Ciência Rural, Santa Maria, v.53:12, e20220450, 2023 http://doi.org/10.1590/0103-8478cr20220450
ISSNe 1678-4596
FOOD TECHNOLOGY

# Physicochemical characterization, phenolic composition and antioxidant activity of genotypes and commercial cultivars of blueberry fruits

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**ABSTRACT**: The physicochemical characterization, antioxidant potential and phenolic composition of fruits of six genotypes (BB3, BB4, BB6, PW1, PW2 and PW5) and seven commercial cultivars (Bluebelle, Bluegem, Briteblue, Climax, Delite, Powderblue and Woodard) were carried out of blueberry, from the 2019/2020 production cycle. Color, soluble solids content, pH, titratable acidity, antioxidant activity (DPPH and ABTS), total phenolic compounds, total flavonoids, total anthocyanins and individual anthocyanins were analyzed. In general, all genotypes and cultivars are rich in phytochemicals. The genotypes PW1, PW2, PW5, BB3 and the cultivars Bluegem, Briteblue, Climax and Delite stand out. Thus, the blueberry genotypes in this study are considered as promising for the food industry and also for fresh consumption as commercial cultivars already consolidated in the fruit market, in view of the excellent phenolic composition present in these small fruits.
**Key words**: *Vaccinium ashei* Reade, genetic variability, small fruits, phytochemicals, anthocyanins.

# Caracterização físico-química, composição fenólica e atividade antioxidante de genótipos e cultivares comerciais de frutos de mirtileiro

**RESUMO**: Fez-se a caracterização físico-química, potencial antioxidante e composição fenólica de frutos de seis genótipos (BB3, BB4, BB6, PW1, PW2 e PW5) e sete cultivares comerciais (Bluebelle, Bluegem, Briteblue, Climax, Delite, Powderblue e Woodard) de mirtileiro, oriundos do ciclo de produção 2019/2020. Analisou-se a cor, teor de sólidos solúveis, o pH, a acidez titulável, atividade antioxidante (DPPH e ABTS), compostos fenólicos totais, flavonoides totais, antocianinas totais e antocianinas individuais. De modo geral, todos os genótipos e cultivares são ricos em compostos fitoquímicos. Como destaque, estão os genótipos PW1, PW2, PW5, BB3 e as cultivares Bluegem, Briteblue, Climax e Delite. Assim, os genótipos de mirtileiro do presente estudo, são considerados tão promissores para a indústria alimentícia e também para o consumo *in natura* quanto as cultivares comerciais já consolidadas no mercado da fruticultura, tendo em vista a excelente composição fenólica presente nestes pequenos frutos.
**Palavras-chave**: *Vaccinium ashei* Reade, variabilidade genética, pequenos frutos, antioxidantes, fenólicos, antocianinas.

## INTRODUCTION

Blueberries are fruits of the *Vaccinium* genus, belonging to the Ericaceae family, which have been widely studied (WANG et al., 2015), due to their economic (TAN et al., 2018), nutritional (SCALZO et al., 2013; UPADHAYA & DWIVEDI, 2019) and functional (GALLARDO et al., 2018; KRAUJALYTĖ et al., 2015) importance. Due to their small dimensions (approximately 1.0 to 1.8 cm in diameter and average mass ranging from 1.0 to 2.2 g), blueberries are often called small fruits (FREIRE, 2004), and due to their phytochemical richness, they are also called "superfruits" (STEVENSON & SCALZO, 2012). Thus, the "small superfruits" appear.

