

# Physico-chemical characterization and bioactive compounds of blackberry fruits (*Rubus* sp.) grown in Brazil

*Caracterização físico-química e de compostos bioativos em amora-preta (*Rubus* sp.) cultivada no Brasil*

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## Abstract

Five blackberry cultivars (*Rubus* sp.) were evaluated for antioxidant capacity, bioactive compounds and composition. Ascorbic acid levels, consisting of dehydro-ascorbic acid, ranged from 9.8 to 21.4 mg.100 g<sup>-1</sup> fresh weight. Cyanidin (66 to 80% of total flavonoids), epicatechin, quercetin and traces of kaempferol were the main flavonoids found in all cultivars. The five cultivars presented high antioxidant capacity in the  $\beta$ -carotene/linoleic acid system, with inhibition similar to the synthetic antioxidant BHT, at a 50  $\mu$ M concentration. Caingangue cultivar presented high vitamin C and total phenolics content, while Guarani had the highest cyanidin, total anthocyanin and total flavonoids levels and also the highest antioxidant capacity. These cultivars also presented good TSS/TA ratios. From the data, at a quantitative level, blackberry can be considered a good source of bioactive compounds, as well as potentially beneficial to human health.

**Keywords:** *Rubus*; flavonoids; anthocyanin; antioxidant capacity.

## Resumo

Cinco cultivares de amora-preta (*Rubus* sp.) foram avaliadas quanto a sua capacidade antioxidante, perfil de compostos bioativos e composição físico-química. Os níveis de ácido ascórbico total, presentes na forma de ácido desidroascórbico, variaram entre 9,8 a 21,4 mg.100 g<sup>-1</sup> (b.u.). Os principais flavonóides presentes nas cinco cultivares foram: a antocianina cianidina (66 a 80% do total de flavonóides); o flavan-3-ol epicatequina; e os flavonóis quercetina e traços de caenferol. As cinco cultivares apresentaram alta capacidade antioxidante quando avaliadas pelo sistema de co-oxidação  $\beta$ -caroteno/ácido linoléico, similar ao antioxidante sintético BHT, na concentração de 50  $\mu$ M. A cultivar Guarani apresentou os maiores teores de flavonóides totais, antocianina total, cianidina e de capacidade antioxidante, enquanto que a cultivar Caingangue apresentou alto conteúdo de vitamina C e de fenólicos totais. Estas duas cultivares também apresentaram uma boa correlação TSS/TA. Assim, a amora-preta pode ser considerada uma boa fonte de compostos bioativos e assim contribuir com a manutenção do estado de saúde.

**Palavras-chave:** *Rubus*; flavonóides; antocianinas; capacidade antioxidante.

## 1 Introduction

Fruit and vegetable intake has proven to reduce the development of a considerable number of chronic diseases, such as cancer and cardiovascular diseases. The protective effect evidenced in laboratory and epidemiological studies has been associated with a variety of nutrient and non-nutrient constituents, being many of them characterized by their antioxidant properties (PIETTA, 2000). Among the compounds with high antioxidant capacity are ascorbic acid, tocopherols,  $\beta$ -carotene and phenolic compounds, especially flavonoids (DREWNOWSKI; GOMEZ-CARNERO, 2000).

Anthocyanins, the glycoside form of the anthocyanidins, belong to a class of flavonoids which present water-soluble pigments responsible for the orange, red and blue colors of many fruits, vegetables, leaves, flowers and roots. Anthocyanidins vary with different hydroxyl or methoxyl substitutions in their basic structure, flavylium (2-phenylbenzopyrillium). Only six of the 16 anthocyanidins identified in natural products occur

frequently and in many different vegetables and fruits: cyanidin, pelargonidin, delphinidin, peonidin, petunidin and malvidin (WU; PRIOR, 2005a; b). Anthocyanins are highly studied compounds because of their effects on human health as antioxidant compounds, as shown in a number of in vitro and in vivo studies (KÄHKÖNEN; HEINONEN, 2003), for their anti-inflammatory effect modulating cyclooxygenase 1 and 2 enzymes (TALL et al., 2004) and their inhibitory effect on the development of some cancer cells (HAGIWARA; YOSHINO; ICHIBARA, 2002).

