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Antioxidant activity of hydrophilic and lipophilic extracts of Brazilian blueberries

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ABSTRACT

Hydrophilic and lipophilic extracts of ten cultivars of Highbush and Rabbiteye Brazilian blueberries (*Vaccinium corymbosum* L. and *Vacciniumashei* Reade, respectively) that are used for commercial production were analysed for antioxidant activity by the FRAP, ORAC, ABTS and β-carotene–linoleate methods. Results were correlated to the amounts of carotenoids, total phenolics and anthocyanins. Brazilian blueberries had relatively high concentration of total phenolics (1622–3457 mg gallic acid equivalents per 100 g DW) and total anthocyanins (140–318 mg cyanidin-3-glucoside equivalents per 100 g DW), as well as being a good source of carotenoids. There was a higher positive correlation between the amounts of these compounds and the antioxidant activity of hydrophilic compared to lipophilic extracts. There were also significant differences in the level of bioactive compounds and antioxidant activities between different cultivars, production location and year of cultivation.

1. Introduction

Blueberries are one of the most widely consumed fruits in the world, so many studies have been done on their properties. The work Prior et al. (1998) is of special interest. They reported that blueberries had the highest antioxidant activity of any of the 42 fruits and vegetables that they evaluated. However, most of the research has focused on the phenolic compounds, flavonoids and anthocyanins, which are the major pigments in blueberries (Moyer, Hummer, Finn, Frei, & Wrolstad, 2002; Wang, Chen, Camp, & Ehlenfeldt, 2012). Moreover, blueberries are widely cultivated in North America, but can only be harvested during the summer. In contrast, they are not yet as widely cultivated in Brazil, even though they can potentially be grown in all seasons (Pertuzatti et al., 2012). Moreover, some tropical blueberries have higher antioxidant contents than cultivars that are grown in temperate climates (Dastmalchi, Flores, Petrova, Pedraza-Peñalosa, & Kennedy, 2011). So, there is special interest in being able to grow and export Brazilian blueberries, especially from October through May, which is between the harvest season in the USA, Canada and Europe, where the demand is huge.

Pertuzatti et al. (2012) found small amounts of carotenoids and ascorbic acid, along with elevated levels of tocopherols in the fruits, which was also reported by others (Barcia, Jacques, Pertuzatti, & Zambiazi, 2010). So, compounds other than phenolics can affect the antioxidant activity of blueberries. Due to this, the objective of this study was to analyse hydrophilic and lipophilic extracts of ten blueberry Brazilian cultivars used in commercial production for antioxidant activity by four different methods.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals, 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulphonate) (ABTS, 99%), β-carotene, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ, 99%), gallic acid (97.5–102.5%), (±)-6-hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®, 97%), 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH) and fluorescein disodium were purchased from Sigma–Aldrich (Steinheim, Germany), linoleic acid was obtained from Fluka (Deisenhofen, Germany).

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2.2. Sample

Seventeen samples (Table 1) from 10 blueberry cultivars in two harvests, 2010/2011 and 2011/2012 were cut in half, frozen and lyophilized in a Terroni Freeze-dryer, model LS-3000E, São Paulo, Brazil. Liquid nitrogen was added to them, and they were crushed. Then they were placed in aluminium bags, evacuated and stored at −20 °C until analysed.

2.3. Preparation of extracts

The lipophilic extract was prepared by a slight modification of the method described by Rodriguez-Amaya (2001). That is, 15 mL of acetone +7 g of lyophilized sample was sonicated for 10 min in an Ultrasound SX-20 (Arruda, Ultra-Sons LTDA, Brazil) and filtered. This was repeated three times and the filtrates combined. This was partitioned between petroleum ether and water. The petroleum ether phase was taken as the lipophilic fraction and analysed immediately, to prevent any decomposition of the analytes. The hydrophilic extract was prepared by the method of Bochi et al. (2014), adding extraction solution consisting of 50 mL of 0.35:20:80 formic acid/acetone/water (v/v/v) to 250 mg of lyophilized product, followed by stirring for 20 min. The mixture was filtered and the filtrate collected and another 50 mL extraction solution was added, followed by filtration. The filtrates were combined.

A UV–Vis 1600 spectrophotometer from Shanghai Mapada Instruments was used to measure UV–Vis absorbances.

2.4. Determination of total phenolics (TPH)

The concentrations of phenolic compounds were determined by the method of Singleton, Orthofer, and Lamuela-Raventos (1999). That is, aliquots of the hydrophilic extract were added to 2.5 mL of a solution of the Folin–Ciocalteu reagent (diluted 1:10 in water). After 5 min, 2.0 mL of 7.5% Na_2CO_3 were added. After 2 h the absorbance at 760 nm was read. A calibration curve was constructed using gallic acid and results were expressed as mg of gallic acid equivalents per 100 g of sample (mg GAE/100 g).

2.5. Determination of total anthocyanins (ACY)

Anthocyanins in the hydrophilic extracts were quantified by the pH differential method of Giusti and Wrolstad (2001) by measuring absorbances at 520 and 700 nm. The molar extinction coefficient and molecular weight of cyanidin-3-glucoside (CYD-3-G) were used (26,900 M^{-1} cm^{-1} and 449.2 g/mol), so results were expressed as mg CYD-3-G per 100 g dry weight (mg CYD-3-G/100 g DW).

Table 1
Cultivars, species and producers of Brazilian blueberries, with their respective cities.

