Original article

Bioactive compounds, antioxidant activity and mineral composition of fruits of raspberry cultivars grown in subtropical areas in Brazil

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Bioactive compounds, antioxidant activity and mineral composition of fruits of raspberry cultivars grown in subtropical areas in Brazil.

Abstract – Introduction. Raspberry is an economically important berry crop; its fruit contains numerous phenolic compounds with potential health benefits. It is known that the chemical content of the fruit is affected by processing factors, but limited information is available on the influence of production factors or impact of genotypes. **Materials and methods**. The raspberry cultivars tested in our study were Autumn Bliss, Batum, Heritage, Polana (red fruits) and Golden Bliss (yellow fruits); a hybrid boysenberry and a black raspberry (black fruits). The plants were grown in two subtropical areas in Brazil. Harvested fruits were used to determine the contents of bioactive compounds (anthocyanins, ascorbic acid, β-carotene, lycopene and total phenols), antioxidant activity and mineral composition (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, manganese, zinc and iron). **Results**. Our results revealed a strong influence of the site on the parameters evaluated and indicated the existence of variation among the cultivars. In general, colored raspberries are a rich source of nutrients, especially nitrogen, potassium, iron and manganese, and contain significant amounts of bioactive compounds.

Brazil / *Rubus idaeus* / *Rubus niveus* / raspberries / antioxidants / proximate composition

Composés bioactifs, activité antioxydante et composition minérale des fruits de divers cultivars de framboisier cultivés dans les zones subtropicales du Brésil.

Résumé – Introduction. La culture du framboisier est économiquement importante; son fruit contient de nombreux composés phénoliques présentant divers avantages potentiellement bénéfiques pour la santé. Il est connu que la composition chimique du fruit est affectée par des facteurs d'industrialisation, mais peu d'informations sont disponibles sur l'influence des facteurs de production ou l'impact du génotype sur cette composition. **Matériel et méthodes**. Les cultivars de framboisier testés dans notre étude ont été Autumn Bliss, Batum, Heritage, Polana (framboises rouges) ; Golden Bliss (framboises jaunes), un hybride de mûrier de Boysen ; et un framboisier à fruit noir. Les plantes ont été cultivées dans deux zones subtropicales du Brésil. Les fruits récoltés ont été utilisés pour déterminer les teneurs en composés bioactifs (anthocyanines, acide ascorbique, β-carotène, lycopène, phénol total) des fruits, ainsi que leur activité antioxydante et leur composition minérale (azote, phosphore, potassium, calcium, magnésium, soufre, bore, cuivre, manganèse, de zinc et de fer). **Résultats**. Nos résultats ont révélé une forte influence du site sur les paramètres évalués ; ils ont mis en évidence des variations entre les cultivars. En général, les framboises colorées sont riches en nutriments, notamment en azote, potassium, fer et manganèse ; elles contiennent des quantités significatives de composés bioactifs.

Brésil / *Rubus idaeus* / *Rubus niveus* / framboise / antioxydant / composition globale

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1. Introduction

In recent decades, cultivation of temperate fruit species has been characterized by a significant increase in specific regions, with cultivation no longer being practiced only in the coldest areas but moving into non-traditional growing regions that experience mild winters and summers with higher temperatures. A recent example in Brazil is the cultivation of raspberries in subtropical regions [1].

The fruits of the raspberry plant have a high free radical-scavenging capacity, containing a number of phenolic compounds that offer significant health benefits to consumers [2]. In addition, these compounds can show the natural pigments, mainly anthocyanin, which confers an attractive coloration when fruits are processed to make dairy products, jams and fruit preserves [3].

The fruit chemical composition of a specific cultivar may be affected by several factors, with the environmental conditions being among the most important. Anttonen and Karjalainen reported a variation in fruit chemical composition due to the site of cultivation in fruits from distinct regions in Northern Europe [4]. The fruit yield and quality also vary among raspberry cultivars [5].

