

# Phenolic compounds and antioxidant activity of blueberry cultivars grown in Brazil

## *Compostos fenólicos e atividade antioxidante de cultivares de mirtilo produzidas no Brasil*

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### Abstract

The aim of this study was to evaluate the content of the phenolic compounds and anthocyanins and the antioxidant activity of blueberry (*Vaccinium* sp.) cultivars grown in Brazil. The Folin-Ciocalteu method was applied in order to quantify the phenolic compounds and ABTS, DPPH, FRAP, and  $\beta$ -carotene/linoleic acid methods were applied in order to evaluate antioxidant activity. The phenolic compounds content ranged from 274.48 to 694.60 mg GAE.100 g<sup>-1</sup> of fresh weight (FW). Anthocyanins content ranged from 40.62 to 378.31 mg.100 g<sup>-1</sup> FW for Bluecrop and Tifblue cultivars, respectively. Antioxidant activities assessed by ABTS, DPPH and FRAP methods presented significant differences among the studied cultivars ranging from 1238.48 to 2445.96, 1014.24 to 2055.06 and 699.78 to 1740.25  $\mu$ mol TEAC.100 g<sup>-1</sup> FW, respectively. The results confirm the blueberry as a source of phenolic compounds with high antioxidant activity and also show that there are different levels of concentrations of phenolic compounds and antioxidant activity according to the cultivar and production location.

**Keywords:** blueberry; cultivars; antioxidant activity; phenolic compounds.

### Resumo

O objetivo deste trabalho foi avaliar a concentração de compostos fenólicos totais, antocianinas monoméricas totais e a atividade antioxidante in vitro das principais cultivares de mirtilo (*Vaccinium* sp.) produzidas no Brasil. O método Folin-Ciocalteu foi aplicado para quantificar os compostos fenólicos totais e os métodos ABTS, DPPH, FRAP e  $\beta$ -caroteno/ácido linoleico para avaliar a atividade antioxidante. O teor de compostos fenólicos totais encontrado variou de 274,48 a 694,60 mg de equivalente a ácido gálico (EAG).100 g<sup>-1</sup> em peso fresco (PF). A concentração de antocianinas monoméricas variou da cultivar Bluecrop até a Tifblue, com valores de, respectivamente, 40,62 a 378,31 mg.100 g<sup>-1</sup> PF. A atividade antioxidante medida pelos métodos ABTS, DPPH e FRAP mostrou grande amplitude entre as cultivares estudadas, com valores variando de, respectivamente, 1238,48 a 2445,96, 1014,24 a 2055,06 e 699,78 a 1740,25  $\mu$ mol TEAC.100 g<sup>-1</sup> PF. Os resultados confirmam o mirtilo como fonte de compostos fenólicos com elevada atividade antioxidante. Demonstram ainda que existem diferentes níveis de concentrações de compostos fenólicos e atividade antioxidante de acordo com a cultivar e o local de produção desta fruta.

**Palavras-chave:** mirtilo; cultivares; atividade antioxidante; compostos fenólicos.

## 1 Introduction

Free radicals and other reactive species can cause oxidation and biomolecular damage when the oxidative species exceed the anti-oxidative defenses of the organism resulting in oxidative stress. This is associated to aging and to the development of pathologies such as cancer, cardiovascular disease, neurodegenerative disorders, diabetes, and inflammation (DUFFY et al., 2007; MATEOS; BRAVO, 2007; LU; FINKEL, 2008). However, evidence has shown the relationship between a diet rich in fruit and vegetables and a decrease of cardiovascular disease and certain types of cancer, which, hypothetically, is due to the antioxidant contents (GERMAN; WALZEM, 2000; KAUR; KAPOOR, 2001; YANG et al., 2001; GARCIA-ALONSO et al., 2004). This relationship has stimulated research on the antioxidant capacity of fruit and vegetables.

Among the compounds with antioxidant properties found in fruits and vegetables, phenol compounds stand out. They

account for the largest part of the antioxidant activity of many plants (DUTHIE; CROZIER, 2000). They occur naturally in plants as secondary metabolites, and are present in fruits, vegetables, leaves, nuts, seeds, and flowers. They are an integral part of the human diet and have also been intentionally added to some medication preparations (WU et al., 2004). Small fruits, known as berries, are very rich in phenol compounds and present high antioxidant activity (SEERAM, 2008; WOLFE et al., 2008), and are interesting as ingredients for use in juices, jams, ice cream, and cake icing, in addition to being used successfully in the development of functional foods with the objective of enhancing health (POTTER et al., 2007). Among the berries, the blueberry (*vaccinium* sp.), represented by various species and cultivars, stands out, and some of them present antioxidant activity significantly greater than others (PRIOR et al., 1998; MOYER et al., 2002).