Cultivar	Specie	Producer	Cities
Elliot	Highbush	1	Campestre da Serra (28° 47′ S, 51° 05′ W)
Florida	Rabbiteye	2	Vacaria (28° 30′ S, 50° 56′ W)
Bluecrop	Highbush	2	Vacaria (28° 30′ S, 50° 56′ W)
Elliot	Highbush	2	Vacaria (28° 30′ S, 50° 56′ W)
Climax	Rabbiteye	2	Vacaria (28° 30′ S, 50° 56′ W)
Powderblue	Rabbiteye	2	Vacaria (28° 30′ S, 50° 56′ W)
Bluegem	Rabbiteye	2	Vacaria (28° 30′ S, 50° 56′ W)
Coville	Highbush	2	Vacaria (28° 30′ S, 50° 56′ W)
Powderblue	Rabbiteye	3	Erechim (27° 38′ S,52° 16′ W)
Briteblue	Rabbiteye	3	Erechim (27° 38′ S,52° 16′ W)
Woodard	Rabbiteye	3	Erechim (27° 38′ S,52° 16′ W)
Florida	Rabbiteye	3	Erechim (27° 38′ S,52° 16′ W)
Climax	Rabbiteye	3	Erechim (27° 38′ S,52° 16′ W)
Bluegem	Rabbiteye	3	Erechim (27° 38′ S,52° 16′ W)
Elliot	Highbush	4	Vacaria (28° 30′ S, 50° 56′ W)
Darrow	Highbush	4	Vacaria (28° 30′ S, 50° 56′ W)
Bluecrop	Highbush	4	Vacaria (28° 30′ S, 50° 56′ W)

2.6. Determination of total carotenoids

Total carotenoids in lipophilic extracts were determined by the method of Rodriguez-Amaya (2001) by measuring the absorbance at 450 nm. The results were expressed as μg of carotenoids per g of dry weight (μg β-carotene/g DW), using the molar extinction coefficient of β-carotene in petroleum ether (2592 M^{-1} cm^{-1}).

2.7. Determination of the antioxidant activity

2.7.1. Ferric reducing antioxidant power (FRAP)

The reduction of ferric tripyridyltriazine (Fe^{+3}-TPTZ) was measured by the method of Benzie and Strain (1996), in which the absorbance at 593 nm was measured. Results were expressed as Trolox equivalents per gram of dry weight, TE/g DW.

2.7.2. Free radical capture ABTS

This was done as described by Re et al. (1999). A stock solution of 7 mM ABTS in 2.45 mM potassium sulphate was stored refrigerated and in the dark. Prior to doing the analyses, this was diluted in ethanol until the absorbance at 734 nm was 0.70 ± 0.02. For the analysis of the lipophilic extract, this was dried under a stream of nitrogen gas, and then re-suspended in isopropanol/acetonitrile 1:1 (v/v). Next, 30 μL of the hydrophilic or lipophilic extract of the sample was added to a test tube, along with 3 mL of dilute ABTS solution. After being incubated for 25 min at 30 °C, the absorbance was read and compared to that of Trolox. Results were expressed as Trolox equivalents per g of dry weight, or TE/g DW.

2.7.3. Oxygen radical absorbance capacity (ORAC)

The method of Dávalos, Gómez-Cordovés, and Batolomé (2004) was used to measure the ORAC values of hydrophilic extracts. That is, 20 μL of the hydrophilic extract was diluted 8-fold with the extraction solution, then placed on microplates, along with 120 μL of fluorescein (0.387 mg/L) and 60 μL of AAPH (0.108 g/L). The plates were incubated in an automated microplate reader (BMG Labtech) at 37 °C and read every min for 80 min using a fluorescence detector with an excitation wavelength of 485 nm and emission at 520 nm. A calibration curve was prepared using Trolox and the results were expressed as Trolox equivalents per g of dry fruit, or (TE)/g DW.

A modification of the method of Dávalos et al. (2004) was used to determine the ORAC values of lipophilic extracts. The lipophilic extract was dried and then dissolved in 250 μL of acetone and diluted with 750 μL of 7% (v/v) β-methyl cyclodextrin (1:1 acetone/water, v/v). This same solution was used as a blank and to dissolve the Trolox standard. Next, 20 μL of the extract was added to microplates, along with 120 μL of fluorescein (0.387 mg/L) and 60 μL of AAPH (0.108 g/L). The fluorescence was read, as described for the lipophilic extracts.

2.7.4. β-Carotene/linoleic acid model system

The method of Marco (1968) was used with modifications. First, an emulsion was prepared by adding 300 μL of a solution of β-carotene in chloroform (1 mg/mL), 22 μL of linoleic acid and 200 μL of Tween 40. Next, the chloroform was evaporated off and distilled water was added until the absorbance at 470 nm was between 0.6 and 0.7. Then, 10 μL of extract and 250 μL of the emulsion were placed on the microplate at 45 °C and the absorbance at 470 nm was read every 5 min for 120 min. The results were expressed aspercentage of inhibition of oxidation.

2.8. Statistical analysis

Data analysis was carried out with ANOVA and Tukey' test focusing on significant differences in means. Correlations among data obtained were calculated using Pearson's correlation coefficient (r). Statistic 7.0 software programme was employed with significance level between mean differences at 5% ($p < 0.05$). Principal component analysis (PCA) was done using the Pirouette 3.11 program. Data were auto-scaled before doing PCA. All analyses were made in triplicates and the results were given as means.

