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Characterization of a new blackberry cultivar BRS Xingu: Chemical composition, phenolic compounds, and antioxidant capacity *in vitro* and *in vivo*

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ABSTRACT

The cultivar BRS Xingu was launched by EMBRAPA in 2015 with the intention of presenting higher productivity. Due to the lack of studies on this cultivar, the objective was to present the physical–chemical, centesimal, and phenolic composition of the BRS Xingu blackberry, its antioxidant capacity, protection against ROS generation, and compare it with other commercialized cultivars such as Guarani, Tupy, and Xavante. The BRS Xingu was prominent regarding anthocyanin and condensed tannin content and superior to the other cultivars. Moreover, BRS Xingu presented higher antioxidant capacity, protection of *C. elegans* from ROS generation, and soluble solid content when compared to Tupy, which is the most cultivated variety in the world. In the new cultivar, five anthocyanins, five phenolic acids, and ten non-anthocyanin flavonoids were identified. BRS Xingu is presented as an alternative blackberry with potential for industrialization and *in natura* consumption.

1. Introduction

The daily intake of 400 g of fresh fruit brings many health benefits (WHO, 2015), such as reduced chance of infarction, ischemia, cerebral hemorrhage, lower blood pressure and blood glucose levels (Du et al., 2016). Blackberry is a good choice of fruit for *in natura* consumption or processing because of its small size. Moreover, it is appropriate for cultivation by small family farms due to the favorable conditions in practically all of southern Brazil, where temperatures are lower (Antunes, 2002). In addition to its high nutritional value, blackberry is rich in dietary fibers, vitamins, minerals, and a source of bioactive compounds such as phenolic compounds, including anthocyanins (Hirsch, Facco, Rodrigues, Vizzotto, & Emanuelli, 2012).

Studies have shown that the total content of phenolic compounds, ashes, and soluble solids (Hirsch et al., 2012) of blackberry vary according to the cultivar, making it essential to characterize the chemical composition of individual cultivars.

The BRS Xingu blackberry was launched in 2015 and developed for 11 years by Embrapa Clima Temperado (EMBRAPA, 2015). This blackberry is a cross between Tupy and Arapaho and presents medium to large size, reddish-black coloration, and sweet-acid flavor (EMBRAPA, 2015). It was developed with the purpose of prolonging maturation time and, consequently, increasing sugar content and productivity when compared to the Tupy cultivar (EMBRAPA, 2015), which is the most cultivated in the world (SNA, 2017).

As the Tupy cultivar, the BRS Xingu cultivar stands out for its productivity, reaching approximately 800 g per plant, resulting in the production of 3264 tons/ha more than its predecessor. Additionally, its

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harvest period (late November through early January) lasts fifteen days longer than the Tupy cultivar, leading to economic advantages to the farmers and greater blackberry supply in a period in which this fruit is not abundant (SNA, 2017).

In this context, the agronomic and production advantages of this new cultivar advocates the need for its individual characterization. Notably, there are no known studies in the national and international literature on the characterization of blackberry fruits of the BRS Xingu cultivar. Therefore, the present study aimed to present the composition of the centesimal, physical–chemical, and phenolic compounds, antioxidant capacity, and effect of *C. elegans* in preventing or protecting against ROS generation of the BRS Xingu fruit and three other cultivars (Guarani, Tupy, and Xavante) produced in Rio Grande do Sul State (Brazil).

2. Materials and methods

2.1. Chemicals and reagents

The analytical reagents sulfuric acid, potassium sulfate, and copper sulfate were purchased from Synth (Diadema, Brazil). Sodium hydroxide, ethyl alcohol, methyl red, monobasic phosphate buffer, sodium sulfate, chloroform, methyl alcohol, sodium sulfate, butyl hydroxy toluene (BHT), acetone, formic acid (85%), boric acid, and chromatographic grade acetonitrile were purchased from Dinâmica (Jóia, Brazil). The enzymes α-amylase, protease, and amyloglucosidase were obtained from Tecnoglobo (Curitiba, Brazil). The dibasic phosphate buffer was acquired from Neon (Suzano, Brazil). Methylcellulose with viscosity 1500 CP and epicatechin (≥90%) were purchased from Sigma-Aldrich (Jurubatuba, Brazil). Chromatographic grade formic acid and methanol were purchased from Fluka, Sigma-Aldrich (Steinheim, Germany), and Fisher Scientific (Madrid, Spain), respectively. Double-deionized water with conductivity lower than 18.2 MΩ was obtained from the Milli-Q system (Millipore, Bedford, MA, USA). The phenolic standards cyanidin-3-O-glucoside (≥95%) and quercetin-3-glucoside (≥90%) were purchased from Sigma-Aldrich (Jurubatuba, Brazil),while epicatechin (≥97%) and chlorogenic acid (≥98%) were purchased from ChromaDex (Irvine, USA) and Chem-Impex International (Wood Dale, USA), respectively.

The trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (≥97%) and AAPH (2,2′-azobis-2-amidinopropane dihydrochloride) were purchased from Sigma-Aldrich (Jurubatuba, Brazil) and the fluorescein from Êxodo científica (Sumaré, Brazil). The bacto agar, cholesterol, ethyl alcohol, streptomycin sulfate, and CM-H2DCFDA were purchased from Sigma-Aldrich (Jurubatuba, Brazil). Sodium chloride, calcium chloride, magnesium sulfate, dibasic sodium phosphate, and sodium hydroxide were obtained from Vetec (São Paulo, Brazil), with phosphate monopotassium and DMSO (dimethyl sulfoxide) being purchased from Próquimica (Canoas, Brazil) and bacto peptone and luria broth from Kasvi (São José dos Pinhais, Brazil).

2.2. Samples

Blackberry (*Rubus spp*) fruits of cultivars BRS Xingu, Tupy, Guarani, and Xavante from the 2016 and 2017 harvests were evaluated. The fruit were provided by Embrapa Clima Temperado, which is located in the municipality of Pelotas (Latitude: −31.776, Longitude: 52.3594 31°46′34 "South, 52°21′34″ West), Rio Grande do Sul State, Brazil. The collected samples were selected with no physical injuries. The fruits (n = 10) presented an average of 5.88; 5.58; 4.95; 3.53 g, 2.91; 2.34; 2.21; 2.07 cm in length, and 2.18; 1.87; 1.89; 1.75 cm of width for the cultivars Tupy, Guarani, Xavante, and BRS Xingu, respectively (coefficient of variation < 13%, with pachymeter accuracy of 0.05 mm).