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Blueberry production is concentrated mainly in the United States and Canada, and the former is responsible for 66% and the latter for 33% of world production (STRIK, 2005). Brazil has recently become a blueberry producer with a small production concentrated in the south and southeastern regions of the country, in the municipalities of Vacaria and Caxias do Sul (Rio Grande do Sul, RS), Barbacena (Minas Gerais, MG), and Campos do Jordão (São Paulo, SP) (SANTOS, 2004). Considering the lack of information on the phenolic compounds content and antioxidant activity of the blueberries produced in Brazil, the objective of this study was to assess the phenolic compounds content, anthocyanins content, and *in vitro* antioxidant activity of the main blueberry (*Vaccinium* sp.) cultivars grown in Brazil using the ABTS, DPPH, FRAP and  $\beta$ -carotene/linoleic acid methods.

## 2 Materials and methods

### 2.1 Chemicals

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH),  $\beta$ -carotene, linoleic acid, BHT (2,6-di-tert-butyl-4-methylphenol), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and gallic acid were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Folin-Ciocalteu reagent, 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ), and Tween 40 were purchased from Fluka Chemie AG (Buchs, Switzerland). Methanol, chloroform, ferric chloride, sodium carbonate, chloridric acid and potassium persulphate were purchased from Vetec (São Paulo, Brazil).

### 2.2 Samples

The blueberry samples were collected directly from the producers (2008 harvest) in Rio Grande do Sul state - RS (southern region), in the municipalities of Pelotas (31° 45' S, 52° 22' W), Caxias do Sul (31° 45' S, 52° 20' W), and Vacaria (28° 29' S, 50° 57' W); in São Paulo state - SP (southeastern region), municipality of Campos do Jordão (22° 44' S, 45° 35' W); and Minas Gerais state - MG (southeastern region), municipality of Barbacena (21° 18' S, 43° 48' W). The following cultivars were used: Bluecrop (*Vaccinium corymbosum*); Delite, woodard, climax, bluegem, flórida M, powderblue, briteblue, bluebelle, and Tifblue (*Vaccinium ashei* Reade). The samples were collected randomly resulting in 3 (three) samples of 500 g for each cultivar. The samples were transported to the laboratory at  $5.0 \pm 1.0$  °C, where they were separated into plastic packages of 100 g portions. These packages were de-oxygenized with nitrogen for five minutes, frozen, and stored at  $-18.0 \pm 0.5$  °C until analysis. Special care was taken while selecting to avoid damaged, unripe, or very ripe fruits.

### 2.3 Preparation of extracts

Samples of ground blueberry (2.000 g) were extracted with methanol (60 mL  $\times$  3) in an ultrasonic water bath (USC-1400 Unique) for 15 minutes at room temperature (20 °C). The extract was centrifuged at 2000 g for 10 minutes and the supernatant

was stored in an amber flask. This extract was used to determine the total phenolic compounds (TP) and for the antioxidant activity (AA) analysis.

Samples of ground blueberry (1.500 g) were extracted with acidified (HCl 0.1%) methanol (15 mL  $\times$  3) in an ultrasonic water bath (USC-1400 Unique) for 30 minutes at room temperature (20 °C). The extract was centrifuged at 2000 g for 10 minutes, and the supernatant was used to assess the total anthocyanin contents (TA).

### 2.4 Determination of total phenolic compounds (TP)

The total phenolic content of each extract was determined spectrophotometrically (Hewlett-Packard 8452 A Spectrophotometer) according to the Folin-Ciocalteu method (SINGLETON; ROSSI, 1965). Absorbance was read at 765 nm and results were expressed, in fresh weight, as mg.100 g<sup>-1</sup> gallic acid equivalent (GAE).

### 2.5 Determination of total anthocyanins (TA)

Total anthocyanins content was determined by the pH-differential method (GIUSTI; WROLSTAD, 2001). Absorbance was read at 700 nm and at the wavelength of maximum absorption. Results, in fresh weight, were expressed as the concentration of anthocyanins in mg.100 g<sup>-1</sup> cyanidin-3-glucoside equivalent ( $\epsilon = 26900$ , MW = 449.2).

### 2.6 Antioxidant activity

#### ABTS method

The ABTS method (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) is based on the deactivation of the antioxidant radical cation ABTS<sup>•+</sup>, which is measured by the decrease in absorbance at 734 nm. The ABTS method was performed as described by Re et al. (1999). Absorbance was read at 734 nm, 7 minutes after the extract addition. The total antioxidant activity of the blueberry, in fresh weight, was expressed in  $\mu$ Mol.100 g<sup>-1</sup> of TEAC (Trolox-equivalent antioxidant capacity).

