Journal of Pharmaceutical Research International

33(29A): 218-223, 2021; Article no.JPRI.67855
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Polyphenols Content and Antioxidant Activity of Bilberry Juice Obtained from Different Altitude Samples

Stoyan I. Papanov[1*], Ekaterina G. Petkova[2] and Ivan G. Ivanov[3]

[1]Faculty of Farmacy Medical, University Plovdiv, 15A Vasil Aprilov Blvd., Plovdiv, 4000, Bulgaria.
[2]Medical Collage, Medical University Plovdiv, 120 Br. Bakston Str., Plovdiv, 4004, Bulgaria.
[3]Technological Faculty, University of Food Technologies, 26A Maritza Blvd., Plovdiv, 4002, Bulgaria.

Authors' contributions

This work was carried out in collaboration among all authors. Author SIP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EGP and IGI managed the analyses of the study. Author IGI managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i29A31581
Editor(s):
(1) Dr. Syed A. A. Rizvi, Nova Southeastern University, USA.
Reviewers:
(1) Patricio Orellana-Palma, Universidad Tecnológica Metropolitana, Chile.
(2) N. Suganthy, Alagappa University, India.
(3) César Leyva-Porras, Centro de Investigación en Materiales Avanzados S.C. (CIMAV), México.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67855

Original Research Article

Received 12 March 2021
Accepted 18 May 2021
Published 22 May 2021

ABSTRACT

There are about 30 different types of blueberries. In Bulgaria, there are 4 species of genus Vaccinium - red (vitis-idaea), black (myrtillus), blue (uliginosum) and Caucasian (arctostaphylos). Bilberries are a source of carotene, vitamin B1, vitamin B2, potassium, sodium, calcium, magnesium, phosphorus, iron, vitamin D, manganese, soluble and insoluble fibres like pectin. Blueberries contain significant amounts of anthocyanidins, antioxidants and ellagic acid. The main wealth of blueberries are the antioxidants that bind free radicals and thus prevent malignant tumors. This is why blueberry is an excellent remedy for oncology.

Objective of study was to investigate the content of total polyphenols, antioxidant activity and total anthocyanins of bilberry depending on altitude.

Systematic approach and critical analysis of the available scientific periodicals and DPPH method for determining the antioxidant activity (radical trapping activity).

**Corresponding author: E-mail: stoyan.papanov@abv.bg;*

It has been established a direct relationship between altitude and antioxidant activity. In the range of 900 to 1450 m the relationship between altitude and total anthocyanins is inversely proportional. After 1500 m, the dependency becomes proportional. There is no correlation between pH, total polyphenols and titratable acidity.

Keywords: Blueberry; altitude; antioxidant activity.

ABBREVIATIONS

AOA : Antioxidant Ability;
TA : Titratable Acidity;
TAA : Total Anthocyanins;
TPC : Total Polyphenols Content.

1. INTRODUCTION

There are about 30 different types of blueberries grown in different regions. In Bulgaria, there are 4 species of genus Vaccinium - red (vitis-idaea), black (myrtillus), blue (uliginosum) and Caucasian (arctostaphylos). The total anthocyanin content of bilberries is generally in the range of 300-700 mg/100 g fresh fruit, although this range varies with cultivar, growing conditions, and degree of ripeness of the berry [1]. Along with anthocyanins, 100 g of fresh bilberries contains small quantities of vitamin C (3 mg), quercetin (3 mg), and catechin (20 mg) [2]. Bilberries are a source of carotene, vitamin B1, vitamin B2, potassium, sodium, calcium, magnesium, phosphorus, iron, vitamin D, manganese, soluble and insoluble fibres like pectin. Blueberries contain significant amounts of anthocyanidins, antioxidants and ellagic acid. Bilberries (*Vaccinium myrtillus* L.) is the richest natural sources of anthocyanins. These polyphenolic components give bilberries its blue/black colour and high antioxidant content, and they are believed to be the key bioactive responsible for the many reported health benefits. Although bilberries is promoted most commonly for improving vision, it has been reported to lower blood glucose, to have anti-inflammatory and lipid-lowering effects, and to promote antioxidant defence and lower oxidative stress [2]. Therefore, bilberry is of potential value in the treatment or prevention of conditions associated with inflammation, dyslipidemia, hyperglycemia or increased oxidative stress, cardiovascular disease, cancer, diabetes, and dementia and other age-related diseases. There are also reports that bilberries has antimicrobial activity. Most studies have been focused on the antioxidant properties of anthocyanins [2,3]. The fruits possessed DNA repair effects, improved cell adhesion, as well as observed the antineoplastic and antimicrobial effects [3,4,5]. Commercial bilberries products about a 25% anthocyanidins (equivalent to 36% anthocyanins). Recommended daily dosages also vary greatly, for example, 20-60 g of dried berries and 160-480 mg of powdered extract [1,5]. In the available scientific periodicals there are many studies on antioxidant activity, polyphenols, anthocyanins. Of interest is the question of whether growing conditions affect the properties of the fruit. The main such parameter is the altitude of cultivation.