Blueberry production stands out in countries like the United States and Canada, the world's largest producers (CANTUARIAS-AVILÉS et al., 2014). In Brazil, the production of blueberry on a commercial scale started approximately in the 1990s (MORAES et al., 2007) and it is estimated that the production grew reaching approximately 400

Received 08.09.22 Approved 02.21.23 Returned by the author 04.18.23
CR-2022-0450.R1
Editors: Leandro Souza da Silva Mara Fernandes Moura

ha in 2014 although, there are no updated official statistics (CANTUARIAS-AVILÉS et al., 2014). This production takes place mostly in regions with sub-tropical and temperate climates or microclimates (PERTUZATTI et al., 2021). The most expressive cultivars are from the rabbiteye (*Vaccinium ashei* Reade) and high bush (*Vaccinium corymbosum* L.) group (PERTUZATTI et al., 2016). Among them, those belonging to the high bush group are more demanding of chill hours for full production, needing around 650 to 800 hours, while the cultivars from the rabbiteye group are less demanding at low temperatures, budding and flowering well with only 360 chill hours (FREIRE, 2004), due to its rusticity, thus providing excellent adaptation to temperate climate conditions, favoring plant growth and development; consequently, generating fruits of high nutritional and commercial quality (SCHUCHOVSKI et al., 2020).

In addition to their good appearance and flavor, blueberries stand out for their richness in nutritionally and functionally important compounds (CONNOR et al., 2002; PRIOR et al., 1998). There is a consensus that these are fruits rich in phenolic compounds, especially phenolic acids, flavonoids and anthocyanins (GAVRILOVA et al., 2011; MARTÍN-GÓMEZ et al., 2020; PERTUZATTI et al., 2016). As a result, these fruits are characterized as a significant source of antioxidants (STEVENSON & SCALZO, 2012), either consumed in processed or fresh form (GOLDMEYER et al., 2014).

Several studies addressing chemical composition, antioxidant potential, and nutritional and functional characteristics, *in vitro* and *in vivo*, point to the qualitative potential of blueberries in human nutrition (BELL et al., 2017; DEBNATH-CANNING et al., 2020; GIACALONE et al., 2011; GÜNDÜZ et al., 2015; HUANG et al., 2016; KLIMIS-ZACAS et al., 2016; LI et al., 2017; LIN et al., 2020; MCANULTY et al., 2019; MIRAGHAJANI et al., 2020; SUN et al., 2020; TRAVICA et al., 2020; ZHU et al., 2017). For this reason, associated with production cost, it makes blueberries *in natura*, or in their processed form into juices, pulp, creams, ice cream, cookies, extracts and others, fruits of international relevance.

Although; most studies highlights benefits to human health, the consolidated knowledge on the subject was built by inductive and deductive strategies, based on studies that reported that there really is interference with *in vivo* experiments (BELL et al., 2017; HUANG et al., 2016; KLIMIS-ZACAS et al., 2016; MCANULTY et al., 2019; MIRAGHAJANI et al., 2020; ZHU et al., 2017). In these studies, it was observed that the consumption of blueberries contributes to attenuating the occurrence of some chronic diseases, such as neurodegenerative (TRAVICA et al., 2020), diabetes (BELL et al., 2017) and cardiovascular (HUANG et al., 2016; KLIMIS-ZACAS et al., 2016; MCANULTY et al., 2019; ZHU et al., 2017). These benefits have been attributed to compounds such as polyphenols, particularly due to the presence of anthocyanins (LI et al., 2017).

When identifying the main phytochemicals in blueberries, it was observed that the most frequent and in the highest concentrations are phenolic acids, including gallic, caffeic, ferulic, syringic, chlorogenic and hydroxybenzoic acid (FIGUEIRA et al., 2016; SKREDE et al., 2000; WANG et al., 2012a), flavonoids such as catechin, epicatechin, quercetin, myricetin and kaempferol (PERTUZATTI et al., 2021) and anthocyanins, which are derived from five major anthocyanidins, such as delphinidin, cyanidin, petunidin, peonidin and malvidin (PERTUZATTI et al., 2016). Furthermore, it has been shown that there are significant variations between groups, cultivars and in places where blueberries are planted (SPINARDI et al., 2019), both in terms of agronomic and phytochemical behavior (MIKULIC-PETKOVSEK et al., 2012). Also, it has been shown that the composition varies when the same genotype is cultivated in different regions (SKROVANKOVA et al., 2015).