#### DPPH method

2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) radical is one of the few stable and commercially available organic nitrogen compounds. The method is based on the deactivation of the DPPH<sup>•</sup> radical by compounds with antioxidant properties present in fruit extracts, and this deactivation is monitored at 515 nm. The DPPH method was carried out as described by Kim et al. (2002). The decrease in the absorbance of 100  $\mu$ M DPPH<sup>•</sup> radical (2.9 mL) dissolved in 80% methanol was evaluated at 515 nm, 30 minutes after the addition of each extract. The total antioxidant activity of blueberry, in fresh weight, was also expressed in  $\mu$ Mol.100 g<sup>-1</sup> of TEAC.

#### FRAP method

As described by Benzie and Strain (1996), with modifications by Arnous, Makris and Kefalas (2002), the FRAP method

is based on the direct measurement of the antioxidant (reducing) ability through the reduction of the complex Fe<sup>3+</sup>/tripirydyltriazine (TPTZ) to Fe<sup>2+</sup> at acidic pH (3.6). Absorbance was read at 620 nm and the reducing power, in fresh weight, was expressed in µMol.100 g<sup>-1</sup> of TEAC.

#### Co-oxidation of β-carotene/linoleic acid method

Oxidation inhibition power was evaluated by the discoloration of the β-carotene/linoleic acid system as described by Marco (1968) and modified by Miller (1971). A 20 µL aliquot of β-carotene solution (20 mg.mL<sup>-1</sup> in chloroform) was placed in a 250 mL erlenmeyer flask with 40 µL of linoleic acid, 1 mL chloroform, and 20 mg of Tween 40. After homogenization, the chloroform was completely evaporated with nitrogen. Deionised water (previously submitted to oxygen atmosphere for 30 minutes) was then added until formation of a clear emulsion with absorbance ranging from 0.6 to 0.7 at 470 nm. Aliquots of the extracts (100 µL) and 100 mg.L<sup>-1</sup> of BHT were added to 5 mL of linoleic acid emulsion in glass cuvettes (optical path of 10 mm). After the initial reading, the absorbance was monitored every 15 minutes for 2 hours. During this period, the cuvettes were kept at 50 °C in a water bath. The decrease in absorbance was compared to the control (without antioxidant). The antioxidant activity was expressed as percent inhibition, in relation to the control, according to the following Equation 1:

$$\text{inhibition \%} = 100 - \left( \frac{A_i - A_f}{C_i - C_f} \times 100 \right) \quad (1)$$

$A_i$  = extract initial absorbance

$A_f$  = extract final absorbance

$C_i$  = control initial absorbance

$C_f$  = control final absorbance

#### 2.7 Statistical analysis

The descriptive analyses, linear regression (R<sup>2</sup>), linear correlation (R), analysis of variance (ANOVA), and the Tukey test were performed using Statistica<sup>®</sup> software (2004), 7.0 version. The data are presented as mean ± standard deviation (SD). Three samples (n = 3) of each cultivar were analyzed, and all assays were performed in triplicate. Differences at p < 0.05 were considered significant.

### 3 Results and discussion

#### 3.1 Total phenolic compounds (TP) and total anthocyanins (TA)

Table 1 shows the phenolic compounds concentration in the blueberry cultivars studied. The phenolic compounds concentration ranged from 274.48 to 694.60 mg GAE.100 g<sup>-1</sup> FW. The Clímax, cultivar, from Barbacena (MG), presented the highest TP concentration. Sellappan, Akoh and Krewer (2002) studied different blueberry cultivars in the state of Georgia (USA) and reported a maximum TP concentration 34% higher than that found in this study, 929.62 mg.100 g<sup>-1</sup>

FW, for the Brightblue cultivar. The same authors reported a minimum concentration of 261.95 mg.100 g<sup>-1</sup> FW, a value close to that found for the Bluecrop cultivar in the present study, 274.48 mg.100 g<sup>-1</sup> FW. This concentration was higher than that reported by Prior et al. (1998), which was 189.90 mg.100 g<sup>-1</sup> FW, for the Bluecrop cultivar produced in North Carolina (USA), but which was lower than those reported by Kalt et al. (1999), 386.17 mg.100 g<sup>-1</sup> FW, and Giovanelli and Buratti (2009), 299.00 mg.100 g<sup>-1</sup> FW, assessing the same cultivar produced in Canada and Italy, respectively.

Samples of the Powderblue, Clímax, Bluebelle, and Bluegem cultivars collected in different production locations in Brazil (Table 1) showed significant difference (p < 0.05) for TP concentration. These results confirmed the effect of the production location on the phenolic compounds concentration. Similarly, Connor, Luby and Tong (2002) observed a significant influence (p < 0.05) in two harvests of the cultivation location on the TP content present in 16 blueberry cultivars.