Among the small fruits group, including strawberry, raspberry, blueberry and blackberry crops, blackberry appears as the richest source of anthocyanins, with cyanidin content ranging from 93 to 280 mg.100 g<sup>-1</sup> fresh weight depending on the cultivar (CONNOR et al., 2002), followed by black raspberry (up to 197 mg), blackberry (153 mg) and strawberry (32 mg) (WANG; LIN, 2000). Blackberry plants were introduced in Brazil by the Estação Experimental de Pelotas (RS), renamed

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EMBRAPA Clima Temperado in the 1970s, and since then its production has increased in the states of Rio Grande do Sul, São Paulo and Minas Gerais, with the introduction and adaptation of new cultivars. Blackberry (*Rubus eubatus*) has a shrub frame with erect or trailing stems. The blackberry fruits, weighing around 4 to 7 g, have an acid to sweet-acid taste and are not berries, but an aggregate fruit composed of many smaller fruits called drupes. Blackberry, being easy to handle and having low production costs even concerning chemicals, is an alternative crop for family agriculture. The fresh fruits, besides being very nutritious, rich in minerals, pro-vitamin A, vitamin B and calcium, also contain bioactive compounds, such as ellagic acid and anthocyanins (ANTUNES et al., 2003).

The antioxidant capacity of the dietary intake of fruits and vegetables changes considerably according to the presence of diverse compounds with antioxidant capacity, but the amount of anthocyanins present in them seems to be important (HASSIMOTTO; GENOVESE; LAJOLO, 2005). Among the Brazilian vegetables already studied, mulberry (*Morus nigra*), açai (*Euterpe oleracea*), jambolão (*Syzygium cumini*), purple lettuce (*Lactuca sativa*), purple cabbage (*Brassica oleracea*) and commercial frozen blackberry (*Rubus* sp.) pulp appear as important anthocyanin sources (HASSIMOTTO; GENOVESE; LAJOLO, 2005).

Species of the *Rubus* genus are known as natural sources of anthocyanins with high antioxidant effect (DEIGHTON et al., 2000). Due to the increasing interest in blackberry consumption and the knowledge that the nutritional composition varies according to the cultivar, this work aimed at evaluating the phenolic and anthocyanin content, antioxidant capacity and physicochemical quality of five blackberry cultivars grown in Brazil.

## 2 Materials and methods

Blackberry fruits (*Rubus eubatus*) from the cultivars Tupy, Guarani, Caingangue, Brazos and Seleção 97 were harvested at the germplasm bank of Estação Experimental de Caldas (EPAMIG/FECD), Minas Gerais, Brazil. The ripe fruits (black color) were harvested during the 2003/2004 harvest season. After harvest, the fruits were selected, separating those with injuries. They were then washed in chlorinated water, drained and frozen, and stored at  $-20\text{ }^{\circ}\text{C}$  until analyses. Each sample consisted of fruits harvested at the beginning, middle and end of the season. Frozen fruits from each sample were macerated with liquid nitrogen using a mortar and pestle and immediately submitted to analyses of ascorbic acid, flavonoids, total phenolics and the in vitro antioxidant assay.

Physicochemical characteristics: Frozen fruit samples were thawed at room temperature and homogenized in a mixer. Following seeds removal, 10 g of pulp from each sample were poured into a 150 mL vial and 80 mL of distilled water was added. The vials were placed in a water bath at  $80\text{ }^{\circ}\text{C}$  for 2 hours. The extract was transferred to 100 mL volumetric flasks and filtered through a cheese cloth. Titratable acidity was determined by titration with 0.1 N NaOH to a pink color using 1% phenolphthalein as indicator and expressed as  $g\cdot 100\text{ g}^{-1}$  citric

acid. The pH of the diluted pulp of each sample was determined using a pH-meter and soluble solids ( $^{\circ}\text{Brix}$ ) were determined using a handheld refractometer. Moisture and ashes were determined in the undiluted pulp, according to AOAC methods (1995). Total carbohydrates were determined in the diluted pulp according to Dubois et al. (1956). Soluble sugars were extracted three times with 80% ethanol at  $80\text{ }^{\circ}\text{C}$ . After centrifugation, the supernatants were combined and the ethanol was evaporated under vacuum. The soluble sugar content was analyzed by high performance liquid chromatography with pulse amperometric detection (HPLC-PAD – Dionex, Sunnyvale, California, USA), using a PA<sub>1</sub> column (Dionex, Sunnyvale, California, USA) in an isocratic run of 18 mM NaOH during 25 minutes. Total soluble sugars were taken as the sum of glucose, fructose and sucrose values. All samples were analyzed in triplicate and the results were subjected to statistical analysis of variance. Treatment means were separated using the Tukey test ( $p < 0.05$ ).