The objective of our work was to analyze and compare the bioactive compounds, antioxidant activity and mineral content of the fruits of different raspberry cultivars produced in subtropical areas in Brazil.

Table I.
Raspberry cultivars analyzed to study the bioactive compounds, antioxidant activity and mineral composition of their fruit in subtropical areas in Brazil.

Rubus species	Cultivar name	Fruit color
Rubus idaeus	Autumn Bliss	Red
	Batum	
	Polana	
	Heritage	
	Golden Bliss	Yellow
Rubus niveus Thunb.	Black raspberry	Black
Rubus loganbaccus × *R. baileyanus* Britt.	Boysenberry	Purple

2. Materials and methods

Fruits of seven raspberry cultivars were analyzed (*table I*).

Approximately 500 g of fruit from each cultivar were collected at the stage of physiological maturation at two sites: the first one was a commercial orchard situated in the Mantiqueira Mountains in Campos do Jordão City, São Paulo State, lat. 22°44' S, long. 45°35' W, alt. 1,628 m, Cwa climate, mesothermal with a dry winter, commonly named a tropical highland according to the Köppen classification; the second one was in Alto do Rio Grande in Lavras town, Minas Gerais State, lat. 21°14' S, long. 45°00' W, alt. 918 m, Cwb climate, with a dry winter and temperate summers.

Both orchards were managed following the recommendations of Gonçalves *et al.* [1]. The average maximum and minimum temperatures and the accumulated rainfall for the period of development and collection of the fruits (October to December of 2011) were 22.5 °C, 8.0 °C and 157.5 mm, respectively, in Campos do Jordão, and 27.1 °C, 15.1 °C and 72.6 mm, respectively, in Lavras.

Fruits were collected soon after dawn, when the ambient temperature was relatively low. They were carefully placed onto transparent polyethylene terephthalate trays with a capacity of 125 g (11-cm length × 10.5-cm width × 4.5-cm height). Small circular holes in the lids of the containers allowed gas circulation, and moisture-absorbing paper was placed in the interior. The trays were put into a plastic foam box with ice and transported to the Biochemistry Laboratory of the Chemistry Department where the fruits were examined for uniformity of color and the absence of mechanical or physiological disorders.

The fruits were then utilized for the following analyses:

– Anthocyanins: the determination of monomeric anthocyanins was performed using the pH differential method according to Giusti and Wrolstad [6]. The results were expressed in mg cyanidin-3-glucoside·100 g^{-1} fresh weight.

– Vitamin C: the extraction of vitamin C was conducted using 2 g of sample in 20 mL oxalic acid and approximately 0.1 g of Kieselguhr, and the mixture was stirred for 15 min in a horizontal stirrer. The solution was filtered through Whatman n° 40 paper, and the content of ascorbic acid was determined using the colorimetric method with 2,4 dinitrophenylhydrazine, according to Strohecker and Henning [7]. The results were expressed in mg ascorbic acid·100 g^{-1} fresh weight.

– Carotenoids: for the determination of the carotenoids β-carotene and lycopene, the samples were homogenized with a mixture of acetone and hexane (2:3), and the absorbance was measured using a spectrophotometer at four wavelengths [(453, 505, 645 and 663) nm]. The results were expressed in µg·100 g^{-1} fresh weight, as reported by Nagata and Yamashita [8].

– Total phenolic compounds: the extraction of the phenolic compounds was performed using reflux in 50% methanol for three consecutive extractions at 80 °C; the extracts were combined and evaporated to 25 mL. After extraction, the total phenolics were measured using Folin-Denis reagent [9]. The results were expressed in mg gallic acid Eq·100 g^{-1} fresh weight.

– Total antioxidant activity: utilizing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method, extracts were obtained according to the method of Marinova *et al.* [10]. The results were expressed in IC_{50}, which is the value defined as the fresh weight of fruit necessary to reduce the initial concentration of DPPH by 50%. The anti-radical activity (A_{Ar}) was defined as [$1/IC_{50}$].