Table 1 shows that, in general, the cultivars representative of the *Vaccinium ashei* Reade species presented greater richness in phenolic compounds than the Bluecrop cultivar, the only representatives of the *Vaccinium corymbosum* species assessed. This greater richness in phenolic compounds was also observed in other studies that assessed cultivars representing the two species (PRIOR et al., 1998; MOYER et al., 2002).

Table 1 shows the TA content in the blueberry cultivars. The concentration of Bluecrop and Tifblue cultivars ranged from 40.62 to 378.31 mg.100 g<sup>-1</sup> FW, respectively. There was

**Table 1.** Total phenolic compounds (TP) and total anthocyanins (TA) of blueberry cultivars (*Vaccinium* sp.) grown in Brazil.

Cultivar	tp (mg.100 g <sup>-1</sup> ) <sup>1</sup>	ta (mg.100 g <sup>-1</sup> ) <sup>2</sup>	TA/TP
<i>Vaccinium corymbosum</i> L.			
Bluecrop <sup>b</sup>	274.48 ± 15.83 <sup>g</sup>	40.62 ± 3.17 <sup>g</sup>	0.15
<i>Vaccinium ashei</i> Reade			
Delite <sup>d</sup>	418.67 ± 5.63 <sup>d</sup>	231.47 ± 5.92 <sup>c</sup>	0.55
Powderblue <sup>a</sup>	675.57 ± 10.25 <sup>a</sup>	245.48 ± 19.71 <sup>c</sup>	0.36
Bluegem <sup>a</sup>	336.57 ± 1.92 <sup>f</sup>	265.99 ± 23.95 <sup>b</sup>	0.79
Clímax <sup>a</sup>	288.03 ± 8.10 <sup>g</sup>	240.50 ± 37.51 <sup>c</sup>	0.83
Flórida M <sup>a</sup>	332.96 ± 24.67 <sup>f</sup>	278.76 ± 19.92 <sup>b</sup>	0.84
Woodard <sup>a</sup>	378.55 ± 26.91 <sup>e</sup>	254.89 ± 18.39 <sup>b</sup>	0.67
Bluebelle <sup>d</sup>	339.39 ± 18.28 <sup>f</sup>	238.85 ± 15.56 <sup>c</sup>	0.70
Powderblue <sup>c</sup>	436.69 ± 13.91 <sup>d</sup>	220.29 ± 18.19 <sup>d</sup>	0.50
Briteblue <sup>c</sup>	482.64 ± 14.36 <sup>c</sup>	258.17 ± 30.38 <sup>b</sup>	0.53
Flórida M <sup>c</sup>	340.41 ± 6.98 <sup>f</sup>	69.97 ± 4.82 <sup>f</sup>	0.21
Bluebelle <sup>c</sup>	557.33 ± 18.58 <sup>b</sup>	162.85 ± 14.56 <sup>e</sup>	0.29
Bluegem <sup>c</sup>	542.45 ± 21.36 <sup>b</sup>	186.66 ± 9.61 <sup>e</sup>	0.34
Clímax <sup>e</sup>	694.60 ± 47.39 <sup>a</sup>	234.08 ± 19.69 <sup>c</sup>	0.34
Tifblue <sup>e</sup>	450.62 ± 6.63 <sup>d</sup>	378.31 ± 16.23 <sup>a</sup>	0.84
<b>Mean ± SD</b>	<b>436.60 ± 131.82</b>	<b>220.46 ± 81.99</b>	<b>0.54</b>

Values are expressed in fresh weight of blueberry and as mean ± standard deviation (SD), n = 3. Different letters in the same column indicate significant difference (p < 0.05).

<sup>1</sup>Expressed as GAE (gallic acid equivalent). <sup>2</sup>Expressed as equivalent to cyanidin-3-glucoside. <sup>a</sup>Caxias do Sul (RS), <sup>b</sup>Vacaria (RS), <sup>c</sup>Pelotas (RS), <sup>d</sup>Campos do Jordão (SP), <sup>e</sup>Barbacena (MG).

no great variability in the TA content among the cultivars studied, except for the Flórida M (Pelotas, RS) and Bluecrop cultivars that presented a much lower concentration and the Tifblue cultivar that presented a much higher concentration than the others. The TA values detected for the blueberries (*Vaccinium* sp.) produced in Brazil were greater than those reported by Sellappan, Akoh and Krewer (2002) for blueberry cultivars (*Vaccinium corymbosum* and *Vaccinium ashei* Reade) and blackberry cultivars (*Rubus* L.) produced in Georgia (USA).