Total anthocyanins: An aliquot of 2 g of undiluted pulp was extracted with 20 mL acidified methanol (1% HCl, v/v), in a shaker, for 30 minutes, at room temperature, and stored overnight at  $4\text{ }^{\circ}\text{C}$ . The extract was filtered and washed several times until complete removal of pigments and the volume was adjusted to 100 mL in volumetric flasks. After appropriate dilution with acidified methanol, samples were stored in the dark for 2 hours and the absorbance was determined at 532 nm in a spectrophotometer. Anthocyanin concentration (expressed as mg pigment.g<sup>-1</sup> pulp) was determined using the molecular weight (449.2) and molar absorbance (26,900) values for cyanidin-3-glucoside (LIMA et al., 2003; PERKINS-VEAZIE; COLLINS, 2002).

Ascorbic Acid (AA) content was determined according to the method of Rizzolo et al. (1984). AA was extracted with metaphosphoric acid (0.3% w/v) and analyzed by reversed-phase HPLC, in a Hewlett-Packard 1100 system, with an auto-sampler and a quaternary pump, coupled to a diode array detector. The column used was a  $\mu$ -Bondapack (300 mm x 3.9 mm i.d., Waters, Milford, MA, USA) column, and elution (flow rate of 1.5 mL/minute) was performed in isocratic conditions with 0.2 M sodium acetate/acetic acid buffer (pH 4.2), monitored at 262 nm. Total AA was estimated after reduction of dehydroascorbic acid (DHA) with 10 mM dithiothreitol.

Flavonoid identification and quantification was performed in duplicate according to the method of Hassimotto, Genovese and Lajolo (2007), with slight modifications. The sample was extracted three times in 100 mL methanol/water/acetic acid (70:30:5 v/v), for 2 minutes (Brinkmann homogenizer, Polytron-Kinematica GmbH, Kriens-Luzern, Sweden), in an ice bath. The homogenate was filtered under reduced pressure through filter paper (Whatman n° 06). The extract was concentrated until methanol elimination, under vacuum, at  $40\text{ }^{\circ}\text{C}$ , in a rotary evaporator (Rotavapor RE 120, Büchi, Flawil, Sweden) and made up with distilled water for application in solid-phase extraction (SPE) columns. An aliquot of the extract was passed through polyamide (CC 6, Macherey-Nagel, Germany) columns (1 g/6 mL), previously conditioned with methanol and distilled water. Impurities were washed out with distilled water and retained flavonoids were eluted with methanol containing

0.1% HCl. The flow rate through the columns was controlled by means of a vacuum manifold Visiprep 24 DL (Supelco, Bellefonte, PA, USA). The eluate was evaporated to dryness under reduced pressure at 40 °C, redissolved in methanol: acetic acid (99:5 v/v), and filtered through a 0.22 µm tetrafluoroethylene (PTFE) filter (Millipore Ltd., Bedford, MA, USA), prior to HPLC analysis. Identification and quantification of flavonoids were achieved using analytical reversed-phase HPLC, in a Hewlett-Packard 1100 system, with an auto-sampler and quaternary pump, coupled to a diode array detector (DAD). The column used was a Prodigy 5 µ ODS-3 reversed-phase silica (250 mm 4.6 mm i.d., Phenomenex Ltd.) column. The solvents were (A) water/tetrahydrofuran/trifluoroacetic acid (98:2:0.1 v/v) and (B) acetonitrile, and the solvent gradient consisted of 8% B at the beginning, 10% at 5 minutes, 17% at 10 minutes, 25% at 15 minutes, 50% at 25 minutes, 90% at 30 minutes, 50% at 32 minutes, 8% at 35 minutes (run time, 35 minutes). Eluates were monitored at 270, 370 and 525 nm. Flow rate was 1 mL/minute, column temperature was 30 °C, and injection volume was 5-20 µL. Samples were injected in triplicate, and flavonoids were quantified using external standards. Peak identification was performed by comparison of retention times and spectra with the standards and the library spectra. Results were expressed as milligrams aglycone per 100 g Fresh Weight (FW) (quercetin and cyanidin, Extrasynthese, Genay, France; epicatechin, Sigma, St. Louis, MO, USA), as mean ± standard deviation (SD).