– Macro- and micronutrients: the analytical determinations were performed according to Nour *et al.* [3]. Nitrogen was determined using the semi-micro-Kjeldahl method; phosphorus and boron, by colorimetric methods; sulfur, by turbidimetry; potassium, by flame and emission photometry; calcium, magnesium, copper, iron, manganese and zinc, by atomic absorption spectrophotometry.

The experimental design utilized was completely randomized, with four replicates. The data were subjected to an analysis of variance, and the means were compared using the Scott-Knott test at the level of 5% of probability, with the aid of the SISVAR program [11].

3. Results and discussion

The analysis of variance of the data obtained from our analyses of the raspberry fruits showed significant effects for the (site × cultivar) interaction for all of the bioactive compounds considered (*table II*).

3.1. Fruit anthocyanin content

Regarding the anthocyanin content of the raspberries, only the black raspberry cultivated in the Mantiqueira Mountains presented higher content (43% higher) than those produced in Alto do Rio Grande, whereas boysenberry exhibited higher values of anthocyanins when cultivated in Alto do Rio Grande. At both of the cultivation sites, the highest anthocyanin content was found in black raspberries and boysenberry fruits, whereas 'Golden Bliss' presented the lowest values. There were no differences in the anthocyanin content among the cultivars of red raspberry in each cultivation place.

The effect of the site on the anthocyanin content in raspberries was also reported by Salinas-Moreno *et al.* in fruits of the cultivar 'Autumn Bliss' grown in two towns in Mexico [12]. Regarding the variation in the anthocyanin contents among cultivars, Pantelidis *et al.* ascribed different values to the range of color of the raspberries, with values of 49.2 mg·100 g^{-1} for 'Heritage', 39.1 mg·100 g^{-1} for 'Autumn Bliss' and only 3.4 mg·100 g^{-1} for 'Fall Gold' (yellow colored) [13]. The contents of anthocyanins found in our work are higher than those reported by Pantelidis *et al.*, who collected fruits in Northern Greece [13]. Ancos *et al.* collected fruits in Cáceres, Spain; they found values of 9.05 mg anthocyanins·100 g^{-1} for 'Autumn Bliss' and 14.0 mg anthocyanins·100 g^{-1} for 'Heritage' [14]. The difference among the cultivars in relation to the cultivation site in our study may be related to the higher temperature in the subtropical

Table II.
Bioactive compounds of different-colored raspberry fruits of cultivars grown in the Mantiqueira Mountains and Alto do Rio Grande (UFLA, Lavras, MG, Brazil in 2012).

Cultivars	Anthocyanins (mg·100 g^{-1} fresh weight)		Vitamin C (mg·100 g^{-1} fresh weight)		β-carotene (µg·100 g^{-1} fresh weight)		Lycopene (µg·100 g^{-1} fresh weight)		Total phenolics (mg GAE·100 g^{-1} fresh weight)		Antioxidant activity/IC_{50} (mg·100 g^{-1} fresh weight)	
	Mantiqueira Mountains	Alto do Rio Grande	Mantiqueira Mountains	Alto do Rio Grande	Mantiqueira Mountains	Alto do Rio Grande	Mantiqueira Mountains	Alto do Rio Grande	Mantiqueira Mountains	Alto do Rio Grande	Mantiqueira Mountains	Alto do Rio Grande
Autumn Bliss	431.03 Ac	426.17 Ab	47.22 Ac	51.58 Ab	28.89 Aa	22.70 Bc	2.49 Ad	0.35 Be	415.99 Ba	553.23 Aa	280.75 Ab	354.01 Ab
Batum	403.05 Ac	402.16 Ab	50.86 Ac	58.11 Ab	18.19 Bb	26.92 Ab	3.78 Ac	3.78 Ab	395.80 Aa	314.64 Ac	238.15 Ba	377.92 Ab
Heritage	268.17 Ac	334.35 Ab	54.22 Ac	43.96 Ab	10.58 Ac	7.69 Ad	4.06 Ac	2.42 Bc	393.22 Aa	446.79 Ab	348.32 Ab	326.12 Ab
Polana	368.81 Ac	377.41 Ab	66.36 Ac	51.92 Ab	18.57 Ac	7.29 Bd	3.10 Ad	3.08 Ac	316.72 Bb	489.28 Ab	253.90 Ba	443.75 Ac
Golden Bliss	29.06 Ad	27.18 Ac	41.73 Ac	38.53 Ab	9.31 Bc	21.92 Ac	4.52 Ac	1.59 Bd	287.61 Bb	491.69 Ab	282.35 Ab	246.75 Aa
Black raspberry	3,321.10 Aa	1,895.36 Ba	137.49 Aa	103.62 Ba	17.16 Bb	51.63 Aa	7.90 Aa	6.89 Ba	300.71Ab	315.07 Ac	237.27 Aa	187.27 Aa
Boysenberry	1,435.74 Bb	1,813.42 Aa	93.28 Ab	93.01 Aa	16.66 Ab	8.40 Bc	5.48 Ab	1.77 Bd	381.84 Aa	319.75 Ac	163.57 Aa	223.22 Aa
Coefficient of variation (%)	11.39		5.85		12.85		14.23		16.15		13.42	