The objective of the study was to investigate the content of total polyphenols, antioxidant activity and total anthocyanins (TA) of bilberries harvested at different alttitude.

2. METHODS AND MATERIALS

2.1 Fruit Sample

Bilberries varieties from Bulgaria were grown respectively: The altitudes of the grown were as well 900 m, 1200 m, 1450 m, and 2000 m respectively in the area of Rakitovo, Nova Mahala village, Yundola village and Batashki snowfall - Rodopi mountain.

2.2 Juice Extraction, pH and Titratable Acidity Analysis

To obtain the juice, bilberry fruits were liquefied using a Philips food processor. The obtained juice was pre-filtered and then centrifuged at 3000 rpm for 15 min. pH was measured with a pH meter (WTWinoLab pH 7110, Germany). Titratable acidity, expressed as percentage of malic acid, was performed by titrating 10 mL of pomegranate juice with a 0.1 M NaOH to a pH point of 8.1. The results were expressed in g citric acid per 100 g juice.

2.3 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 of mL sample and 0.8 of mL 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 by the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per 100g juice, according to calibration curve, built in range of 0.02 - 0.10 mg gallic acid (Sigma) used as a standard.

2.4 Total Monomeric Anthocyanins Analysis

The total monomeric anthocyanins content was determined using the pH differential method described by Papanov et al., [6]. 2 g juice were extracted with 8 mL ethanol at ultrasound bath for 15 min. The pH of juice samples was brought to 1.0 by adding 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 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 (cyanidin-3-glucoside) equivalents with a molecular weight of 449.2 and an extinction coefficient of 26 900 L/(cm.mol).

2.5 Antioxidant Activity (DPPH Assay)

Each analyzed extract (0.15 mL) was mixed with 2.85 mL freshly prepared 0.1 m mol solution of 1,1-diphenyl-2- picrylhydra-zyl 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 m mol Trolox equivalents (TE) per 100 g juice by using calibration curve, build with 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 m mol 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®, Fluka) dissolved in methanol (Sigma).

3. RESULTS AND DISCUSSION

The samples of bilberries were harvested at 4 locations in Rodopa mountain Southern Bulgaria. Berry samples were subjected to chemical analyses in order to assess quality differences based on altitude. The values indicated strong effects of population origin and potentially genotype on berry quality. The results for titratable acidity, and pH for bilberry juice obtained from different samples grown in different altitude are presented in *Table 1*. They were not found significant differences between the obtained juices for investigated parameters (titratable acidity and total acidity). The pH values ranged between 2.5 and 2.8 with average value – 2.7. The titratable acidity values ranged between 1.4 and 3.1% with average value - 1.4%. Rohloff et al. [7] reported similar results for pH (from 2.6 to 2.8) and titratable acidity (from 1.0 to 1.4 g/100g) for bilberry grown in Norway. The main organic acids in bilberry fruit were citric malic acid and quinic acids Combined, these acids represented approximately 90% of the total analyzed organic acids [8]. Significant differences in titratable acidities have been detected in sample harvested in Yundola village about 2 times higher (3.1 g/100 mL) than other samples (mean 1.4 g/100 mL). pH and titratable acidity does not depend significantly on altitude.

Main phenolic compounds of bilberry fruit were presented on anthocyanins and ellagitannins [9]. It is evident from *Table 2*, that the highest content of total phenols has the juice obtained from bilberry fruit collect from Batashki snowfall 2000 m. (411.15 ± 0.41 mg GAE/ 100 mL), followed by Yundola village 1450 m., Nova Mahala village 1200 m. and Rakitovo 900 m. Mikulic-Petkovsek et al., [8] reported that in bilberry fruit were identified a high amount of hydroxycinnamic acids - p-coumaric acid derivatives, caffeic acid derivative, trans- and cis-5-caffeoylquinic acid, caffeic acid, 5-hydroxyvanillic acid, 5-coumaroylquinic acid and 5-feruloylquinic acid. In accordance have been established that the total polyphenol content (TPC) of investigated berry juice were from 200 to 500 mg GAE/ 100 g [8]. In contrast, bilberries originating in Norway have a higher content of polyphenols (from 500 to 600 mg GAE/100g) than the Bulgarian ones [7].