The Flórida M, Bluegem, Powderblue, and Bluebelle cultivars, grown in different locations, presented significant difference ( $p < 0.05$ ) in TA concentration. The Flórida M cultivar from Caxias do Sul (RS) was stood out because it had a concentration approximately 4 times greater than that presented by the same cultivar produced in Pelotas (RS).

Table 1 shows the TA/TP ratio as an estimate of the representativeness of the TA in relation to the phenolic compound concentration present in the fruit. As can be observed, on average, the anthocyanins represented 54% of the TP concentration present in the cultivars assessed. Thus, data found in the present study corroborated the results obtained by other authors (MOYER et al., 2002; TARUSCIO; BARNEY; EXON, 2004; GIOVANELLI; BURATTI, 2009), who reported the anthocyanins as accounting for a large proportion of the phenolic compounds present in blueberries. These flavonoids are responsible for a large part of the AA presented by blueberries, as shown in the study of Zheng and Wang (2003), in which the anthocyanins represented 55.4% of the TP and contributed to 56.3% of the antioxidant activity presented by the fruit.

The blueberries (*Vaccinium* sp.) produced in Brazil, in spite of the differences observed among cultivars, are rich in phenolic compounds. This statement was confirmed by the higher concentration of TP compared to other fruits such as apple, avocado, plum, raspberry, strawberry, banana, blackberry, grape, cherry, mango, lemon, orange, nectarine, peach, pear, and pineapple (WU et al., 2004; WOLFE et al., 2008).

### 3.2 Antioxidant activity (AA) – ABTS, DPPH, and FRAP methods

Table 2 presents the results of AA assessment in the blueberry cultivars using the ABTS, DPPH, and FRAP methods. These methods presented high positive correlation ( $p < 0.05$ ) of ABTS/FRAP ( $R = 0.94$ ), DPPH/FRAP ( $R = 0.86$ ), and ABTS/DPPH ( $R = 0.92$ ). This means that the analytical methods used presented a very similar response and can be used without distinction to quantify AA in this fruit.

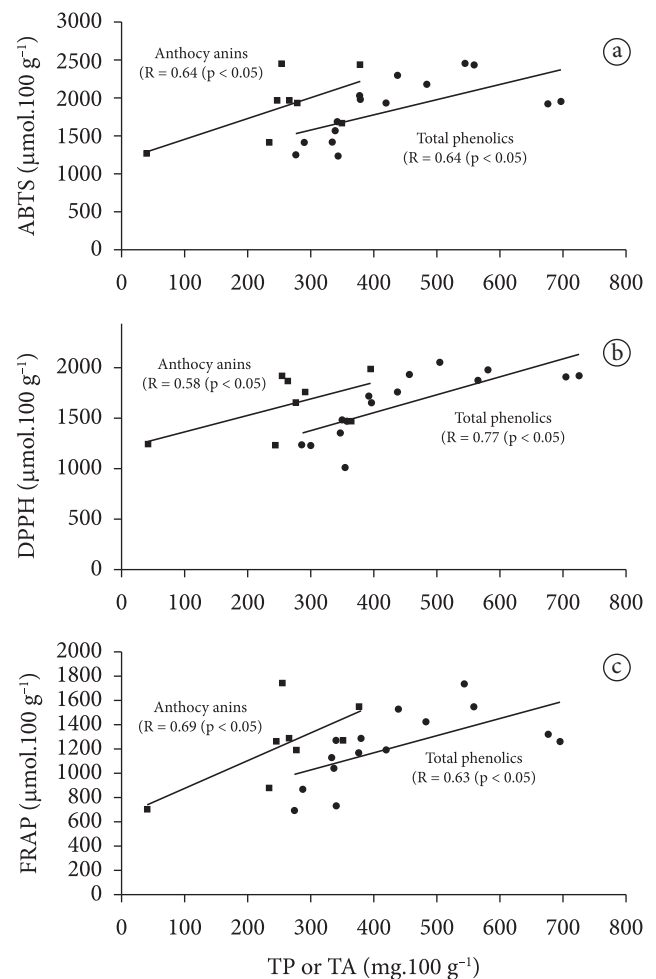
Moderate correlation ( $p < 0.05$ ) was detected between the mean AA by the ABTS, DPPH, and FRAP methods and the TP and TA concentration in the cultivars studied (Figure 1). This moderate correlation may be the result of the presence of compounds that present AA but are not phenolic compounds or the presence of phenolic compounds with greater efficiency than others (LIEN et al., 1999; NATELLA et al., 1999; GIOVANELLI; BURATTI, 2009). Furthermore, this may be the result of antagonistic or synergistic effects among the phenolic compounds present in the matrix. Thus the TP concentration