* Means followed by the same upper-case letter in the row and lower-case letter in the column do not differ by the Scott-Knott test ($P \leq 0.05$).

region of Brazil, that stimulated anthocyanin biosynthesis.

As an economically viable source, the high concentration of anthocyanin pigments in the fruits obtained in our study indicates their potential use as natural dyes in the food industry. Zozio *et al.* suggested that anthocyanins have been shown to be promising alternatives to the use of synthetic dyes [15].

3.2. Fruit ascorbic acid content

Black raspberries grown in the Mantiqueira Mountains presented higher ascorbic acid contents than the fruits of Alto do Rio Grande area. It is likely that larger variations in temperature due to the higher elevation of the Mantiqueira Mountains stimulated the biosynthesis of ascorbic acid in this species.

This species differed significantly from the other raspberries at both of the sites, with average values of 120.5 mg·100 g^{-1}. No differences were found among the cultivars of red and yellow raspberries. The ascorbic acid content of black raspberry is ten times greater than that reported by Jin *et al.*, who found only 19.88 mg ascorbic acid·100 g^{-1} for *R. niveus* fruits grown in China [16].

The recognition of raspberries as a source of vitamin C is reported in the literature [14]. Pantelidis *et al.* reports that 100 g of raspberries supply 16.8 mg vitamin C·100 g^{-1} ('Fall Gold', a yellow-colored raspberry) to 37.7 mg vitamin C·100 g^{-1} ('Heritage', a red-colored raspberry), namely, the equivalent of more than 30% of the daily recommended dose [13]. Nevertheless, the values found in our work are higher, at 137.49 mg vitamin C·100 g^{-1} for black raspberries, 41.73 mg vitamin C·100 g^{-1} for 'Golden Bliss' (yellow) and 66.36 mg vitamin C·100 g^{-1} for 'Polana' (red). Thus, raspberry fruits grown in subtropical climatic conditions tend to produce increased amounts of vitamin C.

3.3. Fruit carotenoid content

The colored raspberries presented varying carotenoid contents according to both the site and cultivar. β-carotene was notable in relation to lycopene, with maximum and minimum contents of (51.63 and 7.29) μg·100 g^{-1}, respectively. The highest values of β-carotene were found in the red raspberry 'Autumn Bliss' grown in the Mantiqueira Mountains and in black raspberry grown in Alto do Rio Grande. The low values found for the β-carotene content in the raspberries in our study may be associated with the low content of fat present in the fruits, as also indicated by Jacques *et al.* in quantifying the carotenoid content of blackberry, another small fruit [17]. Although the lycopene content was low, black raspberry presented the highest values at both of the sites.