Total phenolics were measured in duplicate samples of each extract according to the method of Zielinski and Kozolowska (2000), using the Folin-Ciocalteu reagent and gallic acid as standard. The results were expressed as Gallic Acid Equivalents (GAE).

*In vitro* antioxidant assay: The antioxidant capacity of the extracts obtained from the analysis of total phenolics was determined according to the β-carotene bleaching method (HASSIMOTTO; GENOVESE; LAJOLO, 2005). An aliquot (20 µL) of β-carotene solution (2 mg.mL<sup>-1</sup> in chloroform) was added to a flask containing 40 µL linoleic acid, 1.0 mL chloroform and 0.4 mL Tween 40, and mixed. The chloroform was evaporated to dryness under nitrogen. After this, oxygenated distilled water (100 mL) was added and the mixture was shaken. Aliquots (100 µL) of the extract were added to 2.9 mL of the β-carotene solution in a cuvette and mixed. The absorbance of the solution at 470 nm was measured immediately and after 2 hours of incubation in a water bath at 50 °C using a Hewlett Packard 8453 spectrophotometer. The control consisted of 100 µL methanol instead of the sample extract. Antioxidant capacity was calculated as percent inhibition relative to the control.

### 3 Results and discussion

Physicochemical characteristics of the five blackberry cultivars analyzed are described in Table 1. Moisture content was around 90%, with differences among cultivars being lower than 3%. Ash was close to 0.3% of total fresh fruit weight in all cases. In terms of total soluble solids (°Brix), Guarani and Seleção 97 cultivars had the highest values, while Brazos and

Tupy cultivars presented the lowest soluble solids contents, which resulted in low TSS/TA ratios (4.0 and 5.2, respectively), when compared to the mean TSS/TA ratio of 5.8 obtained for the other cultivars (Table 1). The best TSS/TA ratio (7.4) was observed for Seleção 97 cultivar.

Soluble sugars ranged from 2.7 to 4.8%, and were composed mainly of fructose and glucose (Table 2), except for Seleção 97 cultivar that showed small amounts of sucrose (0.7 g.100 g<sup>-1</sup> fresh fruit). Glucose and fructose concentrations varied among cultivars, but the glucose/fructose ratio was between 1.2 and 1.4 for all cultivars. The values for glucose and fructose were similar to other soft fruits (SEYMOUR, 1996).

Vitamin C, total phenolics and total anthocyanins: The two forms of ascorbic acid with vitamin C biological activities were analyzed, the reduced form (L-ascorbic acid – AA) and the oxidized form (L-dehydro-ascorbic acid – DHA). In general, DHA content in vegetables represents less than 10% of total vitamin C (LEE; KADER, 2000), but in the five blackberry cultivars studied, the reduced form was not detected, only the oxidized form. Total ascorbic acid content (TAA) ranged from 9.86 mg.100 g<sup>-1</sup> FW (cv. Brazos) to 21.36 mg.100 g<sup>-1</sup> FW (cv. Caingangue) (Table 3), which represent amounts high enough to consider blackberry as a good source of vitamin C. The absence of AA in all cultivars under study may be related to the fast oxidation of vitamin C and absence of *de novo* synthesis of ascorbic acid during development or ripening. Total phenolics ranged from 341 to 499 mg.100 g<sup>-1</sup> FW and Caingangue cultivar stood out with the highest values. Total anthocyanins determined by the colorimetric method ranged from 116 to 194 mg.100 g<sup>-1</sup> FW (Table 3).