Additionally, the anthocyanins were other bioactive compounds with mainly meaning for human health effect. Bilberry fruit contained high levels of anthocyanins such as cyanidin, delphinidin, malvidin, petunidin and peonidin glycosides [8,10,11]. In this base we investigated the influence of altitude on biosynthesis of anthocyanins the results are presented in Fig. 1. The highest content of total anthocyanins has the juice obtained from bilberry fruit collect from Batashki snowfall area with the highest altitude (Fig. 1) (341 ± 0.06 mg /100 g juice), followed by Racitovo and Nova Mahala village. It has been found that bilberries harvested from the Batashki Snowfall, 2000 m have the highest antioxidant activity 4158.47 ± 7.91 mmol TE/100 g, followed by bilberry harvested from Yundola,1450 m, Nova Mahala village, 1200 m and Rakitovo, 900 m.

The total polyphenols content depends on a moderate to significant decreasing (inverse) of the linear relationship (r^2 = -0.86 and r^2 = -0.42) with total acidity and altitude respectively. With an increase in pH and altitude, the amount of polyphenols decreases. The total anthocyanins content depend on altitude with high direct (increasing) linear relationship r^2 = 0.75. Antioxidant activity in bilberry juice mainly depend on total anthocyanins content (r^2 = 0.96). The titratable acidity decrease when the content of total anthocyanins increase (r2 = -0.51). The antioxidant activity significantly increase when increase altitude i.e. they have significantly correlation (r^2 = 0.89). (Table 3). Similar results have been reported for raspberry Papanov et al., [6]. There is no correlation between common phenols and common anthocyanins and pH, total phenols and titratable acidity.

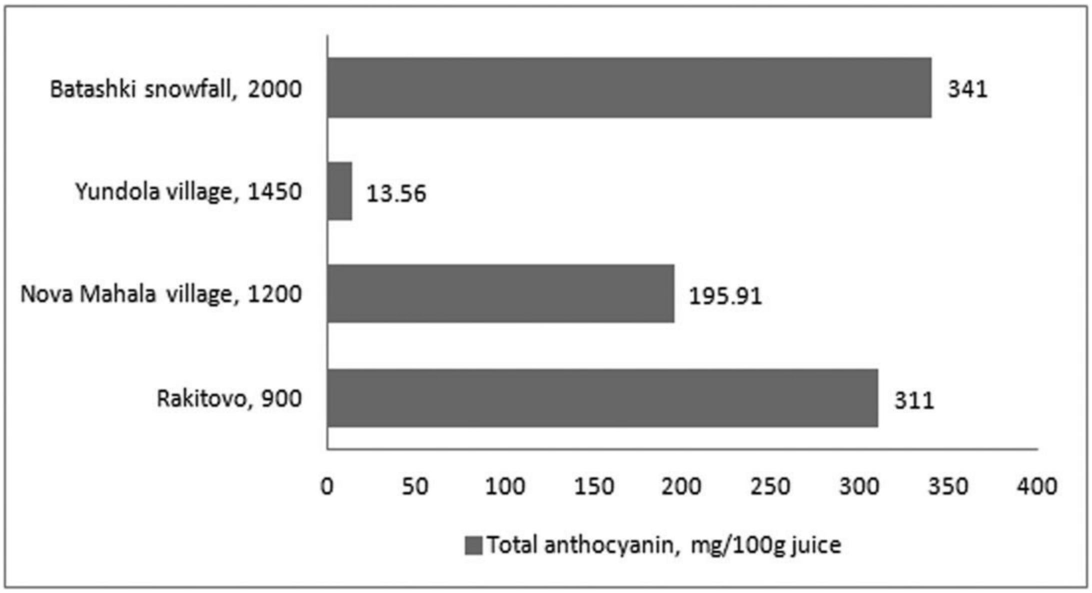

Fig. 1. Total anthocyanins in different altitude areas

Notes: - Total anthocyanins, mg/100g juice

Table 1. Total acidity (pH) and titratable actidity of different samples of bilberry juice