Thus, descriptive researches that reveal the composition of universal cultivars in each production region become relevant (GÜNDÜZ et al., 2015; PERTUZATTI et al., 2021; SCALZO et al., 2015; SPINARDI et al., 2019; WANG et al., 2017; YOUSEF et al., 2013; ZENG et al., 2020; ZHANG et al., 2020). Thus, in the studies mentioned above, it was observed that when analyzing several cultivars of blueberry in different countries, significant differences were obtained in the phenolic composition of these fruits. Although, this is mostly a descriptive research, it is hypothesized that the blueberries of all cultivars and genotypes studied have phytochemical richness, regardless of their genetic particularities (WANG et al., 2012b). Therefore, this study analyzed and compared the phenolic composition and antioxidant activity of fruits from six selected genotypes, in relation to seven commercial blueberry cultivars.

## MATERIALS AND METHODS

*Origin, collection and storage of samples*

The fruits used in the study come from a hatchery formed by seven 18-year-old commercial

cultivars (Bluebelle, Bluegem, Briteblue, Climax, Delite, Powderblue and Woodard) and six 13-year-old genotypes (BB3, BB4, BB6, PW1, PW2 and PW5), from an experimental area, located in the third district of Pelotas, RS, at 31º 33' 4.13" S, 52º 23' 54.13" W and 120 m altitude. Two of these being 'Bluebelle' and 'Powderblue' used for the extraction of seeds that originated the genotypes, through free pollination in a previous study, selected through mass selection, in an initial population composed of 3.554 propagated plants, implanted in a area composed of five more cultivars (Bluegem, Briteblue, Climax, Delite and Woodard) being therefore, also their possible parents and objects of this study.

During the 2019/20 production cycle, in the middle of the first half of December, approximately 1 kg of fruits were harvested in three clones of each cultivar and in the genotypes, in full maturation stage, characterized by the dark blue color of the epidermis, inside of properly identified plastic bowls, with 100 g of sample destined for the performance of physical-chemical analyzes (color, pH, titratable acidity, soluble solids), which were separated by three repetitions. For the other analyzes of the study (antioxidant activity by means of DPPH and ABTS radicals, total and individual monomeric anthocyanins, total phenolic compounds and total flavonoids) 200 g of sample were separated per repetition, which were placed in polyethylene packaging (0.10 microns), stored in an ultrafreezer; subsequently, lyophilized in a lyophilizer (Liobrás - L101) and stored in Falcon tubes with a capacity of 45 mL.

*Instrumental determination of color*

For the instrumental determination of the color, a colorimeter (CR300, Minolta Chromamater) was used, through the CIELab color system. The parameters evaluated were $a^*$ and $b^*$, where the hue angle h starts on the $+a^*$ axis and is given in degrees; 0 would be $+a^*$ (red), 90 would be $+b^*$ (yellow), 180 would be $-a^*$ (green) and 270 would be $-b^*$ (blue). The following equation ($^o$Hue=tan -1 $b^*/a^*$) was used to calculate the Hue angle, which indicates the observed color. The tests were carried out with three repetitions, and in each repetition three fruits were used.

*pH and titratable acidity*

The pH was determined from 1 g of sample previously macerated in a 100 mL beaker, 40 mL of distilled water was added, the content was then stirred until the particles were uniformly suspended and then the pH was determined by potentiometry in a pH meter (K392014B, Kasvi®). To evaluate the titratable acidity (TA), the volumetric method with 0.1 M NaOH was used, 1 g of sample was weighed and 40 mL of distilled water was added, stirred and the pH of the sample was observed. Afterwards, the sample was titrated until pH 8.2 was reached. Three repetitions were performed for each variable and the results were expressed in mg of malic acid per 100 g of wet mass sample, according to the adapted method proposed by Instituto Adolfo Lutz (2008).

*Soluble solids*

The soluble solids (SS) content was determined according to the Instituto Adolfo Lutz (2008), at 20 °C, using 1 drop of pure juice in a digital refractometer (PR-32α, Atago®), and the results expressed in °Brix.