Flavonoids and antioxidant capacity: Flavonoid content in blackberry cultivars ranged from 123 to 213 mg.100 g<sup>-1</sup> fresh fruit (Table 4). Anthocyanins were the main flavonoids in blackberry fruits composition. Cyanidin derivatives (3 or 4 peaks) were the pigments detected in the five cultivars, representing 66-80% of the flavonoids. Using a standard, the main cyanidin derivative peak was identified as cyanidin-3-O-glucoside. The highest cyanidin concentrations were detected in Guarani and Caingangue cultivars (about 150 mg.100 g<sup>-1</sup>), values higher than those detected for domestic and wild species grown in China (1-118 mg.100 g<sup>-1</sup>) (DEIGHTON et al., 2000). Also, data shown for blackberry fruits are superior to anthocyanin contents found in other red fruits, such as strawberry (30 to 60 mg.100 g<sup>-1</sup>, CORDENUNSI et al., 2003), purple Surinam cherry (16.23 mg.100 g<sup>-1</sup>, LIMA et al., 2005) or acerola (3.79 to 59.74 mg.100 g<sup>-1</sup>, LIMA et al., 2003).

Total anthocyanin content determined by spectrophotometry (Table 3) was higher when compared to cyanidin content determined by HPLC. In general, the different color shades determined by anthocyanins depend on the aglycone type, glycosilation, pH and co-pigmentation with organic anions (STAFFORD, 1990). The combination of these factors contributes to the observed final color, which does not represent the HPLC quantification of anthocyanin. Thus, color and color intensity of each colored vegetable are not necessarily associated with a distinct chromophore (aglycone), but with a combination of different intrinsic and extrinsic molecular characteristics, which

**Table 1.** Physicochemical characteristics of five Brazilian blackberry cultivars. Different letters in columns denote significant differences according to the Tukey test ( $p < 0.05$ ).

| Cultivar   | Ash (%)                    | Moisture (%)              | pH                        | TSS (°Brix)               | TA (% citric acid)       | Carbohydrates (%)         | TSS/TA |
|------------|----------------------------|---------------------------|---------------------------|---------------------------|--------------------------|---------------------------|--------|
| Caingangue | 0.29 ± 0.006 <sup>AB</sup> | 90.88 ± 0.08 <sup>C</sup> | 3.42 ± 0.002 <sup>A</sup> | 7.60 ± 0.47 <sup>C</sup>  | 1.26 ± 0.03 <sup>D</sup> | 5.04 ± 0.06 <sup>B</sup>  | 6.03   |
| Brazos     | 0.31 ± 0.016 <sup>A</sup>  | 93.25 ± 0.38 <sup>A</sup> | 3.31 ± 0.003 <sup>D</sup> | 6.19 ± 0.11 <sup>D</sup>  | 1.54 ± 0.02 <sup>A</sup> | 3.07 ± 0.11 <sup>D</sup>  | 4.01   |
| Tupy       | 0.24 ± 0.009 <sup>D</sup>  | 91.37 ± 0.20 <sup>B</sup> | 3.23 ± 0.005 <sup>F</sup> | 6.93 ± 0.13 <sup>CD</sup> | 1.33 ± 0.02 <sup>C</sup> | 4.72 ± 0.17 <sup>BC</sup> | 5.21   |
| Guarani    | 0.29 ± 0.012 <sup>AB</sup> | 90.47 ± 0.26 <sup>C</sup> | 3.30 ± 0.006 <sup>D</sup> | 9.23 ± 0.15 <sup>B</sup>  | 1.47 ± 0.03 <sup>B</sup> | 5.92 ± 0.16 <sup>A</sup>  | 6.28   |
| Seleção 97 | 0.25 ± 0.007 <sup>CD</sup> | 91.59 ± 0.28 <sup>B</sup> | 3.33 ± 0.004 <sup>C</sup> | 9.32 ± 0.36 <sup>AB</sup> | 1.26 ± 0.01 <sup>D</sup> | 4.90 ± 0.10 <sup>B</sup>  | 7.39   |

n.d.: not detected; and values are means ± SD (n = 3).

