Popov, I.N., Lewin, G. 1996. Photochemiluminescent detection of antiradical activity; IV: Testing of lipid-soluble antioxidant. *Journal of Biochemical and Biophysical Methods*, **31**, 1–8.
- Rubinskienė, M., Viškelis, P. 2002. Accumulation of ascorbic acid and anthocyanins in berries of *Ribes nigrum*. *Botanica Lithuanica*, **8**(2), 139-44.
- Santiya, M., Aluko, R.E., Dhewa, T., Moreno Rojas, J.M. 2021. Potential Health Benefits of Plant Food-Derived Bioactive Components: An Overview. *Foods*, **10**(4), 839.

-
- Šavikin, K., Zdunić, G., Janković, T., Tasić, S., Menković, N., Stević T., Đorđević, B. 2009. Phenolic content and radical scavenging capacity of berries and related jams from certificated area in serbia. *Plant Foods for Human Nutrition*, **64**, 212–217.
- Sellappan, S., Akoh, C.C., Krewer G. 2002. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry*, **50**, 2432–2438.
- Singleton, L.V., Orthofer, R., Lamuela-Raventós, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymology*, **299**, 152–178.
- Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T. 2015. Bioactive compounds and antioxidant activity in different types of berries. *International Journal of Molecular Sciences*, **16**(10), 24673-24706.
- Stanciu, G., Lupsor, S., Tomescu, A., Sirbu, R. 2019. Evaluation of antioxidant capacities and phenols composition of wild and cultivated berries. *Revista de Chimie*, **70**(2), 373-377.
- Woisky, R.G., Salatino, A. 1998. Analysis of propolis: Some parameters and procedures for chemical quality control. *Journal of Apicultural Research*, **37**, 99–105.
- You, Q., Wang, B., Chen, F., Huang, Z., Wang, X., Luo, P. G. 2011. Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars. *Food Chemistry*, **125**, 201–208.