3. Results and discussion

The results for total phenolics and anthocyanins in hydrophilic extracts are shown in Table 2. In general, there were significant differences between the different harvests, with the 2010/2011 having more. This can be attributed to the fact that the incidence of UV radiation was higher then, according to the Brazilian National Meteorological Institute, or Instituto Nacional de Meteorologia Brasileiro (INMET). From Oct., 2010 to Jan., 2011 it was 3092 KJ m^2 in Vacaria, while from Oct., 2011 to Jan., 2012, the maximum was 3017 KJ m^2 in the city of Erechim. Also, there was less rain during the maturation period during the 2010/2011 harvest than in the 2011/2012 harvest. It is well-known that a lack of water and UV stress causes an elevated production of secondary metabolites such as phenolics and anthocyanins.

According to Agati, Azzarello, Pollastri, and Tattini (2012), the biosynthesis of flavonoids increases when plants are grown in bright light or when they are exposed to other types of stress. Excess light on a daily basis is stressful to plants and could reduce the activity of chloroplast antioxidants while up-regulating the biosynthesis of flavonoids, even in the absence of UV irradiance (Agati et al., 2012).

The concentration of total phenolics in the hydrophilic extracts ranged from 1922 to 3457 mg gallic acid equivalents (GAEs)/100 g DW in the 2010/2011 harvest and 1622–2590 mg GAE/100 g DW in the 2011/2012 harvest. In the first harvest, the Florida cultivar produced in Vacaria and the Elliott cultivar produced in Campestre da Serra had the highest concentrations of phenolic compounds. They did not differ significantly from each other ($p \leqslant 0.05$). In the second harvest, almost all the cultivars from producer number 2 (city of Vacaria) produced the highest concentrations of phenolic compounds. They did not differ significantly from each other ($p \leqslant 0.05$), with the exception of Bluecrop and Coville. In general, the cultivars that had the highest concentration of phenolic compounds belonged to the Rabbiteye species (*Vaccinium ashei* Reade), with the exception of the Elliot cultivar, which belonged to the Highbush species (*V. corymbosum* L.). It had the highest concentration of phenolic compounds in both harvests.

The concentration of total anthocyanins in the hydrophilic extracts ranged from 159 to 318 mg CYD-3-G/100 g DW in the 2010/2011 harvest and 140–298 mg CYD-3-G/100 g DW in the 2011/2012 harvest. These results were lower than those reported by others, who found 72–242 mg CYD-3-G/100 g of fresh fruit (Jacques, Pertuzatti, Barcia, & Zambiazi, 2009; Moyer et al., 2002), with a water content >80% (Moraes, Pertuzatti, Corrêa, & Salas-Mellado, 2007). However, the differences may be attributed to the size of the fruits (Prior et al., 1998), because many studies demonstrated that smaller fruits have a high concentration of anthocyanins, because they are more concentrated in the skin than the pulp (Gao & Mazza, 1994; Moyer et al., 2002) and because it can depend on the climactic conditions (Agati et al., 2012) and type of cultivation (Hakkinen & Torronen, 2000).

Anthocyanins have been shown to have human health benefits in many studies, due to their anti-inflammatory (Krikorian et al., 2010), anticancer (Giusti & Jing, 2007; Seeram et al., 2006) and anti-mutagenic activities which are capable of blocking the metabolism of cancer cells and even kill them (Nile & Park, 2014; Smith et al., 2004).

The antioxidant concentrations found in the hydrophilic extracts are shown in Table 3. When the ORAC, FRAP and ABTS methods are compared, it can be seen that they are different for the same blueberry cultivar. These are differences in the assays reflect differences in the abilities of antioxidants to quench the peroxyl radical and reduce $ABTS^+$ and Fe^{3+} *in vitro* (Thaipong, Boonprakob, Crosby, Cisneros-Zenallos, & Byrne, 2006). Given this, the highest antioxidant capacity was found using the ORAC method, followed by ABTS and FRAP, similar results were reported by others (Rebello et al., 2014). Prior et al. (1998) were the first authors to notice that blueberries have higher antioxidant activity by the ORAC method, confirming that, compared to other fruits and vegetables, blueberries has a relatively high antioxidant activity by this method. In this way, the large difference found by ORAC, ABTS and FRAP may be indicative of the compounds present in blueberries that act stronger as a scavenger of peroxyl radicals than as donors of electrons to the $ABTS^+$ radical cation or Fe^{3+}.

Table 2
Total phenolics and anthocyanins in hidrophilic extracts of blueberries.