2.3. Chemical evaluation of the fruits

In the evaluation of soluble solids, pH, titratable acidity, and color, the pulp was crushed *in natura*. The samples were dried in an oven at 55 °C until reaching constant weight and the results expressed as % of fresh fruit (g/100 g). Color measurements were performed in a colorimeter (Minolta CR-300) using the CIELAB scale. The pH, acidity, and soluble solids were determined according to the methodologies described in AOAC (2016).

2.4. Centesimal composition

Moisture, ash, protein, carbohydrate, and dietary fiber content were measured using the methods described in AOAC (2016). Blackberry moisture content was determined by drying in a vacuum oven at 70 °C until reaching constant weight. The ashes were determined by incinerating the sample in a muffle at 550 °C. Total nitrogen was determined by the micro Kjeldahl method and the crude protein content was calculated by multiplying the total nitrogen content by the conversion factor 6.25.

Total dietary fiber and insoluble dietary fiber levels were determined according to the enzymatic–gravimetric method using the α-amylase, protease, and amyloglucosidase enzymes responsible for the enzymatic hydrolysis of starch and proteins. Soluble fiber was obtained through the difference between total dietary fiber and insoluble dietary fiber (AOAC, 2016). The lipid fraction was determined by cold extraction following the guidelines described by Bligh and Dyer (1959).

Carbohydrate content was determined by the difference using the sum of lipid, protein, moisture, and ash content (AOAC, 2016) subtracted from 100.

2.5. Obtaining the extract for phenolic compound analysis

The blackberries used in the extraction of phenolic compounds were frozen, lyophilized, and crushed. After drying and grinding, all samples were subjected to freezing at −18 °C until analysis. The blackberry extracts were obtained using an aqueous solution composed of 20% acetone and 0.35% formic acid as described by Bochi et al. (2014).

2.6. Spectrometry quantification of total condensed tannins

Total condensed tannin content was measured by the difference in absorbance of total soluble phenolic compounds in water and ammonium sulfate less precipitated by methylcellulose. The results were expressed in mg of epicatechin per 100 g of fresh fruit (mg of epicatechin/100 g) (Sarneckis et al., 2006).

2.7. Anthocyanin separation and identification by HPLC-ESI-MS^n

Anthocyanins were separated using the HPLC-ESI-MS^n method (Agilent Technologies) with a ZORBAX Extend-C18 Rapid Resolution HT (2.1 × 100 mm, 1.8-μm) 600 Bar column (Agilent) at a flow rate of 0.25 mL/min at room temperature. The mobile phases were composed of water, formic acid, acetonitrile (88.5: 8.5: 3, v/v/v) (mobile phase A), and water, formic acid, acetonitrile (41.5:8.5:50, v/v/v, v/v/v) (mobile phase B), with a linear gradient for Solvent B of 0 min, 6%; 10 min, 30%; 20 min, 50%; 25 min, 6% (Barcia et al., 2014). For anthocyanin identification, electrospray ionization mass spectrometry (ESI-MS) (Agilent Technologies, USA) was used with the following parameters: gas temperature of 300 °C, dry gas flow of 5 L/min, nebulizer at 45 psi, capillary voltage of 3500 V and fragment 5 V, and positive mode (Barcia et al., 2014). Mass spectra were extracted, which, along with literature data, provided an attempt to identify the anthocyanins from the blackberries.

2.8. Non-anthocyanin phenolic compound separation and identification by HPLC-TOF-MS

Stock solutions of 5000 mg/L blackberry extracts for each cultivar were prepared by dissolving the appropriate amount of the extract in methanol. The analytical platform applied to identify the phenols in different blackberry extracts was composed of a RRLC 1200 series (Agilent Technologies, Palo Alto, CA, USA), which is an instrument equipped with four blocks: a vacuum degasser, a self-sampler, a pump, and a TOF (time-of-flight) mass spectrometer detector (Bruker Daltonik, Bremen, Germany). The MS detector was coupled to an HPLC system with an orthogonal electrospray interface (ESI) (model G1607 from Agilent Technologies, Palo Alto, CA, USA) operating on negative ion mode.

The phenolic compounds were separated using a Zorbax Eclipse Plus C18 with a column of 150 mm × 4.6 mm id, 1.8 μm (Agilent Technologies, Palo Alto, CA, USA) using a binary mobile phase. Therefore, mobile phase A was prepared with 0.1% formic acid in water while B was prepared in methanol. Analytical separation was performed by injecting 10 μL of each blackberry extract according to the following linear gradient: 0–42 min (5% B), 42–45 min (95% B), and 45–50 min (5% B), where the initial conditions were maintained for 5 min after each analysis, taking 50 min per analysis. The flow rate was 0.4 mL/min and all analyses performed at room temperature (Jiménez-Sánchez et al., 2015).

The ESI source parameters were optimized and implemented as follows: +4 kV capillary voltage; drying gas temperature of 210 °C; drying gas flow of 9 L/min; and nebulizing gas pressure of 2.3 bar. The values of the transfer parameters were: capillary output of −120 V; skimmer 1 of −40 V; hexapole 1 of −23 V; Hexapolo RF of 80 V; and skimmer 2 of −22.5 V. Detection was performed by applying a mass range of 50–1500 *m/z*. To provide good calibration, the TOF mass spectrometer was externally calibrated using a Cole Palmer 74900-00-05 syringe pump (Vernon Hills, IL, USA) directly connected to the interface. The external mass spectrometer was calibrated using sodium formate agglomerate (sodium hydroxide 5 mol/L in water/isopropanol 1/1 (v/v) with 0.1% formic acid) on regression mode (HPC) (Leyva-Jiménez, Lozano-Sánchez, Borrás-Linares, Arráez-Román, & Segura-Carretero, 2018). The DataAnalysis 4.0 analysis software (Bruker Daltonics), which uses a CHNO algorithm that increases the confidence in the suggested molecular formulas, managed the mass data of the molecular ions acquired.

2.9. Non-anthocyanin phenolic compound separation and quantification by HPLC-DAD

To quantify the non-anthocyanin phenolic compounds, 20 μL of the purified sample were injected into the HPLC-DAD (Shimadzu), as described by Giusti and Wrolstad (2001). The Hypersil Gold C-18 reverse phase column (5 μm particle, 150 mm, 2.1 mm) was used with a furnace temperature of 38 °C and two mobile phases composed of 5% methanol, 0.1% formic acid, and milli-Q water (mobile phase A) and acetonitrile acidified with 0.1% formic acid (mobile phase B) at a flow rate of 1.0 mL/min. The linear gradient for mobile phase B was of 0–21 min, 0%; 21–55 min, 4%; 55–70 min, 16%; 70–72 min, 50%; 72–83 min, 100%; and 83–92 min, and 0% until the end of the analysis at 92 min (Quatrin et al., 2019). To quantify the different phenolic classes, calibration curves of epicatechin with a concentration of 10.75 to 107.50 mg/L (280 nm, flavanols), chlorogenic acid (320 nm, hydroxycinnamic acids) of 2.38 to 45.28 mg/L, and quercetin-3-glucoside (360 nm, flavanols) were used in the range of 12.33 to 123.33 mg/L. All external calibration curves present equidistant points and R^2 above 0.99.