3.4. Fruit total phenol content

The highest values of total phenols were found for the cultivar 'Autumn Bliss', followed by 'Golden Bliss', 'Polana' and 'Heritage' grown in Alto do Rio Grande. Black raspberry, 'Golden Bliss' and 'Polana' presented the lowest values when grown in the Mantiqueira Mountains. These results indicate that the climatic conditions can influence the contents of total phenols.

Overall, the total phenol contents in the raspberry fruits were within the range reported by Weber *et al.*, who found phenol contents ranging from (300 to 700) mg·100 g^{-1} in a study of 64 genotypes [18]. The results obtained with the cultivar 'Heritage' were higher than those found by Zhang *et al.*, who reported 293.3 mg·100 g^{-1} in fruits collected in Illinois, USA [19]. Clearly, raspberries cultivated in regions with higher temperatures tend to produce increased contents of total phenols. The content of total phenols of raspberries is above that reported by Ferreira *et al.* in blackberry (241.7 mg·100 g^{-1}) [20].

3.5. Fruit antioxidant activity

The potential of the raspberry extracts to scavenge free radicals was expressed as the concentration of the extract necessary to inhibit the oxidation of the DPPH radical by 50% (*table II*). All of the cultivars presented antioxidant activity; however, the intensity

of action was different among them. When grown in the Mantiqueira Mountains, the cultivars 'Batum' and 'Polana', black raspberry and the boysenberry hybrid displayed the best capacity of scavenging the DPPH radical, with the lowest values of IC_{50} (concentration of extract necessary to inhibit 50% of the free radical formed). Black raspberries and boysenberry produced in Alto do Rio Grande, in addition to yellow raspberries, also showed the best antioxidant capacity.

As compared with other small fruits, such as blackberry, Ferreira *et al.* found that the extract obtained to evaluate the phenolic compounds (methanol 80%) presented antiradical activity in the range of (33.8 ± 1.8) mg sample per mg DPPH [20]. These results demonstrated that an increased amount of extract is necessary for antioxidant action of raspberries. The differences in these results are associated with the fact that antioxidant activity varies in relation to the extraction method utilized [21].

3.6. Mineral composition of raspberry fruits

The analysis of variance for the mineral composition of fruits of the different raspberry cultivars studied revealed a significant effect only for the cultivar factor (*table III*). Because the same fertilization management, according to the recommendations of Gonçalves *et al.*, was adopted at both of the cultivation sites, the mineral contents of the different cultivars were not influenced [1].

The cultivar 'Batum' showed the highest nitrogen and phosphorus contents, and boysenberry showed the greatest values of calcium and magnesium. These cultivars, together with black raspberry, also revealed the highest sulfur contents. Conversely, 'Polana' presented low contents of nitrogen, potassium, calcium and magnesium.

The macronutrients present in the greatest amounts in all of the cultivars were N and K, with average contributions to the recommended daily intake (RDI) of 1.88% and 4.92%, respectively.

The RDI is the amount of vitamins, minerals and proteins that should be consumed daily to meet the nutritional needs of most individuals in a healthy population [22]. For the population to consume nutrients according to the RDI, data regarding the composition of foods and the importance of the addition of minerals in the diet have been widely discussed in nutrition texts. From a nutritional standpoint, raspberry has been highlighted for its contents of mineral salts.

For micronutrients, 'Batum' also presented the greatest contents of boron and zinc. For the elements manganese, iron and copper, boysenberry, 'Polana' and black raspberry demonstrated the highest values, respectively. Among the micronutrients investigated, those presenting the greatest contributions toward the RDI were Fe and Mn, contributing an average of 7.40% and 5.53%, respectively. Therefore, raspberries are as iron-rich as blackcurrants [3].

4. Conclusions

Our experiments revealed that:

1) The contents of bioactive compounds in raspberry fruits are affected by the cultivation site, and the values range according to the cultivar.

2) Raspberry fruits can be considered a naturally rich source of both antioxidants and pigments.

3) Fruits of the cultivars studied constitute a rich source of minerals, such as nitrogen, potassium, iron and manganese.