**Table 2.** Antioxidant activity (ABTS, DPPH, and FRAP) of blueberry cultivars (*Vaccinium* sp.) grown in Brazil.

Cultivar	ABTS <sup>1</sup> ( $\mu\text{mol}.100\text{ g}^{-1}$ )	DPPH <sup>1</sup> ( $\mu\text{mol}.100\text{ g}^{-1}$ )	FRAP <sup>1</sup> ( $\mu\text{mol}.100\text{ g}^{-1}$ )
<i>Vaccinium corymbosum</i> L.			
Bluecrop <sup>b</sup>	1253.90 $\pm$ 87.55 <sup>g</sup>	1244.13 $\pm$ 12.49 <sup>f</sup>	699.78 $\pm$ 16.47 <sup>e</sup>
<i>Vaccinium ashei</i> Reade			
Delite <sup>d</sup>	1931.90 $\pm$ 60.30 <sup>c</sup>	1756.11 $\pm$ 60.11 <sup>b</sup>	1194.15 $\pm$ 30.67 <sup>d</sup>
Powderblue <sup>a</sup>	1929.20 $\pm$ 150.15 <sup>c</sup>	1907.97 $\pm$ 154.77 <sup>a</sup>	1318.41 $\pm$ 2.16 <sup>c</sup>
Bluegem <sup>a</sup>	1571.69 $\pm$ 35.10 <sup>e</sup>	1473.58 $\pm$ 31.71 <sup>d</sup>	1044.20 $\pm$ 37.62 <sup>d</sup>
Clímax <sup>a</sup>	1419.66 $\pm$ 27.70 <sup>f</sup>	1227.88 $\pm$ 15.53 <sup>f</sup>	868.36 $\pm$ 10.09 <sup>e</sup>
Flórida M <sup>a</sup>	1420.42 $\pm$ 84.63 <sup>f</sup>	1348.76 $\pm$ 144.05 <sup>e</sup>	1126.72 $\pm$ 82.68 <sup>d</sup>
Woodard <sup>a</sup>	1975.60 $\pm$ 109.73 <sup>c</sup>	1653.50 $\pm$ 94.22 <sup>c</sup>	1285.94 $\pm$ 42.93 <sup>c</sup>
Bluebelle <sup>d</sup>	1678.12 $\pm$ 94.27 <sup>d</sup>	1478.40 $\pm$ 128.47 <sup>d</sup>	1271.48 $\pm$ 106.67 <sup>c</sup>
Powderblue <sup>c</sup>	2293.20 $\pm$ 150.46 <sup>ab</sup>	1935.84 $\pm$ 47.91 <sup>a</sup>	1530.77 $\pm$ 149.59 <sup>b</sup>
Briteblue <sup>c</sup>	2185.00 $\pm$ 97.93 <sup>b</sup>	2055.06 $\pm$ 134.12 <sup>a</sup>	1429.67 $\pm$ 42.61 <sup>b</sup>
Flórida M <sup>c</sup>	1238.50 $\pm$ 48.45 <sup>g</sup>	1014.20 $\pm$ 81.56 <sup>g</sup>	732.79 $\pm$ 40.63 <sup>e</sup>
Bluebelle <sup>c</sup>	2438.90 $\pm$ 133.90 <sup>a</sup>	1983.00 $\pm$ 121.40 <sup>a</sup>	1544.64 $\pm$ 126.38 <sup>b</sup>
Bluegem <sup>c</sup>	2445.96 $\pm$ 227.75 <sup>a</sup>	1873.19 $\pm$ 55.8 <sup>b</sup>	1740.25 $\pm$ 161.66 <sup>a</sup>
Clímax <sup>c</sup>	1951.61 $\pm$ 22.11 <sup>c</sup>	1917.02 $\pm$ 61.91 <sup>a</sup>	1262.03 $\pm$ 22.20 <sup>c</sup>
Tifblue <sup>e</sup>	2028.80 $\pm$ 128.01 <sup>c</sup>	1727.95 $\pm$ 69.12 <sup>b</sup>	1163.95 $\pm$ 35.62 <sup>d</sup>
<b>Mean <math>\pm</math> SD</b>	<b>1850.83 <math>\pm</math> 404.23</b>	<b>1639.77 <math>\pm</math> 322.78</b>	<b>1214.20 <math>\pm</math> 293.94</b>

Values are expressed in fresh weight of blueberry and as mean  $\pm$  standard deviation (SD),  $n = 3$ . Different letters in the same column indicate significant difference ( $p < 0.05$ ).

<sup>1</sup>Expressed as TEAC (Trolox equivalent antioxidant capacity). <sup>a</sup>Caxias do Sul. <sup>b</sup>Vacaria. <sup>c</sup>Pelotas. <sup>d</sup>Campos do Jordão. <sup>e</sup>Barbacena.