Sample	Altitude, m	pH	Titratable acidity (g citric acid /100 g)
Rakitovo	900	2,79	1,47
Nova Mahala village	1200	2,87	1,45
Yundola village	1450	2,52	3,12
Batashki snowfall	2000	2,87	1,47

Table 2. Total polyphenols and antioxidant activity of different bilberry juice

Sample	Altitude, m	Total polyphenols, mg GAE/100 g juice	Antioxidant ability, DPPH mmol TE/ 100 g juice
Rakitovo	900	375.97 ± 0.22	725.98 ± 0.40
Nova Mahala village	1200	383.93 ± 0.34	737.20 ± 0.20
Yundola village	1450	476.31 ± 0.26	924.98 ± 0.30
Batashki snowfall	2000	411.15 ± 0.41	4158.47 ± 7.91

Table 3. Correlation (r2) between antioxidant activity and investigated parameters altitude, total polyphenols content, total anthocyanins content and titratable acidity ant total acidity

	TPC	TAA	AOA	pH	TA
Altitude	0.41	0.75	0.89	0.07	0.09
TPC (Total Polyphenols Content)	-	- 0.22	0.04	- 0.86	-0.42
TAA (Total Anthocyanins)	-	-	0.96	0.59	-0.51
AOA (Antioxidant Ability)	-	-	-	0.38	-0.27
TA (Titratable Acidity)				- 0.97	

TAA- Titratable Acidity; TAC-Total Anthocyanins Content; TA-Total Acidity; TPC-Total Polyphenols Content

4. CONCLUSION

It has been established a direct relationship between altitude and antioxidant activity. In the range of 900 to 1450 m the relationship between altitude and total anthocyanins is inversely proportional. After 1500 m, the dependency becomes proportional. There is no correlation between pH, total polyphenols and titratable acidity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Upton R editor. American Herbal Pharmacopoeia and Therapeutic Compendium; 2001.
2. Erlund I, Marniemi J, Hakala P, Alfthan G, Meririnne E, Aro A. Consumption of black currants, lingonberries and bilberries increases serum quercetin concentrations. Eur J Clin Nutr. 2003; 57:37–42.
3. Zafra-Stone S, Taharat Y, Bagchi M, Chatterjee A, Vinson J, Bagchi D. Berry anthocyanin sasnovel antioxidant sin human health and disease prevention, Mol Nutr Food Res. 2007;51:675-683.
4. Benzie I, Wachtel-Galor S. Vegetarian diets and public health: Biomarker and Redox Connections, Antioxid Redox Signal. 2010;13:175–191.
5. Chu W, Cheung S, Lau R, Benzie I. Herbal medicine: Biomolecular and clinical aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2011.
6. Papanov S, Petkova Ek, Ivanov I. Polyphenols content and antioxidant activity of various pomegranate juices Bulgarian Chemical Communications. 2019;51(1):113-116.
7. Rohloff J, Uleberg E, Nes A, Krogstad T, Nestby R, Martinussen I. Nutritional composition of bilberries (*Vaccinium myrtillus* L.) from forest fields in Norway – Effects of geographic origin, climate, fertilization and soil properties Journal of Applied Botany and Food Quality. 2015;88:274-287.
8. Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Stampar F, Veberic R. A comparison of fruit quality parameters of wild bilberry (*Vaccinium myrtillus* L.) growing at different locations, J Sci Food Agric. 2015; 95(4):776-785.
9. Burton-Freeman B, Sandhu A, Edirisinghe I. Red Raspberries and their bioactive polyphenols: Cardiometabolic and Neuronal Health Links, Adv Nutr. 2016;7:44–65.
10. Burdulis D, Ivanauskas L, Dirsė V, Kazlauskas S, Ražukas A. Study of diversity of anthocyanin composition in bilberry (*Vaccinium myrtillus* L.) fruits, Medicina (Kaunas). 2007; 43(12):971-977.
11. Burdulis D, Sarkinas A, Jasutien I, Stackeviien E, Nikolajevas L,

Janulis V. Comparative study of anthocyanin composition, antimicrobial and antioxidant activity in bilberry (*Vaccinium myrtillus* L.) and blueberry (*Vaccinium corymbosum* L.) fruits. Acta Poloniae Pharmaceutica ñ Drug Research. 2009;66(4):399-408.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/67855