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Table III.
Mineral composition of fruits of different colored raspberry cultivars in subtropical areas in Brazil and recommended daily intake (RDI [22]) (UFLA, Lavras, MG, Brazil in 2012).

a) Macronutrients

Cultivars	N	P	K	Ca	Mg	S
			mg·100 g^{-1} dry weight			
Autumn Bliss	144.7 c	225.0 a	119.0 c	11.0 c	14.7 b	9.7 b
Batum	169.2 a	220.0 a	132.2 b	11.2 c	14.5 b	12.7 a
Heritage	136.0 d	190.0 b	112.7 d	8.5 e	12.5 d	9.7 b
Polana	139.0 d	172.5 c	103.2 e	7.7 f	12.2 d	8.2 b
Golden Bliss	148.2 c	180.0 c	110.7 d	10.5 d	13.5 c	10.5 b
Black raspberry	160.2 b	177.5 c	152.0 a	16.2 b	14.5 b	12.0 a
Boysenberry	157.2 b	155.0 a	131.7 b	17.5 a	16.2 a	12.0 a
Coefficient of variation (%)	3.63	5.49	1.58	1.96	2.13	23.40

Recommended daily intake (RDI) for macronutrients (mg)

N	P	K	Ca	Mg	S	N
8000	800	2500	800	300	500	8000

b) Micronutrients

Cultivars	B	Cu	Mn	Zn	Fe
			mg·kg^{-1} dry weight		
Autumn Bliss	22.7 a	5.6 c	18.6 f	27.3 c	70.9 d
Batum	26.3 a	5.2 d	38.4 b	30.2 a	63.3 e
Heritage	19.1 b	4.4 e	21.6 e	23.4 e	115.7 c
Polana	19.5 b	5.1 d	15.6 g	23.4 e	169.7 a
Golden Bliss	17.1 b	5.1 d	25.1 d	26.2 d	72.8 d
Black raspberry	9.02 c	8.0 a	33.7 c	23.2 e	112.1 c
Boysenberry	11.2 c	6.8 d	40.6 a	28.4 b	120.4 b
Coefficient of variation (%)	21.34	2.74	2.01	2.46	3.39

Recommended daily intake (RDI) for micronutrients (mg)

B	Cu	Mn	Zn	Fe
180	300	500	1500	1400

* Means followed by same lowercase letter in the column do not differ by Scott-Knott test ($P \leq 0.05$).

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Compuestos bioactivos, actividad antioxidante y composición mineral de los frutos de los cultivares de frambuesas diferentes que crecen en áreas subtropicales en Brasil.

Resumen – Introducción. Frambuesa es un tipo de berry de gran importancia económica que contiene numerosos compuestos fenólicos, con beneficios potenciales para la salud. Se sabe que el contenido de la composición química es afectado por diversos factores, pero pocas son las informaciones sobre la influencia de los factores de producción. **Materiales y métodos**. Los cultivares de frambuesa estudiados en nuestro estudio fueron Autumn Bliss, Batum, Heritage, Polana (frambuesas rojas), Golden Bliss (frambuesas amarillas), el híbrido boysenberry y la frambuesa negra. Las plantas fueron cultivadas en dos áreas subtropicales en Brasil. Los frutos cosechados fueron utilizados para determinar los niveles de compuestos bioactivos (antocianinas, ácido ascórbico, β-caroteno, licopeno, fenoles totales), actividad antioxidante y composición mineral (nitrógeno, fósforo, potasio, calcio, magnesio, azufre, boro, cobre, manganeso, zinc y hierro). **Resultados y discusiones**. Los resultados revelaron una fuerte influencia del local de cultivo sobre la calidad de la fruta, así como la existencia de variación entre los cultivares. En general, frambuesas coloridas son rica fuente de nutrientes especialmente nitrógeno, potasio, hierro y manganeso, así como contienen cantidades significativas de compuestos bioactivos.

Brasil / *Rubus idaeus* / *Rubus niveus* / frambuesa / antioxidantes / composición aproximada