Cultivar	Phenolic compounds (mg GAE/100 g DW)		Anthocyanins (mg CYD-3-G/100 g DW)	
	Harvest 2010/2011	Harvest 2011/2012	Harvest 2010/2011	Harvest 2011/2012
Elliot – 1	2890 ± 206 ab		202 ± 16 def	
Florida – 2	3457 ± 303 aA	2550 ± 139 abB	240 ± 17 bcdA	237 ± 17 bcdA
Bluecrop – 2	2311 ± 506 bcdefA	1622 ± 91 eA	261 ± 21 bA	159 ± 13 fgB
Elliot – 2	2285 ± 131 bcdefA	2424 ± 225 abcA	251 ± 17 bcA	298 ± 26 aA
Climax – 2	2541 ± 137 bcdefB	2792 ± 52 aA	186 ± 12 efB	281 ± 10 abcA
Powderblue – 2	2712 ± 184 bcA	2590 ± 301 abA	310 ± 27 aA	222 ± 18 deB
Bluegem – 2	2668 ± 286 bcdA	2540 ± 32 abA	318 ± 22 aA	285 ± 9.6abA
Coville – 2		2072 ± 182 cde		219 ± 31 de
Powderblue – 3	2555 ± 163 bcdeA	2307 ± 317 bcdA	233 ± 16 bcdA	221 ± 14 deA
Briteblue – 3	2150 ± 39 cdefA	1948 ± 78 deB	159 ± 8.8 fA	142 ± 4.0 fgB
Woodard – 3	2175 ± 108 cdefA	1864 ± 95 deB	163 ± 10 efA	140 ± 3.8 gB
Florida – 3	2489 ± 29 bcdefA	2248 ± 46 bcdB	195 ± 2,8 defA	187 ± 5.7 efA
Climax – 3	2053 ± 205 defA	2182 ± 32 bcdA	173 ± 12 efB	206 ± 4.2 deA
Bluegem – 3	2515 ± 6.3 bcdefA	1931 ± 66 deB	272 ± 5.2 abA	235 ± 16 cdB
Elliot – 4	1949 ± 243 efA	2037 ± 29 cdeA	206 ± 12 cdeA	186 ± 10 efgA
Darrow – 4	1922 ± 88 f		157 ± 9.2 f	
Bluecrop – 4	2008 ± 26 ef		172 ± 10 ef	

Averages followed by the same upper case letters on the same row and lower case letters in different columns do not differ significantly $p \leqslant 0.05$ by the Tukey test.
CYD-3-G = cyanidin-3-glucoside; GAE = gallic acid equivalents; DW = dry weight.
Coefficient of variation <9.0%.

Table 3
Antioxidant activity of hydrophilic extracts of blueberries, using the ABTS, FRAP, ORAC and β-carotene/linoleate model systems.

Cultivar	FRAP (μmol TE/g DW)		Carotene–linoleate (% inhibition)		ABTS (μmol TE/g DW)		ORAC (μmol TE/g DW)	
	2010/2011 Harvest	2011/2012 Harvest	2010/2011 Harvest	2011/2012 Harvest	2010/2011 Harvest	2011/2012 Harvest	2010/2011 Harvest	2011/2012 Harvest
Elliot – 1	273 ± 20 abc		61 ± 5.8 a		112 ± 8.0 g		703 ± 15 def	
Florida – 2	312 ± 28 aA	267 ± 16 abA	38 ± 3.8 defghiB	46 ± 3.5 abA	139 ± 6.6 efB	205 ± 12 aA	697 ± 18 defA	549 ± 21 deB
Bluecrop – 2	247 ± 19 cdeA	178 ± 15 fB	50 ± 7.4 abcdefA	52 ± 7.6 abA	185 ± 11 cA	118 ± 5 cB	600 ± 54 fgA	533 ± 33 eA
Elliot – 2	251 ± 14 bcdeA	253 ± 22 abcA	47 ± 2.4 abcdefgB	57 ± 5.0 aA	157 ± 2.5 deB	194 ± 13 aA	743 ± 40 cdeA	629 ± 48 bcdeB
Climax – 2	266 ± 18 abcdA	273 ± 6.2 aA	46 ± 0.2 bcdefgA	47 ± 2.8 abA	172 ± 11 cdB	217 ± 5 aA	819 ± 19 bcdA	778 ± 21 aA
Powderblue – 2	299 ± 20 abA	253 ± 17 abcB	30 ± 3.6 iB	46 ± 3.0 abA	235 ± 3.2 bA	214 ± 5 aB	930 ± 50 abA	656 ± 60 bcdB
Bluegem – 2	308 ± 24 aA	262 ± 6.4 abB	36 ± 6.3 efghiA	47 ± 7.2 abA	261 ± 11 aA	210 ± 11 aB	646 ± 15 efA	700 ± 42 abA
Coville – 2		215 ± 20 cdef		56 ± 4.3 a		143 ± 6 bc		675 ± 63 abc
Powderblue – 3	172 ± 12 fghiB	217 ± 13 cdeA	52 ± 1.4 abcdA	47 ± 4.5 abA	73 ± 2.7 iB	137 ± 6 bcA	1028 ± 85 aA	636 ± 32 bcdeB
Briteblue – 3	132 ± 7.4 hiB	195 ± 8.0 defA	50 ± 2.3 abcdeA	52 ± 5.3 abA	170 ± 6.7 cdA	140 ± 7 bcB	763 ± 69 cdeA	557 ± 34 cdeA
Woodard – 3	140 ± 13 ghiB	186 ± 7.4 efA	30 ± 7.8 hiB	50 ± 3.4 abA	89 ± 6.8 hiB	126 ± 7 cA	851 ± 69 bcA	549 ± 47 deB
Florida – 3	178 ± 3 fghB	221 ± 5.7 cdeA	35 ± 1.4 ghiB	52 ± 2.1 abA	105 ± 5.5 ghB	143 ± 4 bcA	737 ± 46 cdeA	657 ± 48 bcdA
Climax – 3	207 ± 12 efB	232 ± 5.2 bcdA	35 ± 7.4 fghiB	53 ± 1.4 abA	125 ± 8.5 fgB	164 ± 12 bA	496 ± 31 gA	547 ± 28 deA
Bluegem – 3	188 ± 2 fgB	212 ± 6.9 defA	45 ± 1.6 cdefghA	41 ± 4.0 bA	90 ± 3.8 hiB	143 ± 9 bcA	702 ± 14defA	568 ± 29 cdeB
Elliot – 4	217 ± 18 defA	203 ± 11 defA	60 ± 1.1 abA	57 ± 5.2 aA	141 ± 5.5 efA	119 ± 12 cB	850 ± 25 bcA	567 ± 29 cdeB
Darrow – 4	128 ± 8.5 i		50 ± 1.8 abcdef		139 ± 5.1 ef		801 ± 19 bcd	
Bluecrop – 4	132 ± 4.4 hi		57 ± 9.0 abc		40 ± 3.5 J		723 ± 32 cdef	