2.10. Anthocyanin separation and quantification by HPLC-UV–Vis

The anthocyanins were separated by high performance liquid chromatography (HPLC-Shimadzu). The column used was Zorbax 2.1 mm × 150 mm 3.5μm Elipse XDB particle size with a flow rate of 0.5 mL/min and oven temperature of 38 °C. Both mobile phases were composed of acidified water with 3% formic acid (A) and acetonitrile (B). The linear gradient was of 0 min 8%; 25 min, 32%; 27 min, 90% and 39 min, 8% B, with the test lasting for 50 min. Quantification was performed using a calibration curve with cyanidin-3-O-glucoside standard and readings at a wavelength of 520 nm.

The method was evaluated through the validation parameters described by the Brazilian Health Regulatory Agency (ANVISA, 2003) and the Ministry of Agriculture, Livestock, and Supply (Brasil, 2015). Moreover, the following items were assessed in this analysis: linearity parameters (R^2 = 0.9998), linear range (0.2 mg/L to 45.2 mg/L of cyanidin-3-O-glucoside), and inter-day (coefficient of variation (CV%) of 0.09 to 8.43%) and intraday (CV% of 0.83 to 1.60%) precision, and the detection limit (0.18 mg/L) and quantification limit (0.56 mg/L).

2.11. In vitro and in vivo assays

2.11.1. Oxygen radical absorption capacity (ORAC)

An oxygen radical absorption capacity (ORAC) methodology (Ou, Hampsch-Woodill, & Prior, 2001) was used to measure the antioxidant capacity of the fruits, where the sequestering capacity of an antioxidant is estimated by the formation of a peroxyl radical induced by 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) at 37 °C. Spectrophotometry was measured by excitation wavelength (485 nm) with the emission wavelength (528 nm) of non-fluorescent product fluorescein fluorescence marker, which reacted with the peroxyl radical (Ou, Hampsch-Woodill, & Prior, 2001). The results were expressed in mmol of trolox per 100 g of fresh fruit.

2.11.2. C. elegans strains, maintenance, treatment, and measurement of reactive oxygen species (ROS)

A wild-type *C. elegans* strain N2 (var. Bristol) was provided by the *Caenorhabditis* Genetics Center (University of Minnesota, USA). Wild-type gravid hermaphrodites were synchronized to obtain eggs using bleaching solution (1% NaOCl, 0.25 M NaOH). Then, the eggs were washed three times and stored overnight in an M9 buffer (42 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, and 1 mM $MgSO_4$) to obtain all animals in the L1 stage. The worms were transferred to NGM (Nematode Growth Medium) containing *Escherichia coli* OP50 strain previously seeded as a food source at 20 °C until reaching the young adult stage, which was about 40 h after the L1 stage (Brenner, 1974).

About 1000 worms at young adult-stage were pre-treated acutely for 1 h with Tupy, Guarani, Xavante, BRS Xingu extracts or M9 buffer with control. The concentrations used were based on the total amount of phenolic compounds (sum of the total of anthocyanins, flavonols, flavanols, and hydroxycinnamic acids) of each extract, which were 43.35 μg/mL for Tupy, 49.23 μg/mL for Guarani, 48.35 μg/mL for Xavante, and 49.65 μg/mL for BRS Xingu, with all extracts being dissolved in water. After 1 h in the pre-treatment at 20 °C, the worms were washed three times from the treatments and transferred to the M9 buffer with 50 μM of juglone (5-hydroxy-1,4-naphthalenedione), a superoxide generator (Blum & Fridovich, 1983) or vehicle ethanol (1% final concentration), and incubated under agitation for 1 h at 20 °C. Reactive oxygen species (ROS) generation was quantified using 2.7-dichlorodihydro-fluorescein diacetate (H_2DCFDA) (Schulz et al., 2007) with minor modifications.

After the pre-treatment with Tupy, Guarani, Xavante or BRS Xingu extracts and exposure to 50 μM of juglone, the young adult nematodes were washed three times with the M9 buffer. Afterwards, approximately 40 animals from each group were pipetted into the wells of a 96-well black plate with 20 μM of H_2DCF-DA. Fluorescence intensity was

measured after 2.5 h of incubation using a SpectraMax® i3x microplate reader (Molecular Devices, Sunnyvale, CA) at 20 °C and 488 nm excitation and 510 nm emission. Three experiments were performed in triplicate and the mean values were calculated. Data are expressed as percentual of control.

2.12. Statistical analyses

All analyses and extractions were performed in triplicate (n = 3). The statistical analysis was performed through analysis of variance (ANOVA) followed by the Tukey test (between cultivars) and Student's *t*-test (between years) to evaluate the differences between the groups and Pearson's correlation. Values of $p \leq 0.05$ were considered statistically significant. The principal component analysis (PCA) used to evaluate the contribution of the compounds studied during two years of cultivation in four cultivars of blackberry fruit was also used.

3. Results and discussion

3.1. Physical-chemical, nutritional, and phytochemical composition of the blackberries.

3.1.1. Physical-chemical and centesimal composition

All blackberries showed a dark red color, typical of ripe fruits, possibly due to high anthocyanin content, which are compounds responsible for the red and purple colors of many fruits. However, the presence of these compounds does not always correlate with analysis by colorimetry. The values of L*, which are related to the light axis, were low and tending to black, indicating that blackberries have darker tones (Table 1 and Fig. S1).

The positive values of a* show that the blackberries have a tendency towards red and the positive values of b* show that these fruits have a greater tendency to yellow. Color is an important parameter for producers and consumers since it indicates whether the fruit is in the ideal conditions for commercialization and consumption (Hirsch et al., 2012). Therefore, the BRS Xingu cultivar presents the expected coloration similar to other cultivars already on the market, indicating that consumers would prefer its appearance.

Blackberries harvested in 2017 showed greater tendency to red than those from the 2016 harvest. This may be correlated to fruit acidity and pH since the fruits harvested in 2017 had a higher percentage of organic acids and lower pH than those of 2016. Based on Pearson's correlation, the values of a* are correlated with pH ($p = -0.5$) and with acidity ($p = 0.67$), which is explained by the tendency of anthocyanins to change color when variations occur in the acidity of the medium, resulting in structural and, consequently, color changes (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). The closer to pH 4.0, the greater the predominance of the quinoidal form of the anthocyanins, which presented greater tendency towards blue colors and also explains the lower values of b* in the blackberries of 2016, that is, they have lower tendency to yellow than the cultivars of 2017 (Castañeda-Ovando et al., 2009).

