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Analysis of the changes in the main characteristics and antioxidant properties in raspberries of "Rubin" variety depending on the altitude

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The study aimed at determination and analysis of total acidity (pH), titratable acidity, total polyphenols content, total anthocyanins content and DPPH antioxidant activity (radical scavenging activity) of *Rubus idaeus L cv. Rubin* juice obtained from fruits of a variety grown in different geographical areas and altitudes in Bulgaria. Systematic approach and critical analysis of the available scientific periodicals was apllied; DPPH method was used for determining the antioxidant activity (radical trapping activity). The value of pH index ranges from 2.99±0.03 to 2.75±0.03. The lowest level of common phenols 251.73 ± 0.20 mg GAE/100 mL was observed in raspberry from Banya town. Total anthocyanins were within the range of 13.79 ± 0.13 mg/100 mL. (Dolna Banya town-643 m.) to 86.89 ± 0.06 mg/100 mL (Dobrila hut-1804 m.). Antioxidant activity (DPPH) has values from 37287 ± 030 mmol TE/100 ml (Uzundjovo village) to 2240.20 ± 19.10 mmol TE/100 ml (Dobrila hut-1804 m.). The antioxidant activity of raspberry juice obtained from *R. idaeus cv. Rubin* mainly depends on total anthocyanins content (r^2 = 0.71). The antioxidant activity significantly increases with the increase in altitude, i.e. they have a significant correlation (r^2 = 0.81).

Keywords: raspberry, antioxidant activity, phenols, anthocyanins.

INTRODUCTION

Rubus idaeus L. (raspberry) is a wild or cultural perennial bushy plant from *Rosaceae*, growing to a height of up to 2 meters. It is cultivated in areas with moderate climates, mainly in Europe and the USA. In the wild, raspberries grow in Europe, Asia, South America in temperate, tropical and subtropical climates [1,2]. The international tendency for cultivation and production of small edible fruits shows a permanent increase. Edible berry fruits, wild or cultivated, are proved as a traditional and rich source of bioactive compounds [3]. Many *Rubus* fruits are consumed fresh or as processed products such as juice, jams, jellies, syrups and wines [4]. The leaves and roots have been used in various medicinal applications. Recent studies have revealed that different parts of raspberry have essential positive effects on the human diet and health, which could be mainly ascribed to the presence of several health-related compounds such as organic acids, polyphenolics, tannins and sugars [2]. Fruits contain mainly 4.5 - 4.7% sugars, 1.1-2.0% organic acids (citric and malic acid), vitamin C (up to 45 mg/100 g), carotene and others compounds. In the raspberry fruits several classes of polyphenols have been identified, e.g., hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, pro-anthocyanindins, flavonols, flavones, flavanols, flavanones, isoflavones, stilbenes and lignans. Particularly, identified polyphenols contribute substantially to the antioxidant complement of raspberry fruits, having potential health effects [5].

Raspberry (*Rubus idaeus L.*) is an important berry crop for both the fresh and the processing market. In addition to vitamins and minerals, a range of diverse polyphenols are present in the berries, including flavonoids such as anthocyanins, flavonols and flavanols, ellagitannins and ellagic acid derivatives [6]. Although genetic predisposition is the major factor influencing fruit quality, different agricultural practices, fruit maturity and environmental conditions can also affect berry metabolite amounts and composition [6]. As the harvest season progresses, climatic conditions can significantly impact on fruit quality [7]. Climatic factors such as light conditions (intensity, quality, photoperiod), temperature and precipitation can significantly affect growth and fruit quality, although there are also other abiotic and biotic stress factors [1,6,8].

The aim of the current study was the determination and analysis of total acidity (pH), titratable acidity, total polyphenols content, total anthocyanins content and DPPH antioxidant activity (radical scavenging activity) of *Rubus idaeus L cv. Rubin* juice obtained from fruits variety grown in different geographical areas and altitudes in Bulgaria.

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METHODS AND MATERIALS

Raspberry samples

The present study was carried out on fruits of a set of 7 different areas collected at maturity from the main regions of raspberry cultivation (*Rubus idaeus L cv. Rubin* and wild type) in Bulgaria. They are grown in the following regions of Bulgaria with different altitudes: Pazardzhik, Plovdiv, Sofia and Haskovo. Fruits from Pazardzhik region were raised at an altitude of 1273 m - village of Nova Mahala, 196 m; - village of Kurtovo Konare, 295 m; - town of Banya, 1300 m - Byala cherkva (wild type raspberry); town of Sopot (hut Dobrila - 1804 m, wild type raspberry); Sofia district 643 m; - town of Dolna Banya, Haskovo region 171 m - village of Uzundjovo.