**Figure 1.** Correlation between total phenolic compounds (TP), total anthocyanins (TA), and antioxidant activity (ABTS, DPPH, and FRAP) of blueberry cultivars (*Vaccinium* sp.) grown in Brazil.



alone does not permit a precise indication of the blueberry AA. Similarly, Srivastava et al. (2007) found a moderate correlation between the TP and TA concentration and the AA presented by blueberries. At the same time, these data do not agree with the studies by Prior et al. (1998), Kalt et al. (1999), Moyer et al. (2002), Taruscio, Barney and Exon (2004) and Giovanelli and Buratti (2009) who reported high positive correlation between TP and TA and the AA presented by blueberries.

The AA measured by applying the ABTS method showed great amplitude among the blueberry cultivars studied, with variation of 1238.48 to 2445.96  $\mu\text{mol TEAC} \cdot 100 \text{ g}^{-1}$  FW, respectively, presented for the Flórida M and Bluebelle cultivars, both from Pelotas (RS). Sellappan, Akoh and Krewer (2002) assessed AA using the same analytical method and reported even wider amplitude with values ranging from 811 to 3829  $\mu\text{mol TEAC} \cdot 100 \text{ g}^{-1}$  FW for the blueberry. These wide amplitudes were also observed by Prior et al. (1998) and Moyer et al. (2002) who assessed AA by the ORAC (oxygen radical absorbance capacity) method. The AA measured by the ABTS method for the Powderblue, Clímax, Bluegem, Flórida M and Bluebelle cultivars, produced in two different locations, presented significant difference ( $p < 0.05$ ). Therefore, similarly to the TP content, the differences observed in these values confirmed the effect of production location on AA and were in agreement with the studies conducted by Connor, Luby and Tong (2002) that assessed 16 blueberry cultivars in three regions in the USA (Michigan, Minnesota and Oregon).

The AA assessment by the DPPH and FRAP methods also presented great amplitude among the cultivars studied with values ranging from 1014.20 to 2055.06 and 699.78 to

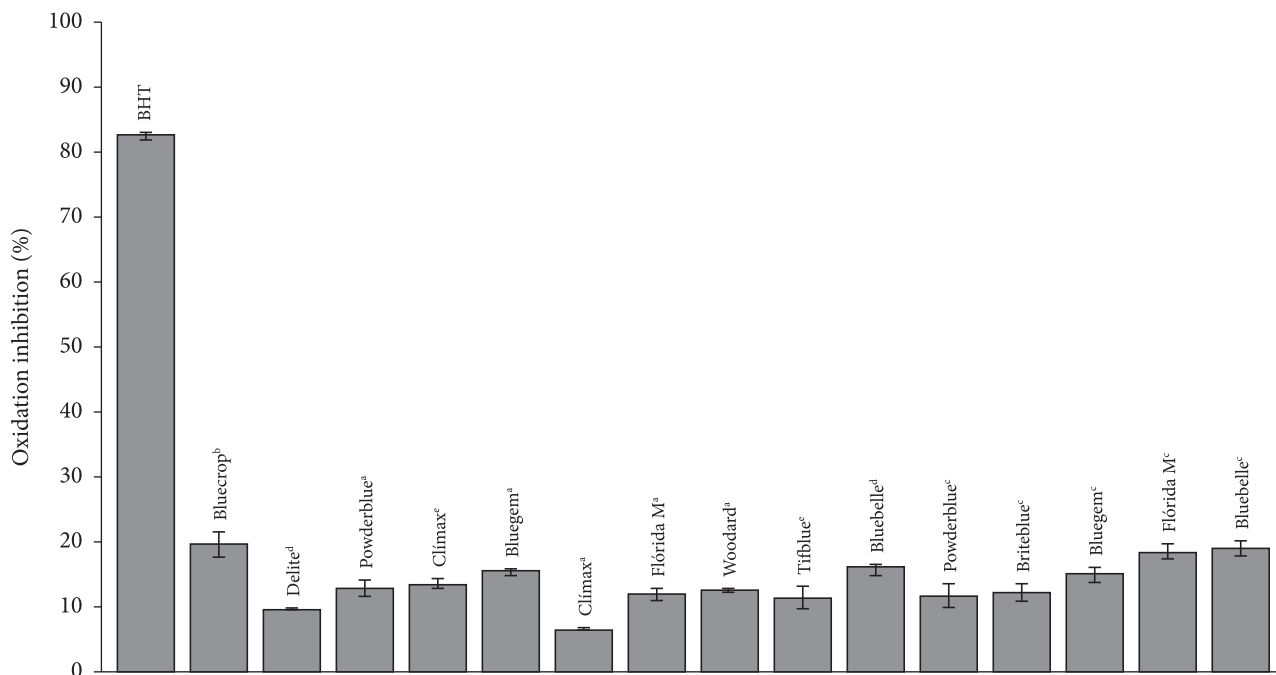
1740.25  $\mu\text{mol TEAC} \cdot 100 \text{ g}^{-1}$  FW, respectively. The Briteblue, Powderblue, Clímax and Bluebelle cultivars (Pelotas, RS) and the Clímax cultivar (Barbacena, MG) presented the highest AA values by the DPPH method, and they did not differ significantly ( $p < 0.05$ ). The Bluegem cultivar (Pelotas, RS) presented the highest AA by the FRAP method. Therefore, like in the ABTS method, some cultivars produced in different locations in Brazil presented significant difference ( $p < 0.05$ ).