The new cultivar presented a higher soluble solids content than Tupy, indicating that BRS Xingu showed higher dissolvable sugar content when compared to Tupy in both years. Moreover, the BRS Xingu cultivar was ideal for producing jam since it presented low pH, which facilitates gel formation and does not require acidulants to form this product. Considering the titratable acidity, BRS Xingu was not distinct from the Guarani cultivar in both years and, in 2017, it resembled the Tupy cultivar. Nevertheless, BRS Xingu showed an intracultivar difference between the years 2016 and 2017. The results may be related to changes in the production of organic acids, such as malic, lacto-isocritric, isocitric, citric, ascorbic, phosphoric, fumaric, shikimic, and quinic acids (Kaume, Howard, & Devareddy, 2012), which differ according to the cultivar (Kafkas, Koşar, Türemiş, & Başer, 2006). Moreover, these changes may also be linked to other external influences such as harvesting time, ripening stage, climate, soil conditions, sun exposure, fruit location on the plant, and post-harvest handling (Acosta-Montoya et al., 2010).

The correlation between soluble solids and acidity is used as a parameter to indicate fruit palatability, which is related to its flavor and an important attribute for *in natura* fruit consumption. BRS Xingu

Table 1
Physical-chemical and centesimal composition of blackberries.

Physical-chemical composition								
Year	2016				2017			
Cultivar	Guarani	Tupy	Xavante	BRS Xingu	Guarani	Tupy	Xavante	BRS Xingu
L*	26.64 ± 0.06^{bA}	26.9 ± 0.1^{aB}	26.74 ± 0.05^{abB}	26.9 ± 0.1^{abB}	27.4 ± 0.5^{cA}	28.84 ± 0.06^{aA}	27.9 ± 0.1^{bcA}	28.2 ± 0.1^{abA}
a*	8.45 ± 0.28^{abB}	8.92 ± 0.30^{aB}	7.77 ± 0.02^{cB}	8.07 ± 0.40^{bcB}	10.59 ± 0.50^{bA}	12.20 ± 0.41^{aA}	10.65 ± 0.10^{cA}	10.9 ± 0.2^{bA}
b*	1.36 ± 0.08^{aB}	1.14 ± 0.05^{bB}	1.35 ± 0.09^{aB}	0.81 ± 0.02^{cB}	2.9 ± 0.3^{aA}	2.8 ± 0.2^{aA}	1.7 ± 0.1^{bA}	2.8 ± 0.1^{aA}
Soluble solids	7.5 ± 0.0^{bB}	7.3 ± 0.6^{bA}	9.0 ± 0.0^{aB}	9.0 ± 0.0^{aB}	10.0 ± 0.0^{aA}	6.8 ± 0.3^{bA}	10.0 ± 0.0^{aA}	10.2 ± 0.3^{aA}
pH	3.72 ± 0.06^{aA}	3.4 ± 0.1^{bA}	3.3 ± 0.1^{bA}	3.22 ± 0.04^{Ba}	3.16 ± 0.02^{abB}	3.13 ± 0.06^{bB}	3.24 ± 0.01^{aA}	3.11 ± 0.02^{bB}
Titratable acid	0.90 ± 0.06^{bcB}	1.00 ± 0.05^{abB}	1.04 ± 0.06^{aA}	0.76 ± 0.06^{cB}	1.276 ± 0.002^{aA}	1.19 ± 0.01^{abA}	1.06 ± 0.06^{bA}	1.24 ± 0.06^{aA}
Centesimal composition								
Year	2016				2017			
Cultivar	Guarani	Tupy	Xavante	BRS Xingu	Guarani	Tupy	Xavante	BRS Xingu
Carbohydrates	10 ± 1^{aA}	9.6 ± 0.2^{aA}	11.5 ± 0.6^{aA}	11.6 ± 0.5^{aA}	8.8 ± 0.2^{bA}	7.5 ± 0.2^{cB}	8.0 ± 0.2^{cB}	9.6 ± 0.3^{aB}
Moisture	87 ± 1^{aA}	87.7 ± 0.2^{aB}	86.6 ± 0.6^{Ab}	85.8 ± 0.5^{aB}	89.3 ± 0.2^{bA}	90.3 ± 0.2^{aA}	90.1 ± 0.3^{aA}	88.4 ± 0.3^{cA}
Total fiber	6.6 ± 0.2^{aA}	5.4 ± 0.2^{bA}	6.2 ± 0.1^{aB}	6.60 ± 0.07^{aA}	6.3 ± 0.4^{aA}	4.9 ± 0.4^{bA}	7.1 ± 0.5^{aA}	5.0 ± 0.3^{bB}
Soluble fiber	1.52 ± 0.01^{bA}	0.62 ± 0.05^{cA}	0.800 ± 0.007^{cB}	3.1 ± 0.4^{aA}	1.5 ± 0.4^{aA}	0.5 ± 0.3^{bA}	1.7 ± 0.1^{bA}	1.2 ± 0.1^{abB}
Insoluble fiber	5.1 ± 0.2^{aA}	4.8 ± 0.3^{aA}	5.4 ± 0.1^{aA}	3.5 ± 0.4^{bA}	4.8 ± 0.5^{abA}	4.4 ± 0.8^{bB}	5.4 ± 0.5^{aA}	3.9 ± 0.4^{cbA}
Protein	1.22 ± 0.02^{bA}	1.47 ± 0.03^{aA}	1.11 ± 0.04^{cA}	1.49 ± 0.04^{aA}	1.00 ± 0.08^{aB}	1.17 ± 0.06^{aB}	1.1 ± 0.1^{aA}	1.17 ± 0.05^{a}
Lipids	0.9 ± 0.2^{abA}	1.00 ± 0.03^{aA}	0.8 ± 0.2^{bA}	0.76 ± 0.05^{bA}	0.485 ± 0.002^{bB}	0.500 ± 0.004^{aB}	0.350 ± 0.006^{dB}	0.432 ± 0.007^{cB}
Ashes	0.27 ± 0.02^{Bb}	0.25 ± 0.02^{bcB}	0.217 ± 0.002^{cB}	0.32 ± 0.01^{aB}	0.42 ± 0.04^{aA}	0.49 ± 0.05^{aA}	0.41 ± 0.02^{aA}	0.42 ± 0.02^{aA}

L *: brightness; a *: tendency to red; b *: tendency to yellow. Soluble solids: Brix. Titratable acid: % citric acid. The results of the centesimal composition are expressed as % (g/100 g of fresh fruit). Mean value ± standard deviation (n = 3). Coefficient of variation < 10%. The means followed by the same lowercase letter in the same year do not differ by the Tukey test ($p \leq 0.05$). The means followed by the same uppercase letter for the same cultivar in different years do not differ by the Student's *t*-test ($p \leq 0.05$).

presented °Brix/Acidity ratio of 11.83 in 2016 and 8.21 in 2017, showing that the new cultivar is balanced since the higher the ratio (> 5), the better the taste of the fruit (Brugnara, 2016).