Averages followed by the same upper case letters on the same row and lower case letters in different columns do not differ significantly $p \leqslant 0.05$ by the Tukey test.
TE = Trolox equivalent; DW = dry weight.
Coefficient of variation <10%.

The ORAC values ranged between 600–1028 μmol TE/g DW for the cultivars analysed in the 2010/2011 harvest and 533–778 μmol TE/g DW for the 2011/2012 harvest. The Powder blue cultivar, produced in Erechim had the highest ORAC value in the 2011/2012 harvest, while the Climax cultivar, produced in Vacaria had the highest ORAC value in the 2010/2011 harvest. Considering that the sample were lyophilized and still retained 4% moisture, the ORAC values can be converted to about 22–41.1 μmol TE/g fresh weight, which can be compared to results reported by others. Moyer et al. (2002) found 18.6–130.7 μmol TE/g fresh weight, Connor, Luby, and Tong (2002) found 10.3–51.9 μmol TE/g fresh weight, and Wang et al. (2012) found 33.8–118.7 μmol TE/g fresh weight. These wide ranges of antioxidant capacities may be due to differences in genotype, climate, sample preparation and analytical techniques. That is, the first two works cited, (Connor et al., 2002Moyer ; et al., 2002) used β-phycoerythrin as the indicator, while Ou, Hampsch-Woodill, and Prior (2001) showed that fluorescein is preferable.

Wang et al. (2012) analysed six of the 10 cultivars analysed in the current study and found higher ORAC values. However, two of the cultivars analyzed, becky blue (33.8 μmol TE/g DW) and delite (39.2 μmol TE/g DW), had similar values as those reported in this study. The same occurred in the study by Prior et al. (1998), who analysed 23 cultivars from four different species. They found 13.9–37.8 μmol TE/g DW, which was similar to those obtained in the current study. However, the only cultivar that was analysed in both this study and in the one by Prior et al. (1998), was Climax, which had higher values (19.9–32.8 μmol TE/g DW) in the current study than in the one by Prior et al. (1998) (13.9 ± 4.1 μmol TE/g DW).

All the methods for determining antioxidant activity of blueberries produced significant differences between cultivars. In the FRAP assay, the values ranged from 128 to 312 μmol TE/g DW (Table 3), which represents 5.1–12.5 μmol TE/g fresh weight. The cultivar that had the largest was Florida-2. When comparing this to literature values, it can be seen that the Bluecrop cultivar showed much difference between different studies. In the present study, this cultivar was produced commercially by two vendors from the city of Vacaria (Rio Grande do Sul, Brazil), as shown in Table 1. Over a two-year period, values ranged from 5.3 to 9.9 μmol TE/g fresh weight, similar to the levels reported by Connor et al. (2002) for the same cultivar (10.6 μmol TE/g fresh weight). Taruscio, Barney, and Exon (2004) found 20.2 μmol TE/g fresh weight and Moyer et al. (2002) obtained higher values (34.4 μmol TE/g fresh weight). However, the large differences observed in these studies can be attributed to different climactic conditions during cultivation, due to the different regions where the blueberries were planted. That is, Connor et al. (2002) analysed fruits found in northern USA, while Taruscio et al. (2004) and Moyer et al. (2002) analysed fruits from northeast USA.

A high positive correlation was found between the FRAP and ABTS values found ($r > 0.8$, $p \leqslant 0.05$), consistent with the results reported by Thaipong et al. (2006). The range of ABTS values was between 40.3 and 260.8 μmol TE/g DW (Table 3). These results were similar to those reported by Arancibia-Avila et al. (2012), who analysed Highbush blueberries that were produced in Chile (197.7 ± 7.2 μmol TE/g DW) and Poland (254.8 ± 11.9 μmol TE/g DW). In the present study the Bluegem cultivar (Rabbiteye species) had the highest ABTS value. Also, the four of the cultivars analysed were in the species *V. Corymbosum* (Elliot, Bluecrop, Coville e Darrow). Their ABTS values ranged from 40.3 μmol TE/g DW (Bluecrop) to 193.9 μmol TE/g DW (Elliot). So, the cultivar Elliot cultivar in this study had about the same antioxidant activity (193.9 μmol TE/g DW) as that reported by Arancibia-Avila et al. (2012) for blueberries from Chile (197.7 μmol TE/g DW). The lower values found for the Bluecrop

