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Studies concerning the impact of the origin region on antioxidant properties of blackberries and blueberries

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Abstract

This study aims to assess the influence of the origin place in climatic terms on the antioxidant properties of blackberries (Rubus fruticosus L.) and blueberries (Vaccinium myrtillus L.). For this purpose, blackberries and blueberries from spontaneous flora were collected in 2019, from the following regions of Romania, as follows: Zugau (Arad County) and Paltinis (Sibiu County) for blackberries, respectively Brad (Hunedoara County) and Paltinis (Sibiu County) for blueberries. The investigated antioxidant features were expressed by antioxidant activity evaluated using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay and total phenolic content evaluated by Folin-Ciocalteu assay. The antioxidant characteristics of the studied berries were determined for hydro-alcoholic extracts obtained by a maceration solvent extraction procedure. The differences recorded in the investigated characteristics of the same species coming from distinct areas were no significant. Nevertheless, a 14% decrease in the total phenolic content of blackberries from Paltinis area compared to blackberries from Zugau area, respectively 12% in blueberries from Paltinis area compared with blueberries from Brad area, was reported. The antioxidant activity varied from 229.91 to 252.43 mg GAE/100 g d.s. being lower in berries from Paltinis area. The collected data highlight that the origin region in climatic terms impacted on the antioxidant properties, a milder climate with higher temperatures and a moderate precipitations regime led to a higher value of antioxidant attributes. The results of this study are important in selecting blackberries and blueberries that show high antioxidant properties for further applications in designing new value-added food products.

Keywords: blackberries, blueberries, DPPH radical scavenging activity, total phenolic content, hydro-alcoholic extracts

1. Introduction

Excellent nutritional profile and proved benefits for human health, makes blackberries (*Rubus fruticosus L.*) and blueberries (*Vaccinium myrtillus L.*) the most usually consumed berries on the world [1]. It is well-known that berries like blackberries and blueberries are valuable sources of natural bioactive compounds with antioxidant activity [2]. Antioxidant properties of berries are due to their high content in polyphenolic compounds [3]. Berry phenolics include phenolic acids, flavonoids (flavonols, flavanols, anthocyanins) and tannins [2,4].

Their high content in bioactive compounds, makes berries appropriate as an alternative source of natural antioxidants and valuable in developing new food supplements, nutraceuticals and novel foods with improved properties [4].

As well, in our days, has been recorded an actual concern in substitute synthetic antioxidants with a healthier alternative such as natural antioxidants of plant origin [5-7]. Natural antioxidants of plant origin exhibit a vast number of biological effects in health promoting including anticancer, anti-aging, anti-diabetic anti-allergic, cardio-protective, antimicrobial and anti- inflammatory effects [6,8-10].

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Various studies highlights the fact that wild fruits, and generally wild plants, contain more natural bioactive compounds and present higher antioxidant values than the commercial cultivars from the same species [11, 12]. It is considered that most of the differences in the bioactive compounds concentration between cultivated plants and the same species grown in wild conditions, are due to the lower moisture content in wild plants [13]. Environmental factors are the main responsible for plant bioactive compounds fluctuations [14,15]. The main abiotic factors that are part of environmental components that induce stress and thereby influence the development of bioactive compounds in plants, are temperature, light and humidity [15,16]. In this regard, the origin area define by local geo-climatic and seasonal changes has an important response on the antioxidant properties. However, the fruits, respectively the berries belonging to the spontaneous endemic flora, are not sufficiently exploit, although they represent an available low costs source of natural antioxidants.

Due to their high content in bioactive compounds with antioxidant properties, blackberries and blueberries and their derivatives may be implement in food, pharmaceutical and cosmetic products [17,18].

The efficient application of berries in industrial production leads to development of new value-added food products with improved features [19, 20]. Applied in food industry, berries and their derivatives, may improve the functional characteristics, oxidative stability and simultaneously shelf life of foods from the category of fruit products, dairy products, bakery and pastries products [4,17,21].

The antioxidant properties of blackberries and blueberries and their fractions have been already explored in several studies, but so far, limited data are available regarding the impact of the origin region on the antioxidant features of fresh berries. Regarding the impact of the origin area on the antioxidant attributes, only a few data are accessible. A wide knowledge on how the origin influence antioxidants concentrations in fresh berries, is relevant for their further applications in various industrial fields.

Related to the above mentioned, the purpose of this study is to explain how climatic terms specific to the origin region, influence the antioxidant activity expressed as DPPH radical scavenging activity and total phenolic content from fresh blackberries and blueberries coming from the endemic spontaneous flora from different regions.

2.Materials and methods

2.1. Plant material and growing conditions

Blackberries (*Rubus fruticosus L.*) and blueberries (*Vaccinium myrtillus L.*) were harvested in 2019 from two areas of Romania. Blackberries were collected from Zugau, Arad County and Paltinis, Sibiu County and the blueberries were harvested from Brad, Hunedoara County and Paltinis, Sibiu County.