*SS/AT ratio*

Determined by the ratio between the two constituents (soluble solids and titratable acidity).

*Antioxidant activity by capturing the DPPH radical*

To determine the antioxidant potential, the method by Brand-Williams et al. (1995) adapted, using the 2,2-diphenyl1-picrylhydrazyl (DPPH) free radical. The quantification was performed in a 96 wells microplate. The absorbance at 515 nm of the DPPH working solution was adjusted to 1.1 ± 0.02. For the reaction, 20 µL of the prepared extract and 280 µL of the use solution were used. The absorbance reading was performed at a 515 nm wavelength in a spectrophotometer (6705 UV/Vis; Jenway®). Methanol was used as a blank. The tests were carried out with three repetitions. Antioxidant content was expressed as trolox equivalents (µmol Eq Trolox $100g^{-1}$), in dry mass samples, according to the linear equation of trolox analytical curve (at concentrations from 10 to 200 µg $mL^{-1}$). The equation for the analytical curve was y=0.0045x + 0.0031, R2= 0.9948, where "y" is the absorbance and "x" is the concentration as trolox equivalents.

*Antioxidant activity by ABTS free radical capture*

Antioxidant capacity was determined according to the method described by Re et al. (1999), by capturing the 2,2-azino-bis(3-ethylbezothiazoline)-6-sulfonic acid radical. A stock solution of ABTS was made (192 mg of ABTS in 50 ml of distilled water). Afterwards, the potassium persulfate solution was prepared, adding 378.4 mg of potassium persulfate in distilled water to complete the volume to 10 mL in a volumetric flask. Subsequently, a use solution was prepared, adding 5 mL of

stock solution and 88 µL of potassium persulfate solution. Quantification was performed in a 96 wells microplate. The absorbance at 734 nm of the ABTS working solution was adjusted to 0.70 nm ± 0.05. For the reaction, 20 µL of the prepared extract and 280 µL of the use solution were used. The samples were vortexed for 30 sec and then allowed to react for 30 min in the dark at room temperature. Ethanol was used as blank. The tests were carried out with three repetitions. The quantification of the antioxidant content was expressed as trolox equivalents (µmol Eq Trolox $100g^{-1}$), in dry mass samples, according to the linear equation of the analytical trolox curve (at concentrations from 10 to 250 µg $mL^{-1}$). The equation for the analytical curve was y=0.0061x + 0.0176, R2= 0.9923, where "y" is the absorbance and "x" is the concentration as trolox equivalents.

*Total phenolic compounds*

The content of total phenolic compounds was determined according to Singleton & Rossi (1965). For extraction, 200 mg of sample were weighed, diluted in 20 mL of P.A. methanol and shaken in Ultra-turax (T18, IKA) at 12,000 rpm for 1 min. Subsequently, the extract was centrifuged (Centrifuge RC5C, Sorvall Instruments) for 15 min at 6,000 rpm. The supernatant was collected into new Falcon tubes and stored in a freezer at -20 °C. For the reaction, 15 µL of the extract, 240 µL of distilled water, 15 µL of Folin Ciocalteau 0.25 N were added, then vortexed for 10 s and left in the dark for 3 min for the reaction to occur. Afterwards, 30 µl of 1 N Na2CO3 were added and left for 2 h in the dark. The tests were carried out with three repetitions. Quantification was performed in a 96 wells microplate. Samples were read using a spectrophotometer (6705 UV/Vis; Jenway®) at a wavelength of 725 nm. The phenolic content was expressed as gallic acid equivalents (mg $100g^{-1}$ gallic acid), in dry mass samples, according to the linear equation of the analytical curve for gallic acid (at concentrations from 10 to 150 µg $mL^{-1}$). The equation for the analytical curve was y=0.0045x + 0.0715, R2= 0.998, where "y" is the absorbance and "x" is the concentration as gallic acid equivalents.