The macronutrients present in most blackberries are carbohydrates (Table 1), of which the cultivars of 2016 presented higher concentrations. Regarding the 2017 harvest, the BRS Xingu presented the highest content, statistically differing from all other cultivars. The highest carbohydrate content in the first year is due to lower moisture content of the fruits, which is probably due to climatic conditions since the annual rainfall in 2016 (1200 to 1400 mm) was lower compared to 2017 (1400 to 1600 mm) (INMET, 2019). Thus, the plants of 2016 received a longer period of insolation than those of 2017, which likely increased the amount of carbohydrates due to the higher rate of photosynthesis. The fruits of plants that receive higher insolation contain higher sugar content because they conduct photosynthesis for more days of the year and are capable of transporting their sugar reserves to the fruits (Taiz & Zeiger, 2006). Blackberries are mainly composed of water (85 to 90%) and showed high values of moisture in both years. High concentrations of water in blackberry fruits are related to their low post-harvest conservation.

The BRS Xingu cultivar presented comparable levels of dietary fiber fractions with the Tupy cultivar in 2017, while the total and soluble fractions were higher in the new cultivar in 2016, showing that BRS Xingu is also rich in dietary fibers when compared to other blackberry cultivars. Dietary fibers provide benefits to human health when consumed frequently since high fiber contents are known for maintaining intestinal health.

The evaluated blackberries presented low protein (1 to 1.50%) and lipid (0.35 to 1%) content, which is common in small fruits (berries) since they do not generally present high lipid and protein content (Hirsch et al., 2012). The ash content (0.22 to 0.50%) is related to the amount of minerals, which are micronutrients necessary to perform vital functions in the development and good health of the human body. Nevertheless, the BRS Xingu cultivar presented a high concentration of ash content (0.32%) when compared to other cultivars in 2016 and an increase of 0.10% in its content in 2017.

3.1.2. Phytochemical composition

The BRS Xingu blackberry stood out among the cultivars regarding anthocyanin and condensed tannin content, presenting higher levels ($p \leq 0.05$) when compared to the other cultivars in both years (Table 2).

The flavonoids were the most commonly found compound class in the studied blackberry cultivars. Anthocyanins, such as cyanidin-3-O-glucoside, flavanols, such as epicatechin and catechin, and flavonols, such as quercetin and kaempferol, were identified and quantified (Lee, Dossett, & Finn, 2012).

The anthocyanin profile of BRS Xingu is similar to Tupy and Guarani. Five anthocyanins (cyanidin-3-O-glucoside, cyanidin-3-O-xyloside, cyanidin-3-O-rutinoside, cyanidin-3-O-(6″-malonyl-glucoside), and cyanidin-3-(6″-dioxalylglucoside)) were identified. However, the last one was not identified in the Xavante cultivar (Table 3). Other anthocyanins, such as cyanidin-3-O-arabinoside, cyanidin-3-O-sophoroside, cyanidin-3-O-glucosylrutinoside, malvidin-3-O-arabinoside, malvidin-3-O-galactoside, and perlagonidin-3-O-glucoside were identified in blackberries (Lee et al., 2012) but not in the same cultivars used here. Anthocyanins are the major phenolic compounds of blackberries, and the higher content of these compounds in BRS Xingu shows that the new cultivar has potential as recent studies have demonstrated that anthocyanins have antidiabetic properties (Gowd et al., 2018) and known antioxidant properties. Furthermore, BRS Xingu has, on average, 27 mg of condensed tannins that can perform antioxidant (Takechi, Tanaka, Takehara, Nonaka, & Nishioka, 1985) and antiviral activities (Chung, Wei, & Johnson, 1998) and reducerisk factors for cardiovascular diseases (Singal, Khaper, Palace, & Kumar, 1998) and diabetes mellitus (Gyamfi & Aniya, 2002). Anthocyanin and condensed tannin content is influenced by plant genetics, with common variations according to the cultivar, environmental conditions, and cultivating season (Acosta-Montoya et al., 2010).

Twelve non-anthocyanin phenolic compounds were identified in the new cultivar (Table 3), all of which were also present in the other cultivars. As mentioned previously, the most abundant phenolic class was flavonoids, and in BRS Xingu, flavonols occurred as quercetin derivatives, in addition to two flavanols (epicatechin and hexuronide catechin) and kaempferol hexoside isomer 1, the last identified in all cultivars. Five phenolic acids were found, among which three were isomers of chlorogenic acid. Glucosylated vanillic acid was identified in all the blackberries analyzed apart from the Guarani cultivar. The ellagic acid pentoside is also present in the Xavante cultivar. Compounds such as apigenin pentoside, purpurascenin, and isorhamnetin hexuronide were identified in the fruits of the Xavante cultivar, and, to date, no such compounds have been reported in the literature for blackberries.

3.1.3. Principal component analysis

PC1 was responsible for explaining 41.68% of the total data variance, with PC2 presenting 23.55%, and PC3 having 13.76%, with the three main components accounting for 78.99% of the result variance (Fig. 1). The first two main components account for 65.23% of the total variance of the results while PC1 and PC3 both account for 55.44%.

In PC1 and PC2, the BRS Xingu from 2016 differ from the others by presenting lower soluble fiber content, which is found in the upper right quadrant. Comparison of PC1 and PC3 show that the variable insoluble fiber is demonstrated in the upper quadrant for cultivars Xavante and Guarani (year 2016 and 2017), while BRS Xingu and Tupy are in the lower quadrant in both years. Separation of the cultivar Tupy of 2016 (PC1 and PC3) is due to the total fiber content being lower for this cultivar.

BRS Xingu of 2017 in PC1 and PC2 showed similar flavonol content when compared to Xavante and Guarani despite appearing separated

Table 2
Phenolic compounds of blackberries in the years 2016 and 2017.