According to their origin place, blackberries were registered as ZBk and PBk for Zugau, respectively Paltinis area, and blueberries were noted as BBl and PBl for Brad and Paltinis area.

In Table 1 are presented the geographical characteristics (altitude, latitude and longitude) of the origin regions of berries taken into study. Altitude shows the most significant difference, Patinis area being at an altitude of fivefold and almost nine fold higher then Brad, respectively Zugau area.

Table 1. Geographical characteristics of Zugau, Paltinis and Brad areas

Area	County	Altitude (m.a.s.l.)	Lat. N (° ' ")	Long. E (° ' ")
Zugau	Arad	162	46° 18' 55"	22° 04' 38"
Paltinis	Sibiu	1442	45° 39' 10"	23° 55' 55"
Brad	Hunedoara	278	46° 07' 48"	22° 47' 24"

m.a.s.l: meters above sea level; Lat. N: latitude North; Long. E: longitude East.

Table 2. Particularities of blackberries and blueberries

Sample	Weight (g)	Size		Color	Harvest period
		Height (mm)	Width (mm)		
ZBk	4.98	18.76	15.42	black-purple	early August
PBk	6.85	22.57	20.15	reddish-black	early August
BBl	0.51	5.08	6.54	dark-blue to black	end of June
PBl	0.58	5.29	6.79	dark-blue to black	end of June

Literature data reported that Zugau area has much higher temperatures and considerably less precipitations then Paltinis. The same pattern is followed for blueberries areas. Brad has a warmer climate and less precipitation then Paltinis area. Paltinis is the region with the highest amount of precipitations and lower temperature due to his high altitude [22].

Table 2 shows particularities of the studied berries regarding their development attributes related to the growing area conditions, in terms of weight, size and color. It present the average of 25 pieces for each sample and also specifies harvest period of blackberries and blueberries. Although the harvesting period is the same for both samples, it can be observed that the main differences are recorded in the two blackberry samples in terms of weight, size and color, due to the fact that origin regions, Zugau, respectively Paltinis, shows large differences in precipitation amount and average temperatures.

For blackberries coming from Zugau area which present higher temperatures and moderate precipitation regime, the size and the weight of berries are smaller and the color is darker then the blackberries from Paltinis area. Blueberries samples taken into study present similar physical attributes due to some resemblance of the climatic parameters.

All berries taken into study were harvested manually at maturity stage, completely ripe, separate of impurities and stored for 24 hours until subsequent extraction procedure at a temperature of 4°C.

2.2. Extraction procedure

In order to perform the DPPH radical scavenging activity and total phenolic content on fresh blackberries and blueberries, hydro-alcoholic extracts using a maceration solvent extraction, were prepared [17, 23]. During extraction procedure, the mixture obtain from solvent and solute represented by fresh berries, was regularly stirred. Extraction solvent was represented by an ethanol/water mixture (1:1, v/v) and the solid:solvent ratio was 1:10 (w/v). Prior to extraction program, fresh blackberries and blueberries were crushed in order to increase the contact surface between solute and solvent. The extraction procedure was performed at ambient temperature (20°C) for 48 hours.

At the end of extraction procedure, the mixture was percolated and before further determinations, the extract was filtered through a 0.45µm teflon hydrophobic membrane.

2.3. Moisture content

Moisture content of the fresh blackberry and blueberry samples was performed according to the Official Methods of Analysis [24]. Water content of the berry studied samples was carried out by the method 925.09 (AOAC, 2005).

2.4. Antioxidant activity

Antioxidant activity of the extracts obtained from fresh berries expressed as capacity to scavenge 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was assessed spectrophotometrically [17,23,25]. In this regard, a DPPH solution, 0.2 mM in ethanol, obtained previously, was mixed with 0.1 mL extract. After the mixture obtained was kept in the dark for 60 minutes, the absorbance was recorded at 517 nm against a blank sample composed of ethanol, using a Specord 200 Analytik Jena Inc. Germany, UV-VIS double-beam spectrophotometer. Gallic acid was used as reference, prepared in ethanol in concentrations ranging from 2.5 to 50 mg/L. The results was expressed as mg gallic acid equivalents (GAE)/100 g dried substance (d.s).

The percentage inhibition (%) of the DPPH radical was calculated according to equation (1):

$$I(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (1)$$

where $A_{control}$ represents the absorbance of DPPH radical solution, and A_{sample} represents the absorbance of the investigated extracts.

All analysis were performed in triplicate and the results were presented as mean values ± standard deviation (SD).

2.5. Total Phenolic Content

Total phenolic content (TPC) was spectrophotometrically assessed according to the Folin-Ciocalteu slightly modified procedure [17,26,27]. Prior other analyses, all berry extracts were diluted with distilled water (1:25) and 1mL from this solution was mixed with 2 mL Na_2CO_3 (20%) and 0.5 mL Folin-Ciocalteu reagent diluted in 5 mL distilled water.