**Table 2.** Total soluble sugars and soluble sugars (g.100 g<sup>-1</sup> FW) of five Brazilian blackberry cultivars. Different letters in columns denote significant differences according to the Tukey test ( $p < 0.05$ ).

| Cultivar   | Glucose                  | Fructose                 | Sucrose    | Total soluble sugars     |
|------------|--------------------------|--------------------------|------------|--------------------------|
| Caingangue | 2.44 ± 0.01 <sup>B</sup> | 1.91 ± 0.09 <sup>B</sup> | n.d.       | 4.35 ± 0.20 <sup>C</sup> |
| Brazos     | 1.69 ± 0.08 <sup>A</sup> | 1.15 ± 0.09 <sup>A</sup> | n.d.       | 2.75 ± 0.17 <sup>A</sup> |
| Tupy       | 2.53 ± 0.07 <sup>B</sup> | 2.02 ± 0.04 <sup>B</sup> | n.d.       | 4.56 ± 0.11 <sup>C</sup> |
| Guarani    | 2.08 ± 0.01 <sup>B</sup> | 1.70 ± 0.09 <sup>B</sup> | n.d.       | 3.77 ± 0.22 <sup>B</sup> |
| Seleção 97 | 2.60 ± 0.02 <sup>B</sup> | 2.11 ± 0.02 <sup>B</sup> | 0.7 ± 0.03 | 4.78 ± 0.35 <sup>C</sup> |

n.d.: not detected; and values are means ± SD (n = 3).

**Table 3.** Total phenolic, vitamin C and anthocyanin contents (mg.100 g<sup>-1</sup> FW) and antioxidant capacity (percentage of inhibition) of five Brazilian blackberry cultivars. Different letters in columns denote significant differences according to the Tukey test ( $p < 0.05$ ).

| Cultivar   | Total phenolics        | Antioxidant capacity | Vitamin C               | Anthocyanins             |
|------------|------------------------|----------------------|-------------------------|--------------------------|
| Caingangue | 499 ± 4 <sup>D</sup>   | 71 ± 4 <sup>A</sup>  | 21.0 ± 2 <sup>C</sup>   | 125.6 ± 0.8 <sup>B</sup> |
| Brazos     | 341 ± 22 <sup>A</sup>  | 66 ± 2 <sup>A</sup>  | 9.9 ± 0.7 <sup>A</sup>  | 133.0 ± 3 <sup>B</sup>   |
| Tupy       | 373 ± 17 <sup>AB</sup> | 71 ± 2 <sup>A</sup>  | 14.0 ± 1 <sup>B</sup>   | 116.0 ± 2 <sup>A</sup>   |
| Guarani    | 427 ± 8 <sup>C</sup>   | 76 ± 3 <sup>A</sup>  | 11.9 ± 0.9 <sup>A</sup> | 194.0 ± 5 <sup>C</sup>   |
| Seleção 97 | 408 ± 14 <sup>BC</sup> | 72 ± 2 <sup>A</sup>  | 15.6 ± 0.5 <sup>B</sup> | 116.0 ± 2 <sup>A</sup>   |

Values are means ± SD.

**Table 4.** Flavonoid content (mg.100 g<sup>-1</sup> FW) of five Brazilian blackberry cultivars, expressed as aglycone. Different letters in columns denote significant differences according to the Tukey test ( $p < 0.05$ ).

| Cultivar   | Quercetin               | Kaempferol | Cyanidin                 | Epicatechin             | Total  |
|------------|-------------------------|------------|--------------------------|-------------------------|--------|
| Caingangue | 19.0 ± 2.0 <sup>C</sup> | Tr         | 142.0 ± 6.0 <sup>C</sup> | 52.0 ± 3.0 <sup>B</sup> | 213.29 |
| Brazos     | 8.0 ± 1.0 <sup>A</sup>  | Tr         | 91.0 ± 2.0 <sup>A</sup>  | 49.0 ± 5.0 <sup>B</sup> | 149.55 |
| Tupy       | 7.8 ± 0.3 <sup>A</sup>  | Tr         | 94.9 ± 0.2 <sup>A</sup>  | 20.7 ± 0.8 <sup>A</sup> | 123.29 |
| Guarani    | 13.3 ± 0.7 <sup>B</sup> | Tr         | 157.0 ± 3.0 <sup>C</sup> | 63.0 ± 2.0 <sup>C</sup> | 233.34 |
| Seleção 97 | 9.0 ± 0.1 <sup>A</sup>  | Tr         | 122.0 ± 1.0 <sup>B</sup> | 52.0 ± 3.0 <sup>B</sup> | 182.97 |

Values are means ± SD; Tr: trace.