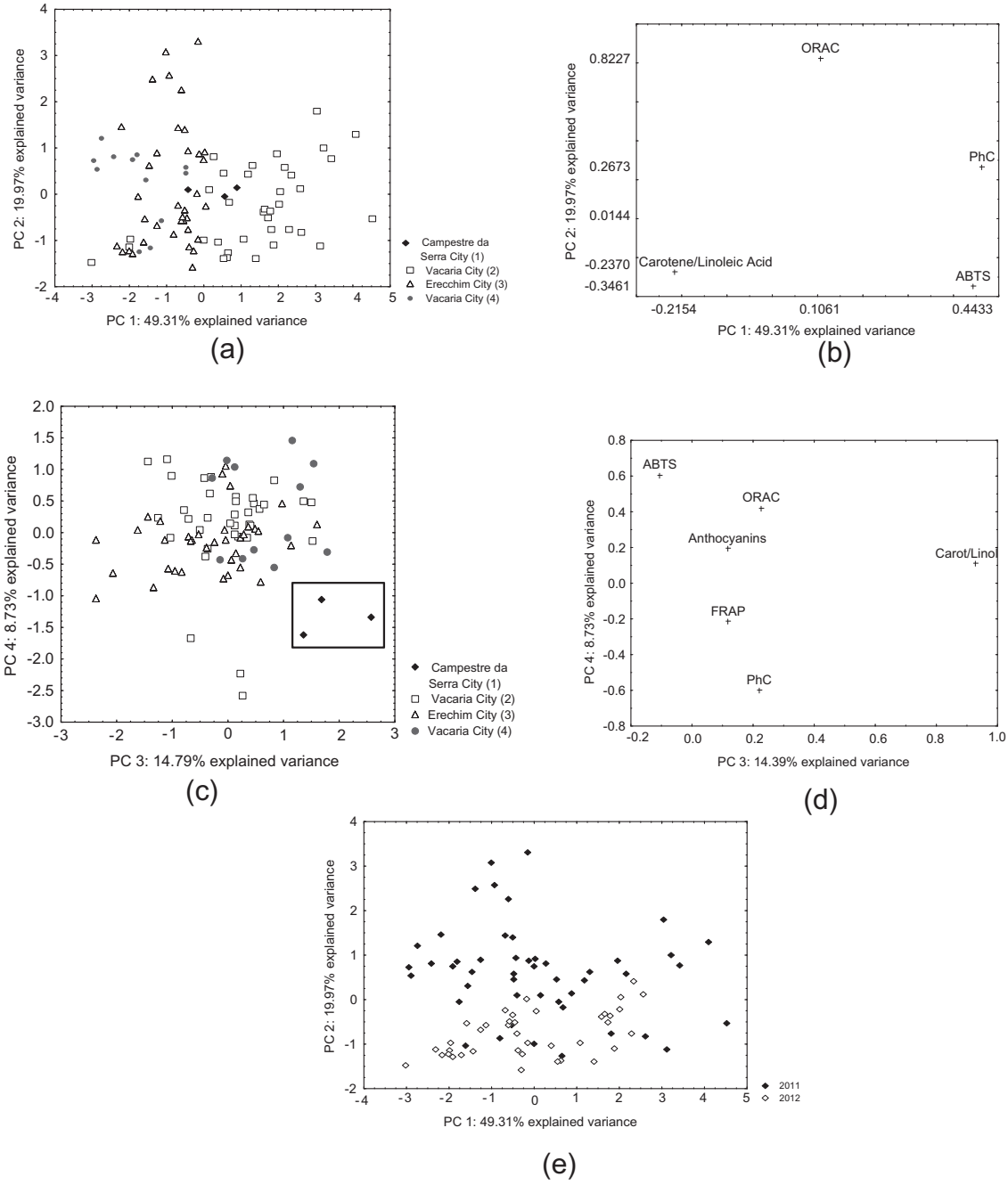

Fig. 1. Principal component analysis (PCA) of hydrophlic extracts of different blueberry cultivars. Percentages of four products and two harvests, (a) Graph of scores for PC1 (factor 1) and PC2 (factor 2), differences between classes (producers); (b) Graph of loadings for PC1 (factor 1) and PC2 (factor 2); (c) Graph of scores for PC3 (factor 3) and PC4 (factor 4); (d) Graph of loadings for PC3 (factor 3) and PC4 (factor 4); (e) Graph of scores for PC1 (factor 1) and PC2 (factor 2), differences between class (harvest). *PhC = Phenolic compounds.

cultivar (Table 3) can be explained by the work of Castrejón, Eichholz, Rohn, Kroh, and Huyskens-Keil (2008), who found that the antioxidant activity of the Bluecrop cultivar decreased during maturation, unlike other cultivars.

Table 3 shows the antioxidant capacity of hydrophilic extracts using the β-carotene/linoleic acid model system. The Elliot cultivar (from Campestre da Serra) had the highest antioxidant capacity (60.9% inhibition) using this assay system. The range for all cultivars was 30.4–60.9% inhibition, with Woodard, produced in Erechim having the lowest. This shows that there are different amounts and/or types of antioxidants in different cultivars. The values found in the current study are similar to those reported by Melo, Maciel, Lima, and Nascimento (2008) who analysed aqueous and acetone extracts of 15 different cultivars and found values from 3.33 to 61.03% inhibition for the aqueous extract and 3.89–67.25% for the acetone extract. Elliot, Bluecrop, Coville, Powderblue, Briteblue, Florida, Climax e Darrow obtained values of >50% inhibition. According to Melo et al. (2008) this can be considered to be a moderate antioxidant level, similar to the aqueous extract of pinha (*Annona squamosa*) and the acetone extract of guava, the fruit with the highest antioxidant values reported by them.