Year 2016					2017			
Cultivar	Guarani	Tupy	Xavante	BRS Xingu	Guarani	Tupy	Xavante	BRS Xingu
Anthocyanins[1]	86 ± 8^{cB}	128 ± 11^{bA}	104 ± 8^{bcB}	158 ± 15^{aA}	138 ± 14^{bA}	91 ± 7^{cB}	126 ± 9^{bA}	168 ± 12^{aA}
Condensed tannins[2]	11.1 ± 0.8^{cB}	20 ± 1^{bA}	17.9 ± 0.9^{bB}	26 ± 2^{aA}	18.2 ± 0.5^{cA}	19 ± 1^{bcA}	22 ± 1^{bA}	28 ± 2^{aA}
Flavanols[2]	4.62 ± 0.09^{cB}	3.3 ± 0.1^{cB}	11 ± 1^{aB}	6.1 ± 0.5^{bB}	29 ± 2^{aA}	8.4 ± 0.8^{cA}	22 ± 2^{bA}	24 ± 2^{bA}
Hydroxycinnamic acids[3]	0.36 ± 0.01^{cB}	0.50 ± 0.03^{bB}	0.59 ± 0.03^{aB}	0.29 ± 0.01^{dA}	0.53 ± 0.03^{bA}	0.81 ± 0.03^{aA}	0.72 ± 0.05^{aA}	0.3 ± 0.1^{cA}
Flavonols[4]	9.33 ± 0.02^{bB}	12.6 ± 0.9^{aA}	10.4 ± 0.9^{bB}	13 ± 1^{aB}	14 ± 1^{aA}	10.0 ± 0.7^{bB}	15 ± 1^{aA}	17 ± 1^{aA}

Anthocyanins [1]: mg of cyanidin-3-O-glucoside/100 g of fresh fruit. Condensed tannins and flavanols[2]: mg of epicatechin/100 g of fresh fruit. Hydroxycinnamic acids[3]: mg of chlorogenic acid/100 g of fresh fruit. Flavonols[4]: quercetin-3-glucoside mg/100 g of fresh fruit. Mean value ± standard deviation (n = 3). The averages followed by the same lower case letter in the same year do not differ by Tukey test ($p \leq 0.05$). The means followed by the same uppercase letter for the same cultivar in different years do not differ by the Student's *t*-test ($p \leq 0.05$). Coefficient of variation < 10%.

Table 3
Retention time (RT) and mass spectra for the tentative identification of anthocyanins and non-anthocyanin phenolic compounds in blackberries.

Anthocyanic phenolic compounds							
RT (min)	Molecular ion and product ion (*m/z*)		Tentative identification	Cultivar			
				Guarani	Tupy	Xavante	BRS Xingu
4.847	449–287		Cyanidin-3-O-glucoside	x	x	x	x
4.848	419–287		Cyanidin-3-O-xyloside	x	x	x	x
6.112	595–287		Cyanidin-3-O-rutinoside	x	x	x	x
9.509	535–287		Cyanidin-3-O-(6″-malonyl-glucoside)	x	x	x	x
10.686	593–287		Cyanidin-3-(6″-dioxalylglucoside)	x	x		x
Non-anthocyanic phenolic compound							
TR (min)	Molecular ion (*m/z*)	Molecular formula	Tentative identification	Cultivar			
				Guarani	Tupy	Xavante	BRS Xingu
4.0	282.0831	$C_9H_{16}NO_9$	UK 1			x	
4.5	353.0878	$C_{16}H_{17}O_9$	Chologenic acid isomer 1	x	x	x	x
4.9	353.0878	$C_{16}H_{17}O_9$	Chologenic acid isomer 2	x	x	x	x
5.4	353.0878	$C_{16}H_{17}O_9$	Chologenic acid isomer 3	x	x	x	x
9.5	180.0666	$C_9H_{10}NO_3$	UK 3	x	x	x	x
13.5	329.0878	$C_{14}H_{17}O_9$	Vanillic acid glucoside		x	x	x
14.2	368.0987	$C_{16}H_{18}NO_9$	UK 4		x	x	x
17.2	357.0827	$C_{15}H_{17}O_{10}$	Dihydrocaffeic acid hexuronide			x	
17.3	341.0878	$C_{15}H_{17}O_9$	Caffeoyl hexoside		x		
17.7	396.0936	$C_{17}H_{18}N_7O_8$	UK 5		x		
17.9	203.0925	$C_9H_{15}O_5$	UK 6	x		x	
18.6	289.0608	$C_{15}H_{13}O_6$	Catechin	x			
21.0	447.0933	$C_{21}H_{19}O_{11}$	Kaempferol hexoside isomer 1	x	x	x	x
21.2	465.1038	$C_{21}H_{21}O_{12}$	Catechin hexuronide	x		x	x
21.8	289.0718	$C_{15}H_{13}O_6$	Epicatechin	x		x	x
22.8	431.1923	$C_{20}H_{31}O_{10}$	UK 7			x	
23.3	401.0878	$C_{20}H_{17}O_9$	Apigenin pentoside			x	
23.8	461.1664	$C_{23}H_{29}O_{10}$	Purpurascenin			x	
24.8	475.0882	$C_{22}H_{20}O_{12}$	Kaempferide hexuronide isomer 1	x	x	x	x
24.8	485.1089	$C_{23}H_{21}O_{11}$	Epigallocatechin 3-O-syringate	x			
28.1	477.0675	$C_{21}H_{17}O_{13}$	Quercetin hexuronide	x	x	x	x
28.2	433.0412	$C_{19}H_{13}O_{12}$	Ellagic acid pentoside			x	x
28.3	463.0882	$C_{21}H_{19}O_{12}$	Quercetin hexoside	x		x	x
28.4	609.1461	$C_{27}H_{29}O_{16}$	Rutin	x		x	x
29.0	607.1305	$C_{27}H_{27}O_{16}$	Quercetin 3-O-[6″-O-(3-hydroxy-3-methylglutaroyl)-β-D-galactoside]		x		
29.3	433.0776	$C_{20}H_{17}O_{11}$	Quercetin pentoside			x	
29.6	491.0831	$C_{22}H_{19}O_{13}$	Isorhamnetin hexuronide			x	
30.0	300.9990	$C_{15}H_5O_8$	Ellagic acid			x	
30.7	447.0933	$C_{21}H_{19}O_{11}$	Kaempferol hexoside isomer 2			x	
31.0	461.0725	$C_{21}H_{17}O_{12}$	Kaempferol hexuronide isomer 2			x	
32.4	711.3786	$C_{33}H_{59}O_{16}$	UK 8	x			
38.4	517.3025	$C_{37}H_{41}O_2$	UK 9	x			
39.3	503.3378	$C_{30}H_{47}O_6$	UK 10	x	x	x	x
40.0	531.3539	$C_{28}H_{51}O_9$	UK 11		x	x	
40.3	503.3237	$C_{26}H_{47}O_9$	UK 12	x	x	x	x
41.1	531.3327	$C_{31}H_{47}O_9$	UK 13		x	x	x
41.6	531.3327	$C_{31}H_{47}O_9$	UK 14		x	x	x