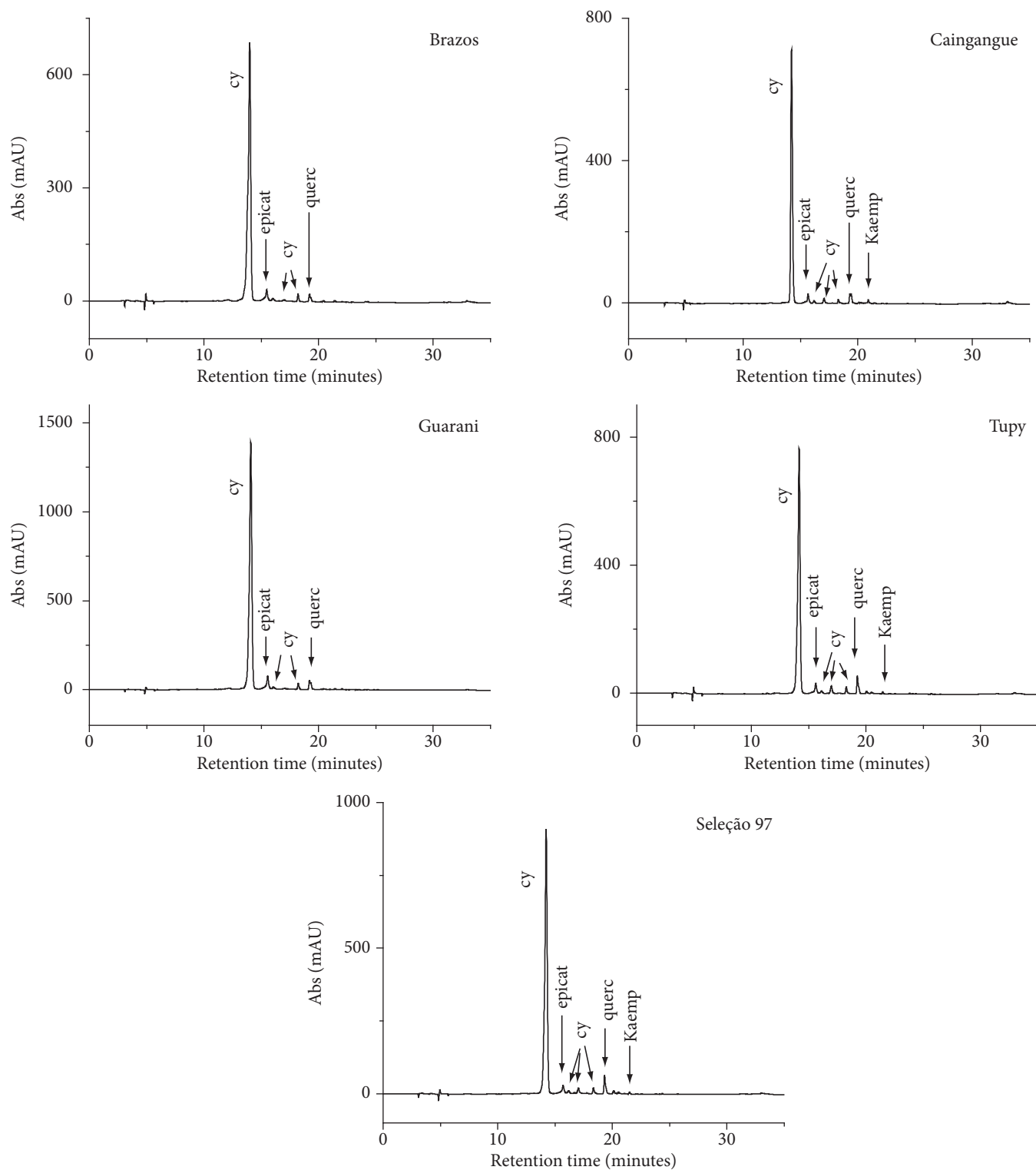
may produce an infinite variety of color shades (HARBORNE, 1988). Moreover, Guarani cultivar showed the highest rates of cyanidin and total anthocyanins (156 and 194 mg.100 g<sup>-1</sup>, respectively).