After a 90 minutes incubation period in the dark, the absorbance was measured at 765 nm against a blank sample prepared under similar conditions using a UV-VIS double-beam spectrophotometer (Specord 200, Analytik Jena Inc., Germany). Calibration curve was prepared using gallic acid solution in concentration range 20-200 mg GAE/L. The results was expressed as mg GAE/100 g d.s. All analysis were performed in triplicate and the results were presented as mean values ± standard deviation (SD).

3. Results and Discussion

3.1. Moisture content: Table 3 shows the moisture content of fresh blackberry and blueberry samples. The moisture content of fresh berries taken into study has been found in the range 83-89% depending on the origin area.

Table 3. Moisture content of fresh blackberries and blueberries

Sample	Moisture content (%)
ZBk	83.01±1.32
PBk	85.49±1.27
BBl	86.57±1.46
PBl	88.52±1.52

As it can be observed from Table 3, depending on the origin place, blackberries recorded a difference of about 3% in moisture content, the water content being higher in the area with a higher precipitations regime. The difference recorded between fresh blueberries samples was around 2%, the amount of precipitation in the two blueberry samples origin area, being closer.

Table 4. DPPH radical scavenging activity and total phenolic content of blackberries and blueberries

Sample	DPPH radical scavenging activity		TPC
	I (%)	mg GAE/100 g d.s	(mg GAE/100 g d.s)
ZBk	91.89±1.85	241.73±0.87	5812.03±18.01
PBk	90.77±2.16	229.91±0.39	4997.86±19.49
BBl	93.95±1.39	252.43±0.47	7214.19±20.19
PBl	92.79±1.44	245.04±1.24	6375.78±19.76

3.2. Antioxidant activity and total phenolic content: In Table 4 are presented data recorded after DPPH and Folin-Ciocalteu assays. DPPH radical scavenging activity is expressed as percentage of inhibition and mg GAE/100 g d.s. and TPC represented on the last column is expressed as mg GAE/100 g d.s.

The lowest inhibition was 90.77% recorded in PBk and the highest was that recorded in BBl extract sample, respectively 93.95%. The inhibition decreased with 1.22% for both blackberry and blueberry samples in berries from Patinis area compared with the other harvesting areas. The antioxidant activity indicated as DPPH radical scavenging activity and expressed in mg GAE/100 g d.s. was reduced with almost 5% for blackberries from Paltinis area compared with those from Zugau area and with about 3% for blueberries from the same area compared with blueberries from Brad.

From the blackberry extracts samples, ZBk extracts showed the highest content in phenols (5812.03 mg GAE/100 g d.s.) and for blueberry extracts, the BBl samples revealed the largest amount in phenols content (7214.19 mg GAE/100 g d.s.). A decreased of 14% in total phenolic content was recorded in blackberries from Paltinis compared with blackberries from Zugau area.

In terms of total phenolic content of blueberry extract samples, was recorded a decrease of about 12% in blueberries coming from Paltinis in comparison with those from Brad area. This findings indicates that berries from areas with a milder climate with higher temperatures and moderate precipitations regime possess higher bioactive potential.

The recorded results are in agreement with the corresponding data reported in other similar studies [17, 23, 27-29]. In this regard, the TPC of wild blackberries reported by Marhuenda *et al.* [28] was about 4500 mg GAE/100 g and for wild blueberries, Bunea *et al.* [29] recorded a TPC value of 672, respectively 819 mg GAE/100 g fresh weight.

Our recorded data showed that TPC was consistent with antioxidant activity, a positive correlation between DPPH radical scavenging activity and TPC being recorded. This results are in agreement with Oszmiański and Lachowicz [30] and Metzner *et al.* [17] findings regarding the fact that bioactive compounds present antioxidant activity and may act as reactive oxygen inhibitors and free radical scavengers. From our results it also may be observed that blueberries shows higher antioxidant characteristics especially in terms of TPC, over 20%, compared with blackberries coming from the same area.

The data recorded on wild blackberries and wild blueberries harvested in 2019 compared with corresponding data reported in similar studies carried out on berries harvested in 2018 by Metzner *et al.* [17,23] showed a slight increase in antioxidant properties that can be attributed to the fact that 2019 was a year with less precipitations compared to 2018. This results lead to the fact that less precipitations may induce an increase in antioxidant attributes of wild berries.

4. Conclusions

Data recorded in this study revealed that both blackberries and blueberries coming from endemic spontaneous flora, present significant deposits of natural bioactive substances with antioxidant capacity. Differences recorded in antioxidant attributes of the same type of berry but with distinct origin area, put in evidence the fact that antioxidant characteristics are related to climatic conditions, especially with temperature and precipitations amount. Concerning the impact of origin region on the antioxidant properties, had been noticed that antioxidant features are more pronounced in berries coming from an area with a milder climate with higher temperatures and moderate precipitations regime. Our results highlights that berries such as blackberries and blueberries, due to their high content in natural bioactive compounds with antioxidant properties, their accessibility and low costs, may be adapted as a valuable ingredients in food, pharmaceutical and cosmetic industry. With a positive impact on the economy and focused on human health, berries from spontaneous flora are convenient, pure and organic fruits that added to various food products, constitute vectors for substances of physiological and nutritional importance.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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