*The presence of the compound was detected in the cultivars where the "x" appears. ** Unidentified (UK).

from these cultivars in PC1 and PC3 due to higher condensed tannin and anthocyanin content. Tupy of 2017 is in the lower left quadrant due to its higher tendency towards red (a*). In PC1 and PC3, the cultivars of 2016 and 2017 are separated by the difference between contents of ash, moisture, carbohydrates, lipids, titratable acidity, and tendency towards yellow (b*). BRS Xingu resembled the Tupy cultivar in both years studied. In 2016, BRS Xingu presented behavior similar to Tupy mainly because of its protein content and color parameter L* in 2017. The PCA showed the relationship between the different cultivars of blackberry studied in both years. The samples tended to resemble the same cultivar and the same years, that is, the cultivar BRS Xingu resembled the cultivar Tupy in 2016 and 2017 while the cultivar Xavante resembled Guarani in both years.

3.2. Phytochemical compound bioactivity

3.2.1. In vitro assay

The results of the antioxidant capacity (Fig. 2) showed that the BRS Xingu blackberry (2.50 mmol/100 g in 2016 and 4.62 mmol/100 g in 2017) presented the same antioxidant capacity as the Guarani cultivar (2.54 mmol/100 g in 2016 and 4.85 mmol/100 g in 2017), with values superior to those of Tupy (1.46 times in 2016 and 1.94 times in 2017) and lower than Xavante (1.19 times in 2016 and 1.34 times in 2017) for both years. The lower antioxidant capacity of Tupy and superior antioxidant capacity of Xavante are explained by their phenolic composition. In the Tupy cultivar, three quercetin derivatives and one catechin derivative were not identified. On the other hand, more compounds derived from kaempferol and quercetin that have high antioxidant capacity were identified in Xavante (Ferreira, Turon, Regina, & Couto, 2015).

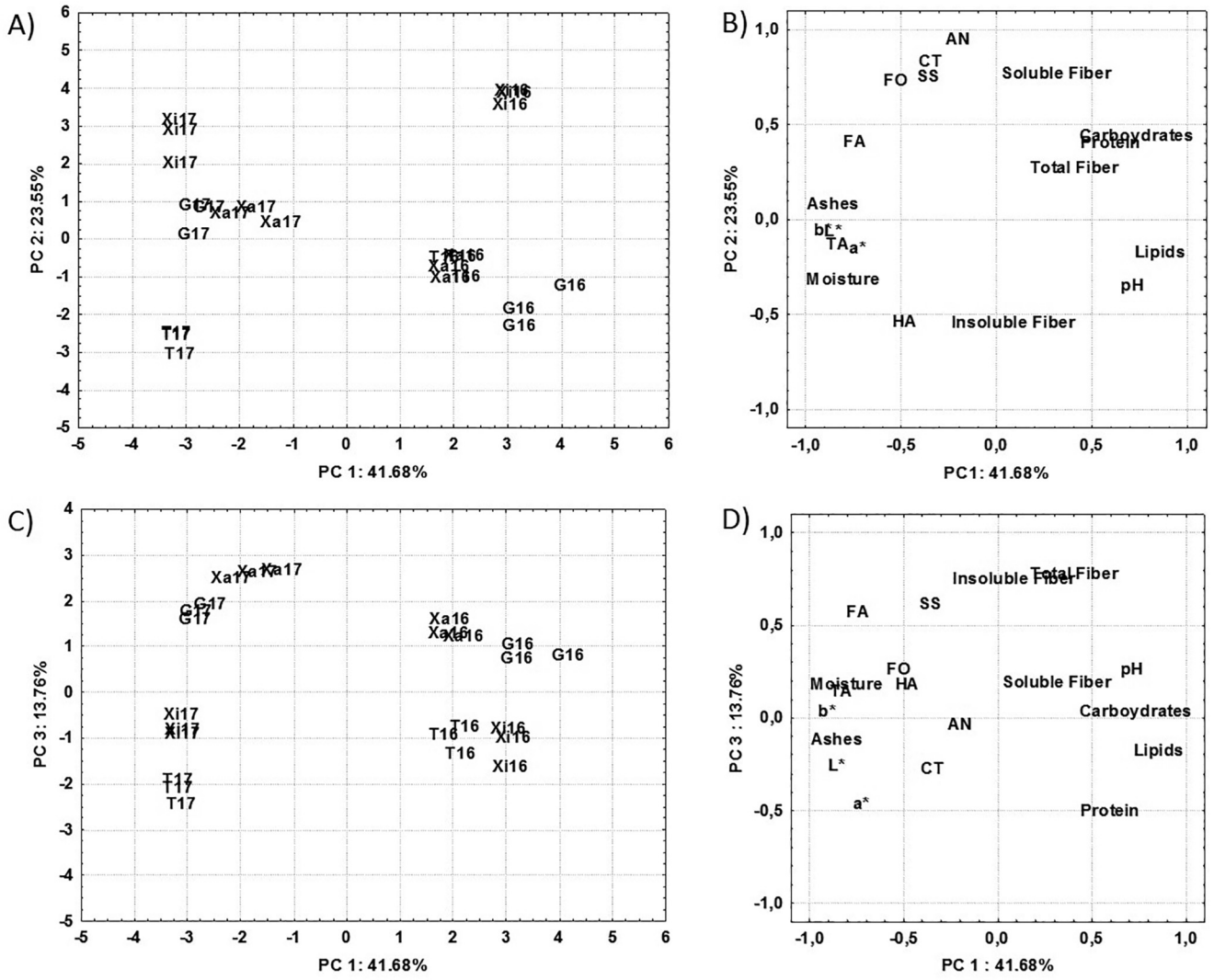

Fig. 1. Principal components analysis (PCA) of blackberries from the 2016 and 2017 harvests. T: Tupy, G: Guarani, Xa: Xavante, Xi: BRS Xingu, 16: 2016; 17: 2017, FA: Flavanols, FO: Flavonols, AN: Anthocyanins, SS: Soluble solids, TA: Titratable acid, CT: Condensed tannins, HA: Hydroxycinnamic acids. Graphs of the samples (A and C) and variables (B and D) demonstrated in the three main components of the variables studied in four different cultivars of blackberry in two years of cultivation.