Juice extraction, pH and titratable acidity analysis

To obtain the juice, raspberry fruits were liquefied using a Phillips food processor. The obtained juice was prefiltered and then centrifuged at 3000 rpm for 15 min. pH was measured potentiometrically with a pH meter (WTWinoLab pH 7110, Germany). Titratable acidity, expressed as percentage of malic acid, was determined by titrating 10 mL of raspberry juice with 0.1 M NaOH to a pH point of 8.2. The results were expressed in g malic acid per 100 g juice.

Total phenolics content (TPC)

The total phenolic contents were measured using a Folin-Ciocalteu assay. Folin- Ciocalteu reagent (1 mL) (Sigma) diluted five times was mixed with 0.2 mL of sample and 0.8 mL of 7.5% Na_2CO_3. The reaction was performed for 20 min at room temperature in darkness. Then the absorbance of the sample was measured at 765 nm against blank sample, developed in the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per 100 g juice, according to calibration curve, built in the range of 0.02 - 0.10 mg gallic acid (Sigma) used as a standard.

Total monomeric anthocyanins analysis

The total monomeric anthocyanins content was determined using the pH differential method described by Lee *et al*. 2005 [9]. 2 g of juice were extracted with 8 mL of ethanol in a ultrasound bath for 15 min. The pH of juice samples was brought to 1.0 with potassium chloride and 4.5 with sodium acetate buffers. The dilutions were then allowed to equilibrate for 5 min at room temperature. The absorbance of the equilibrated solutions at 520 nm for anthocyans content and at 700 nm for haze correction was measured on a VIS spectrophotometer (Camspec M107, UK) with 1 cm path length disposable cuvettes. All absorbance measurements were carried out at room temperature against distilled water as a blank. Pigment content was calculated as cyanidin-3-glucoside equivalents with a molecular weight of 449.2 and an extinction coefficient of 26 900 L/(cm × mol).

Antioxidant activity (DPPH assay)

Each analyzed extract (0.15 mL) was mixed with 2.85 mL of freshly prepared 0.1 mmol solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma) in methanol (Merck). The reaction was performed at 37 °C in darkness and the absorptions at 517 nm were recorded after 15 min against methanol. The antioxidant activity was expressed as mmol Trolox equivalents (TE) per 100 g juice by using a calibration curve, built with 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mmol of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®, Fluka) dissolved in methanol (Sigma).

RESULTS AND DISCUSSION

Currently there is great interest to phytochemicals as bioactive components of food. The roles of fruits, vegetables, and red wine in disease prevention have been attributed, in part, to the antioxidant properties of their constituent polyphenols (vitamins E and C, and carotenoids) [10,11]. A special place in this prevention have fruits and leaves of raspberries.

Raspberries besides red can be black or gold. They are best developed in hilly and mountainous locations and areas with a cool and humid climate. In Bulgaria, two harvests of raspberries are common. They contain easily digestible carbohydrates, rich in dietary fibers, minerals and vitamins. Raspberries quench thirst and improve digestion, so they are used in dietary nutrition, especially for children. Thanks to the salicylic acid found in fruits, raspberries are also used to lower the body temperature.

Consumption of raspberries is associated with broad health benefits - reduced risk for heart disease, diabetes and cancer. The phenolic compounds contained in the raspberries such as flavonoids, phenolic acids, stilbens and procyanidins act synergistically [12].

To analyze *in vitro* the antioxidant activity of samples we used the DPPH method [13]. The antioxidant activity is in the range of 372 mmol TE /100 ml of juice to 2240.20 mmol TE /100 ml of juice (Table 1).

The results for titratable acidity (TA), and pH of raspberry juice obtained from different cultivars grown at different altitudes are presented in Table 1. There are no significant differences found between the obtained juices for the investigated parameters (titratable acidity and total acidity). The pH values ranged between 2.4 and 3.0 with an average value of 2.8 (Table 1). The titratable acidity values ranged between 2.1 and 2.7% with an average value of 2.4%. Citric and malic acids are the main organic acids in raspberry fruit responsible for titratable acidity [3]. Riaz and Bushway (1996) [14] reported a similar result for the mean values of the content of acids for raspberry reported as citric acid, in the range from 1.5 % to 2.6 % (Table 1). The differences between the maximal and minimal content amounted from 10% to 20% of the mean value. The mean contents of acids in the raspberry grown over 1000 m were lower than for those grown under 1000 m altitude. The results of this study showed considerable variation in the content of acids in the fruits of the same cultivar at different altitudes. The possible reason for the variation in the same cultivar could be rainfall and percentage of sunshine during the growing season, which could cause an increase in sugar and a decrease in acid content.