As shown in Table 2, similarly to TP and TA, the cultivars representative of the *Vaccinium ashei* Reade species presented higher AA than that of the representatives of the *Vaccinium corymbosum* species, the Bluecrop cultivar. Moyer et al. (2002) assessed the Bluegem cultivar as the only representative of the *Vaccinium ashei* Reade species and reported AA values approximately twice as great as those found in the samples ( $n = 7$ ) of the *Vaccinium corymbosum* species. Prior et al. (1998) detected AA equivalent to the representative cultivars of the two species.

In general, the AA values in this study were similar to those presented by other authors for blueberry (*Vaccinium sp.*) cultivars and species produced in Spain (GARCIA-ALONSO et al., 2004), and they were slightly lower than those from Italy (GIOVANELLI; BURATTI, 2009) and the USA (MOYER et al., 2002; SELLAPPAN; AKOH; KREWER, 2002; TARUSCIO; BARNEY; EXON, 2004).

### 3.3 Antioxidant activity (AA) – $\beta$ -carotene/linoleic acid method

Figure 2 shows the AA of the blueberry cultivars in the  $\beta$ -carotene/linoleic acid method. All cultivars presented much lower AA than that presented by BHT, which was used as



**Figure 2.** Oxidation inhibition (%) of blueberry cultivars (*Vaccinium sp.*) and BHT at 100  $\text{mg} \cdot \text{L}^{-1}$  in  $\beta$ -carotene/linoleic acid system. Values are expressed in fresh weight of blueberry and as mean  $\pm$  standard deviation (SD),  $n = 3$ . <sup>a</sup>Caxias do Sul, <sup>b</sup>Vacaria, <sup>c</sup>Pelotas, <sup>d</sup>Campos do Jordao, <sup>e</sup>Barbacena.

positive control. The Bluecrop cultivar, representative of the *Vaccinium corymbosum* species, presented the greatest AA in this emulsified system with a value of 19.49% oxidation inhibition. It was followed by the Flórida M and Bluebelle cultivars, both from Pelotas (RS), with values of 18.32 and 18.96% oxidation inhibition, respectively. These two cultivars were not significantly different ( $p > 0.05$ ). The AA values were similar to those reported by Melo et al. (2008) for pineapple, cashew, tangerine, orange, pear, papaya, Hawaiian papaya, mango, watermelon, and melon and were lower than those of guava and pine seeds. In the same study, the fruits were classified as possessing moderate and weak antioxidant activity when they presented oxidation inhibition greater or lower than 50%, respectively. Considering this criterion, all blueberry cultivars presented low antioxidant activity in this emulsified system, similarly to the majority of the fruits mentioned above.

The AA presented by the blueberry extracts in the  $\beta$ -carotene/linoleic acid method was quite different from that measured by the ABTS, DPPH, and FRAP methods. In these three methods, the Bluecrop cultivar was shown as one of the cultivars with smallest AA, but also as the cultivar that presented the greatest capacity to inhibit oxidation in the  $\beta$ -carotene/linoleic acid method. Similarly, Melo et al. (2008) observed that the fruit that presented the highest AA in the DPPH method presented the smallest AA by the  $\beta$ -carotene/linoleic acid method. A possible explanation for these differences is the fact that the ABTS, DPPH, and FRAP methods are not influenced by pro-oxidative substances, unlike the  $\beta$ -carotene/linoleic acid method (DUARTE-ALMEIDA et al., 2006). These differences in the response of these methods show the relevance of applying more than one analytical method, with different principles, when the objective is to assess AA in a food matrix or even of the pure compound.

#### 4 Conclusion

The results confirmed the blueberry (*Vaccinium* sp.) produced in Brazil as a source of phenolic compounds with high antioxidant activity. They also showed that there are different levels of phenolic compounds and antioxidant activity according to the blueberry cultivar and the production location.

Overall, the results of this study show the great potential of blueberries for the development of foods rich in compounds with antioxidant properties.

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