The principal components analysis (PCA) was applied to the hydrophilic extracts of different cultivars (Fig. 1), allowed the grouping of cultivars according to the producer and within each

Table 4
Concentration of total carotenoids and antioxidant activity of lipophilic extracts of blueberries, determined by the ABTS and ORACmethods.

Cultivar	Carotenoides (μg de β caroteno/g DW)		ABTS (μmol TE/g DW)		ORAC (μmol TE/g DW)	
	2010/2011 Harvest	2011/2012 Harvest	2010/2011 Harvest	2011/2012 Harvest	2010/2011 Harvest	2011/2012 Harvest
Elliot – 1	35.2 ± 0.35a		10.0 ± 0.6cde		0.22 ± 0.02cdef	
Florida – 2	2.29 ± 0.11gB	2.84 ± 0.17fgA	12.6 ± 1.1bA	1.26 ± 0.03aB	0.07 ± 0.01hA	0.07 ± 0.00defA
Bluecrop – 2	10.7 ± 0.99eA	7.69 ± 0.44bB	9.0 ± 0.5defA	0.33 ± 0.02efB	0.52 ± 0.09bA	0.11 ± 0.02bcB
Elliot – 2	14.9 ± 1.42dA	7.76 ± 0.28bB	11.8 ± 0.7bcA	0.35 ± 0.05efB	0.58 ± 0.03bA	0.09 ± 0.01bcdB
Climax – 2	15.8 ± 0.05gB	3.71 ± 0.16efA	4.8 ± 0.4gA	0.43 ± 0.06defB	0.12 ± 0.02fghA	0.04 ± 0.01efB
Powderblue – 2	1.65 ± 0.03gB	5.36 ± 0.54cdA	9.8 ± 0.8cdeA	0.99 ± 0.12bB	0.09 ± 0.01ghA	0.05 ± 0.00defB
Bluegem – 2	3.72 ± 0.21fgA	2.30 ± 0.24gB	8.2 ± 0.8efA	0.80 ± 0.11bcB	0.09 ± 0.02ghA	0.08 ± 0.01cdeA
Powderblue – 3	2.87 ± 0.15fgB	5.99 ± 0.41cA	16.2 ± 1.1aA	1.21 ± 0.09aB	0.12 ± 0.01fghA	0.09 ± 0.00cdB
Briteblue – 3	3.48 ± 0.28fgB	5.74 ± 0.31cdA	11.9 ± 1.1bcA	0.28 ± 0.04fB	0.16 ± 0.01efghA	0.07 ± 0.00defB
Woodard – 3	3.33 ± 0.28fgB	4.65 ± 0.49deA	11.0 ± 0.9bcdA	0.41 ± 0.05efB	0.18 ± 0.00defgA	0.04 ± 0.00efB
Florida – 3	1.99 ± 0.17gB	2.59 ± 0.25fgA	16.3 ± 0.8aA	0.63 ± 0.09cdB	0.12 ± 0.01fghA	0.04 ± 0.01fB
Climax – 3	2.49 ± 0.07gB	4.02 ± 0.28eA	6.8 ± 0.7fgA	0.94 ± 0.08bB	0.24 ± 0.01cdeA	0.13 ± 0.01bB
Bluegem – 3	5.36 ± 0.28fA	5.18 ± 0.38cdA	15.8 ± 0.9aA	0.54 ± 0.05deB	0.29 ± 0.03cdA	0.04 ± 0.00fB
Elliot – 4	8.71 ± 0.66eB	24.6 ± 0.61aA	8.7 ± 0.7defA	0.92 ± 0.06bB	0.51 ± 0.02bA	0.26 ± 0.04aB
Darrow – 4	25.8 ± 0.26b		9.9 ± 0.7cde		1.13 ± 0.09a	
Bluecrop – 4	18.0 ± 0.74c		1.9 ± 0.2 h		0.30 ± 0.04c	

Averages followed by the same upper case letters on the same row and lower case letters in different columns do not differ significantly $p \leqslant 0.05$ by the Tukey test.
TE = Trolox equivalent; DW = dry weight.
Coefficient of variation <9.8.

group there is a separation based on the different harvest seasons. The 2010/2011 harvest had higher positive scores in PC2, while the 2011/2012 harvest had negative scores (Fig. 1(e).

The plot of the principal axes (PCs) that are associated with each variable is shown in Fig 1. The PCA showed that the first principal component (PC1) explained 49.31% of the total variance in the data. The second principal component (PC2) explained 19.98%, while the third and fourth principal components explained 14.80% and 8.74% respectively, these observations may be made in the sample score plot (Fig. 1). The PC1 is primarily related to the analysis of phenolic compounds, anthocyanins, FRAP and ABTS, while the second principal component is related to the ORAC value (Fig. 1(b).

The two principal components (PC1 and PC2) explain 69% of the variance in the data. The third and fourth principal components have a high contribution due to the β-carotene/linoleic acid assay and total phenolic compounds (Fig. 1(d), making it possible to distinguish between hydrophilic extracts by producer 1, from the city of Campestre da Serra (Fig. 1(c).