*Total flavonoids*

The content of total flavonoids was determined by the method proposed by Zhishen et al. (1999). Quantification was performed in a 96 wells microplate. For the reaction, 30 µL of the extract, 120 µL of distilled water were added and vortexed for 10s. Afterwards, 9 µL of 10% NaCO2 (m/v) was added, stirred again for 10 s and waited 5 min for the reaction to occur. Subsequently, 9 µL of 20% AlCl3 (m/v) were added, the solution was vortexed for 10 s and the reaction was left for 6 min. Then, 60 µL of 1 M NaOH and 72 µL of distilled water were added. The tests were carried out with three repetitions. Samples were read using a spectrophotometer at a wavelength of 510 nm. Flavonoid content was expressed as catechin equivalents (catechin mg $100g^{-1}$), in dry mass samples, according to the linear equation of the catechin analytical curve (at concentrations from 10 to 200 µg $mL^{-1}$). The equation for the analytical curve was y=0.0024x + 0.0064, R2= 0.9969, where "y" is the absorbance and "x" is the concentration as catechin equivalents.

*Total monomeric anthocyanins*

For the quantification of total anthocyanins, the method proposed by Lee & Francis (1972) was followed, where 950 µL of acidified ethanol at pH 1.0 were added to 50 µL of sample. The solution was vortexed for 1 min, every 15 min, for one hour, and then centrifuged (Centrifuge RC5C, Sorvall Instruments) for 15 min at 5,000 rpm, at 5 ºC. The process was carried out in Falcon tubes wrapped with aluminum foil to avoid contact with light. The reaction was carried out in a 96 wells microplate. The tests were carried out with three repetitions. After centrifugation, the supernatant was read in a spectrophotometer with a wavelength of 520 nm. The content of total anthocyanins was expressed as pelargonidin equivalents (mg $100g^{-1}$ pelargonidin), in dry mass samples, according to the linear equation of the analytical curve of pelargonidin (at concentrations from 2.50 to 50 µg $mL^{-1}$). The equation for the analytical curve was y=0.0782x + 0.0494, R2= 0.9998 where "y" is the absorbance and "x" is the concentration as pelargonidin equivalents.

*Chromatographic analysis of individual anthocyanins by HPLC-MS*

*Preparation of extracts*

For the determination of individual anthocinins, 10 g of fruit were used, then crushed and mixed with 15 mL of a 2% methanol/TFA solution in water (10:90, v/v) and homogenized by an Ultra-Turrax 9.900 xg (IKA-Werke, Staufen, D) for 1 min. The homogenate was extracted for 30 min under stirring in the dark at room temperature. The suspension was centrifuged at 1.000×g for 10 min at 4 °C, and the supernatant was recovered. The residue was extracted again until the red color disappeared (4 x 15 ml) with a solution of methanol/2% TFA in water (10:90, v/v) and treated as described above. The supernatants were pooled

and the volume was adjusted to 200 mL by a 2% TFA solution in water, according to the method proposed by SIEBENEICHLER et al. (2020).