Main phenolic compounds of raspberry fruit were presented as anthocyanins and ellagitannins [15]. It is evident from Table 2 that the highest content of total phenols has the juice obtained from raspberry fruit collect from Banya town area (251.73 ± 0.20 mg GAE/100 mL), followed by Nova Mahala village, Uzundjovo village and Kurtovo Konare village. Additionally, many investigators reported that in raspberry fruit high amounts of sanguiin H-10, casuarictin/potentilin, castalagin/vescalagin, pedunculalagin, and corilagin were identified (about 30 mg/100 g fresh weight) [16-18]. In accordance with Mikulic-Petkovsek *et al*. (2012) [3] it was established that the total polyphenol content of the investigated raspberry juice was from 250 to 180 mg GAE/100 mL. Additionally, the anthocyanins are other bioactive compounds with vital significance for human health. The cyanidin and pelargonidin glycosides: 3-sophoroside, 3-glucoside, 3-rutinoside and 3-glucosyl-rutinoside are dominating in raspberry fruit [1,15]. On this base we investigated the influence of altitude on the biosynthesis of anthocyanins. The results are presented in Table 2. The highest content of total anthocyanins has the juice obtained from raspberry fruit collected from Dobrila hut area with the highest altitude (Table 1) (86.89 ± 0.06 mg/100 mL juice), followed by Byala cherkva village and Uzundjovo village.

The obtained total anthocyanins (Table 2) were in lower concentrations in all samples compared to other cultivars which have about 104–198 mg/100 g fw of total anthocyanins [19]. But our results for total anthocyanins are in accordance with other cultivars cv. Amira and cv. Polka which are similar or higher (35-45 mg/100 g fw) [6].

With greater antioxidant activity are samples that have lower titratable acidity, i.e. these indicators are inversely proportional (r^2 = -0.75) (Table 3).

Similar results have been reported by other researchers. Pantelidis *et al.* (2007) reported a high correlation between total polyphenol and anthocyans content and total antioxidant capacity in different raspberry juices [19].

Table 1. Total acidity (pH) and titratable acidity of different samples of rasperry juice obtained from *R. idaeus cv. Rubin* and wild types.

Raspberry juice samples	Altitude (m)	Total acidity (pH)	Titratable acidity (%)
Uzundjovo village	171	2.99 ± 0.03	2.30 ± 0.01
Kurtovo Konare village	196	3.03 ± 0.03	2.43 ± 0.01
Banya town	295	2.37 ± 0.03	2.51 ± 0.01
Dolna Banya town	643	2.75 ± 0.03	2.70 ± 0.01
Byala cherkva village	1300	2.95 ± 0.03	2.45 ± 0.01
Nova Mahala village	1273	3.00 ± 0.03	2.23 ± 0.01
Dobrila hut (Balkan mountain)	1804	2.75 ± 0.03	2.13 ± 0.01

Table 2. Total polyphenol content, total anthocyanins content and antioxidant activity of investigated raspberry juice obtain from *R. idaeus cv. Rubin* and wild types.

Raspberry juice samples	Total polyphenols content, (g GAE/100 mL)	Total anthocyanins (mg /100 ml)	Antioxidant activity (DPPH method) (mmol TE/100 mL)
Uzundjovo village	219.88 ± 0.22	24.69 ± 0.12	372.87 ± 0.30
Kurtovo Konare village	219.88 ± 0.10	18.84 ± 0.01	402.92 ± 0.28
Banya town	251.73 ± 0.20	15.04 ± 0.11	457.38 ± 0.30
Dolna Banya town	179.26 ± 0.26	13.79 ± 0.13	481.79 ± 0.26
Byala Cherkva village	167.86 ± 0.10	27.95 ± 0.04	513.72 ± 0.21
Nova Mahala village	221.47 ± 0.25	19.56 ± 0.10	1988.52 ± 10.28
Dobrila hut (Balkan mountain)	183.41 ± 0.20	86.89 ± 0.06	2240.20 ± 19.10

Table 3. Correlation (r^2) between antioxidant activity and investigated parameters: altitude, total polyphenols content, total anthocyanins content, titratable acidity and total acidity

Parameters	Total polyphenols content	Total anthocyanins	Antioxidant activity	pH	Titratable acidity
Antioxidant activity	- 0.16	0.69	–	0.07	- 0.75
Altitude	- 0.61	0.71	0.81	0.09	- 0.52
Total polyphenols content	-	-0.40	- 0.16	-0.33	- 0.06
pH	-0.33	-0.03	0.07	-	-0.32
Titratable acidity	-0.06	-0.68	-0.75	-0.31	-

CONCLUSIONS

1. The total polyphenols content depends moderately to significantly decreasing (inverse) by the linear relationship (r^2 = -0.33 and r^2 = -0.61) with total acidity and altitude, respectively. With an increase in pH and altitude, the amounts of polyphenols decrease.

2. The total anthocyanins content depends on altitude with high direct (increasing) linear relationship r^2 = 0.71.

3. The antioxidant activity in raspberry juice obtained from *R. idaeus* cv. *Rubin* mainly depends on total anthocyanins content (r^2 = 0.71). The titratable acidity decreases when the content of total anthocyanins increases (r^2 = -0.68). The antioxidant activity significantly increases when altitude increases, i.e. they have significant correlation (r^2 = 0.81).

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