Table 4 shows the two analyses for antioxidant activity and concentration of carotenoids in lipophilic extracts. The Elliott cultivar had the highest concentration of carotenoids, but there was a difference between different harvest seasons and locations. When harvested in Campestre da Serra in 2010/2011, there were more carotenoids (35.2 μg of β-carotene/g DW) than when harvested in Vacaria in 2011/2012 (24.6 μg of β-carotene/g DW). The range of concentrations of carotenoids for different cultivars and harvest seasons was 1.65 μg of β-carotene/g DW for the Powderblue cultivar and 35.2 μg of β-carotene/g DW for the Elliot cultivar. This low level of carotenoids in blueberries was also verified by others (Jacques, Pertuzatti, Barcia, Zambiazi, & Chim, 2010; Marinova & Ribarova, 2007). Jacques et al. (2009) also found that Powderblue had the lowest level of carotenoids among the seven samples analyzed. This is due to the fact that the primary antioxidants are anthocyanins. Fruits with relatively high concentrations of anthocyanins have a lower concentration of carotenoids during the ripening process.

There was a range of 0.28–16.3 μmol TE/g DW in the ABTS antioxidant assay for lipophilic extracts (Table 4), which is five to 500-fold lower than the hydrophilic extracts (Table 3). This was also seen by Kotíková, Lachman, Hejtmánková, and Hejtmánková (2011), who found much less antioxidant activity in the lipophilic extracts of tomatoes than in hydrophilic extracts. The same authors found <10 μmol TE/g DW for the different varieties analyzed, which is comparable to the levels found in blueberries in the current study.

The low antioxidant values for the lipophilic extracts can be attributed to the antioxidant mechanisms in the different assays done in this study. That is, Rodriguez-Amaya, Kimura, and Amaya-Farfan (2008) found that carotenoids are very efficient in

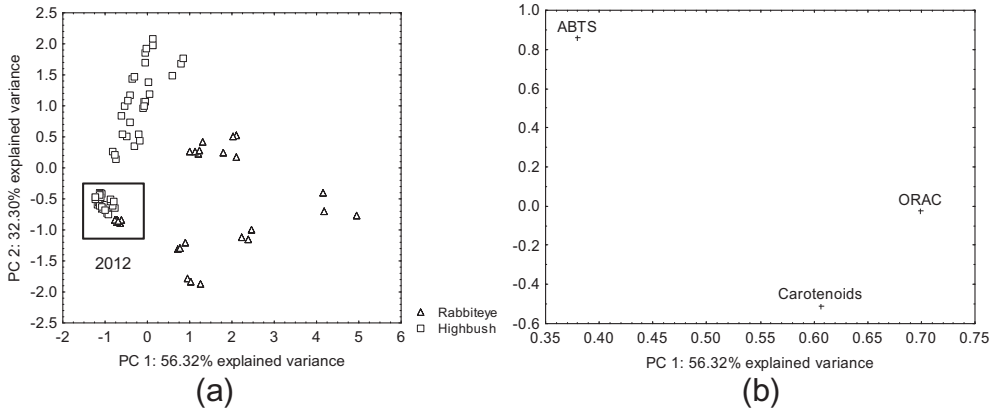

Fig. 2. Principal component analysis (PCA) of lipophlic extracts of blueberry cultivars. Percentages of four products and two harvests, (a) Graph of scores for PC1 (factor 1) and PC2 (factor 2), (b) Graph of loadings.

controlling singlet oxygen, while phenolic compounds can sequester free radicals (ORAC) and transfer of electrons (ABTS) better.

The results from the ABTS assay of lipophilic extracts were considerably higher than the ORAC values for the same extracts (Table 4). The cultivars harvested in 2010/2011 were significantly higher ($p \leqslant 0.05$) than the 2011/2012 harvest. The ORAC values ranged from 0.04 μmol TE/g DW for Climax and 1.13 μmol TE/g DW for Darrow, but were less than those reported by Prior et al. (2003) for pine nuts, sorghum bran and strawberries, which varied from 3.0 to 16.0 μmol TE/g DW. The ORAC values did not correlate well with total carotenoids (0.58). That can be due to the fact that carotenoids are nott the only compounds with antioxidant activities in lipophilic extracts. As reported by Pertuzatti et al. (2012) lipophilic extracts of blueberries have a large amount of tocopherols.

According to the PCA of the lipophilic extracts (Fig. 2), there was a high degree of homogeneity in 2011/2012 harvest. There was a separation between the Rabbiteye and Highbush cultivars in 2010/2011 harvest. This could be due to the fact that Highbush had higher values in the first principal component (Fig. 2(a), which separated from the others due to having the highest concentration of carotenoids and ORAC value. These are the analyses that are distinguished in the principal component (Fig. 2(b). The Rabbiteye cultivar in the upper left quadrant had high positive scores in PC2 and near zero in PC1. When comparing the loadings (Fig. 2(b) it can be seen that this is due to the fact that the samples in this group have lower concentrations of total carotenoids (related to PC1) and the highest values of ABTS (related to PC2). PC1 explains 56.32% of the total variance in the data, while PC2 explains 32.30%. So, these two principal components describe >88% of the variance in the data.

4. Conclusion

The results show that Brazilian blueberries have a relatively high concentration of phenolic compounds and total anthocyanins, along with a small concentration of carotenoids. It was also shown that these compounds have a positive correlation with antioxidant activity, which was higher in the hydrophilic vs lipophilic extracts. Significant differences in the concentrations of bioactive compounds were found in different cultivars, places where they were grown and harvest season. By PCA, it was possible to see that the hydrophilic extracts could be differentiated by producers and harvest seasons. The lipophilic extracts were differentiated according to whether they were Rabbiteye or Highbush.

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