Besides cyanidin glycosides, epicatechin was also detected in concentrations ranging from 20 to 63 mg.100 g<sup>-1</sup>. HPLC-DAD detected the presence of cyanidin, flavan-3-ol epicatechin, quercetin and kaempferol (Figure 1).

According to Wang and Lin (2000), ripe black raspberry and blackberry fruits, on the basis of their wet weight, had higher antioxidant activities than red raspberries or strawberries. According to these authors, black raspberry and blackberry contain high amounts of cyanidin glycosides, a strong antioxidant, whereas strawberries are rich in pelargonidin 3-glucoside and ascorbic acid, which are weak antioxidants, due to structural differences between the two anthocyanidins.

The oxidation of  $\beta$ -carotene was inhibited in all cultivars, with an inhibition rate ranging from 66 to 76% (Table 3). These values are close to those obtained by the synthetic antioxidant butylated hydroxytoluene (BHT) (inhibition of 77.6% at 50  $\mu$ M), which characterizes the blackberry fruits as strong antioxidants. According to these results, Guarani cultivar had the highest antioxidant capacity (inhibition of 76 ± 3%) and anthocyanin content (194 ± 5 mg.100<sup>-1</sup> FW). However, the highest total phenolic and vitamin C contents (499 ± 4 and 21 ± 2 mg.100<sup>-1</sup> FW, respectively) were found in Caingangue cultivar.

In this study, a linear correlation was observed between cyanidin content and antioxidant capacity ( $r = 0.65$ ). According to data by Arabbi, Genovese and Lajolo (2004), the linear correlation between flavonoids and antioxidant capacity was lower than 0.1. This may have happened due to differences in



**Figure 1.** Chromatographic profile of flavonoids from blackberry cultivars obtained by HPLC-DAD (270 nm). Cyanidin derivative (cy), quercetin derivative (querc), kaempferol derivative (kaemp), epicatechin (epicat).

the composition of phenolics between vegetal extracts and their reaction with Folin-Ciocalteu reagent (KÄHKÖNEN et al., 1999). Moreover, values of antioxidant capacity may change according to the methodology chosen.

Flavonoid antioxidant capacity is related mainly to structural characteristics, such as the presence of an *o*-diphenil group in the B ring, the presence of a 3-hydroxyl group connected to a double bond between C<sub>2</sub>-C<sub>3</sub> and adjacent to a 4-oxo function in

the C ring, and the hydroxylation pattern, mainly C<sub>5</sub> and C<sub>7</sub> in the A ring (SEERAM; NAIR, 2002). The absence or replacement of some of these structural characteristics reduces or inhibits antioxidant capacity. According to Rice-Evans et al. (1996), flavonoids showing the highest TEAC values (Trolox equivalent antioxidant capacity) in the ABTS system are: quercetin flavonol, which shows all the structural characteristics mentioned above (4.72 ± 0.10 mM); cyanidin and delphinidin anthocyanidins, which show the same hydroxyl vicinity arrangement of quercetin (4.4 ± 0.1 mM), and catechin esterified to gallic acid (epicatechin gallate and epigallocatechin gallate). In this latter case, the high TEAC values are a reflex of the contribution of gallic acid (4.93 ± 0.02 and 4.75 ± 0.06 mM, respectively). In vegetables, flavonoids are mainly found as glycoside derivatives. Because of this, changes in antioxidant capacity may be expected in relation to their aglycones. Apparently, an important criterion to predict a high antioxidant capacity in vegetable foods is their anthocyanin content. Foods rich in anthocyanins normally show high antioxidant capacity, when compared to those without this class of flavonoids (HASSIMOTTO; GENOVESE; LAJOLO, 2005). Based on these facts, anthocyanins are believed to be the main phenolic compounds responsible for the high antioxidant capacity shown by blackberry fruit extracts, since a high correlation between cyanidin content and antioxidant capacity was observed.

#### 4 Conclusion

Among the five blackberry cultivars studied, Caingangue cultivar showed the highest vitamin C and total phenolics contents. However, Guarani cultivar showed the highest values for antioxidant capacity, total flavonoids, anthocyanins and cyanidin, in addition to physicochemical characteristics adequate for in natura consumption. These data confirm the general affirmation that the regular ingestion of blackberry fruits may contribute to an important intake of antioxidant polyphenols.

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