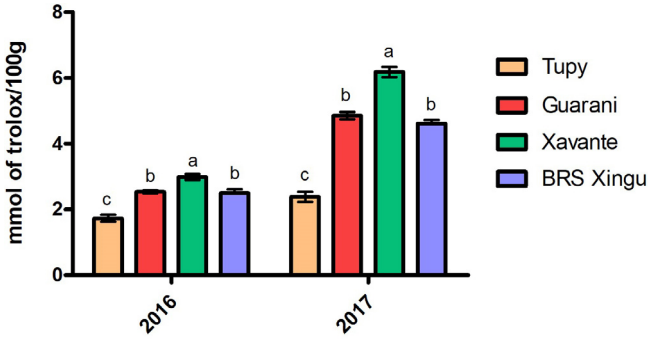

Fig. 2. *In vitro* antioxidant capacity of the blackberries in the years 2016 and 2017. The data are expressed as mmol of trolox/100 g of fresh fruit. The means followed by the same lowercase letter in the same year do not differ by the Tukey test ($p \leq 0.05$). All samples from the same cultivar in different years differ using the Student's *t*-test ($p \leq 0.05$).

Antioxidant capacity was higher in 2017 for all cultivars due to increased flavanol content, such as epicatechin and catechin, which have a synergistic effect with flavonols such as quercetin-3-glucoside, therefore increasing antioxidant capacity (Hidalgo, Sánchez-Moreno, & de Pascual-Teresa, 2010). Phenolic compound content and antioxidant capacity of the fruits is influenced by several factors, including maturation and environmental and climatic conditions that can explain the difference for compounds between different years (Acosta-Montoya et al., 2010).

3.2.2. *In vivo assay*

The production of ROS in wild-type larvae through H2DCF-DA oxidation was measured to analyze if blackberry fruits of the cultivars Tupy, Guarani, Xavante or BRS Xingu extracts can act directly by ROS scavenging. In normal conditions, the extract of Tupy, Guarani, Xavante, and BRS Xingu reduced ROS production of the animals by approximately 34.3, 48.4, 47.0, and 37.6%, respectively (Fig. 3).

The production levels of ROS under normal conditions are generated during basic cellular processes, such as electron transport chain, which is responsible for most of the adenosine triphosphate production (ATP) (Miranda-Vizuete & Veal, 2017). As in other antioxidant-rich flavonoid extracts (Kampkötter et al., 2007), the effects of blackberry cultivars observed are probably due to the antioxidant capacity of the extracts since they have similar phenolic compound concentrations.

Nevertheless, the smallest antioxidant capacity occurred with the

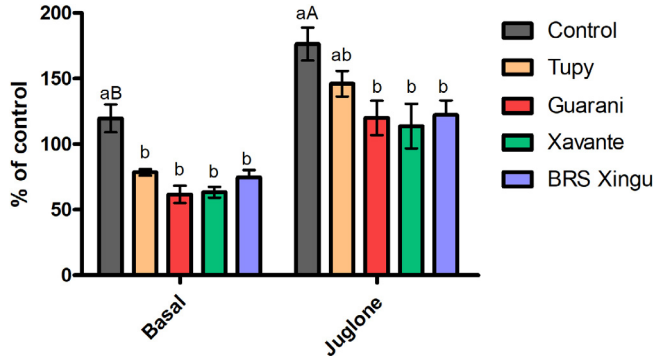

Fig. 3. Effects of four different blackberry cultivars on the production of ROS in *Caenorhabditis elegans*. N2 wild-type pre-treated with four different cultivars of blackberry extract for 1 h at 1-day-old exposed to the presence or absence of Juglone (5-hydroxy-1,4-naphthalenedione) for 1 h. The experiment was performed in triplicate. The data are expressed as percent of control. For each pre-treatment, the same lowercase letters in a same treatment do not differ from each other. Different uppercase letters in the controls show significant differences between each other. Two-way ANOVA followed by Tukey's Post Test.

Tupy cultivar when exposed to oxidant stressors, which is due to the lower antioxidant capacity, also observed in *in vitro* experiments (Fig. 2) since it presented fewer phenolic compounds. Antioxidant effects of isolated flavonoids such as myricetin, quercetin, and kaempferol, were already observed in *C. elegans,* although at higher concentrations (Grünz et al., 2012).

With the exposure to oxidant stressors, other extracts that have antioxidant activity such as *Ilex paraguariensis* assist in detoxifying these reactive species, protecting against oxidative stress (Machado et al., 2018). To analyze whether the Tupy, Guarani, Xavante, and BRS Xingu extracts were able to protect against induced oxidative stress, wild-type worms extracted in the pre-treatment were exposed to juglone, which is a reactive oxygen species generator (Blum & Fridovich, 1983). Juglone, in the concentration of 50 μM, increased ROS production by 47.3% when compared with the control basal group (Fig. 3).

The worms that were pre-treated for 1 h with the different cultivars, Guarani, Xavante, and BRS Xingu, were capable of preventing higher ROS production generated by juglone. Guarani, Xavante, and BRS Xingu presented 31.4, 35.4, and 30.6% less ROS production, respectively, when compared with the juglone control group (Fig. 3).

Despite this, the pre-treatment with Tupy was not effective in preventing the increased ROS levels of acute juglone-induced oxidative stress. These results suggest that the pretreatment with Tupy was not effective in preventing the higher ROS generated by juglone. Apparently, Tupy only exerts antioxidant activity in basal-stress situations.

Nevertheless, the cultivar BRS Xingu showed protective activity against ROS with and without the presence of the oxidant stressors, which proves the protective activity of the compounds of the new cultivar against ROS.

4. Conclusion

This study presents a new blackberry cultivar that stands out due to higher anthocyanin and condensed tannin content. Thus, in addition to the high productivity reported for the new cultivar, BRS Xingu presents higher characteristics to other commercial cultivars as to the Tupy cultivar as the higher content of soluble solids, flavanols, antioxidant capacity *in vitro* and protection *C. elegans* from ROS generation. Therefore, BRS Xingu is a new option of blackberry available with potential for industrialization, *in natura* consumption, and potential benefits associated with phenolic compound consumption.

CRediT authorship contribution statement

Débora P. Moraes: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Project administration. **Jesús Lozano-Sánchez:** Methodology, Validation, Formal analysis, Investigation, Writing - review & editing. **Marina L. Machado:** Methodology, Validation, Formal analysis, Investigation, Writing - original draft. **Márcia Vizzotto:** Conceptualization, Resources, Writing - review & editing, Funding acquisition. **Micheli Lazzaretti:** Methodology, Investigation, Validation. **Francisco Javier J. Leyva-Jimenez:** Methodology, Investigation, Validation. **Tássia L. da Silveira:** Methodology, Investigation, Validation. **Edi F. Ries:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - review & editing, Supervision, Funding acquisition. **Milene T. Barcia:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126783.

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