*Instrumentation and analytical conditions*

For injection, 100 µL of this extract was diluted in 800 µL of HPLC grade methanol (Sigma-Aldrich, St. Louis, MO, USA) and then filtered through a 0.45 µM membrane. With the sample ready, 10 µL of it was injected into an ultra high-efficiency liquid chromatograph (UFLC, Shimadzu, Japan) coupled to a high-resolution quadrupole-time-of-flight mass spectrometer (Maxis Impact, Bruker Daltonics, Bremen, Germany). For the chromatographic separation, the C18 pre-column (2.0 x 4.0 mm) and the C18 Luna column (2.0 x 150 mm, 100 Å, 3.0 µm) (Phenomenex Torrance, CA, USA) were used). The separation process was carried out with the use of two mobile phases (eluents) to promote the interaction with the samples and consequently carry out the chromatographic separation. The mobile phases were: water with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B). The separation process lasted 30 minutes for each sample and the elution gradients used were: 0–2 min, 10% B; 2-15 min, 10-75% B; 15-18 min, 90% B; 18-21 min 90%B; 21-23 min, 10% B and 23-30 min, 10% B. A flow rate of 0.2 ml.min-1 and column temperature of 40 °C were kept constant. The mass spectrometer was operated in positive ESI mode (anthocyanins), having collected spectra in a mass range from m/z 50 to 1200, with capillary voltage at 3.5 kV, nebulization gas pressure (N2) at 2 bar, drying gas at 8 L.min-1, source temperature at 180 ºC, RF collision at 150 Vpp, transfer at 70 mS and prepulse storage at 5 mS. The equipment was calibrated with 10mM sodium formate, covering the acquisition range from m/z 50 to 1200. Automatic MS/MS experiments were performed by adjusting the collision energy values as follows: m/z 100, 15 eV; m/z 500, 35 eV; m/z 1000, 50 eV, using nitrogen as collision gas. MS and MS/MS data were processed using Data analysis 4.0 software (Bruker Daltonics, Bremen, Germany). Anthocyanins were characterized by UV/Vis spectrum (210-800 nm), and exact mass, MSn fragmentation patterns compared to equipment library data, data masses (patterns, Metlin, Mass Bank, Kegg Compound, Chem Spider), standard curve of malvidin 3-O-galactoside, and compared to isotopic standard SIEBENEICHLER et al. (2020).

*Data analysis*

Data analysis was performed using the R program (CORE TEAM, 2019). Data were analyzed using variance analysis (ANOVA) and means comparison test (Tukey's test), taking as mass significance levels greater than 95% ($P \leq 0.05$). In addition, to calculate the correlation coefficient between the results, the Person correlation test was applied.

## RESULTS

*pH, titratable acidity, soluble solid, SS/TA ratio and instrumental determination of color*

Blueberries were characterized as acidic fruits, with pH ranging from 3.23 to 3.76 and total acidity ranging from 0.23 to 0.73 (Table 1). The PW1 genotype was distinguished by having the highest pH (3.76) and the lowest acidity (0.23 mg $100g^{-1}$ malic acid). Similarly, blueberries are characterized by having relatively high contents of soluble solids, which in the present study ranged from 13.33 to 16.89 ºBrix, with Climax being the cultivar with the highest content and the lowest content being the PW1 genotype. Regarding the variable SS/TA, there was great variation between the values (4.56 to 16.83), where the PW1 genotype had a higher value and the cultivar Briteblue had a lower value in the ratio between the two constituents. Finally, the color was similar for all fruits, with no significant differences between cultivars and genotypes, as all were at full maturation stage, characterized by the dark blue color of the fruits.

*Phenolic compounds, flavonoids and total anthocyanins*

In relation to the total phenolic compounds, the blueberries showed excellent amounts of these phytochemicals, as expected, and the contents ranged from 5.00 to 5.87 mg $100g^{-1}$ of gallic acid, especially for the PW1 genotype and for the majority of the samples, which were significantly higher ($P \leq 0.05$), with the exception of the BB4 genotype, which conferred lower phenolic content (Table 2). However, for total flavonoids, the contents ranged from 5.80 to 10.31 mg $100g^{-1}$ of catechin, obtaining statistically similar values in most cultivars and genotypes ($P \leq 0.05$), while the lowest value was verified by BB4 genotype. In the present study, the anthocyanin contents differed between 6.14 and 11.71 mg $100g^{-1}$ of pelargonidin, with emphasis on the PW5 genotype, which presented the highest value and the lowest values were obtained by 'Bluebelle' and by the PW2 genotype.

*Antioxidant activity*

The levels of antioxidant activity through DPPH in blueberry fruits varied between 3.27 and

Table 1 - Physicochemical parameters (pH, TA, SS, SS/TA and color) in blueberry fruits from seven commercial cultivars and six genotypes.

| Cultivars/Genotypes | pH | TA (mg 100g$^{-1}$ malic acid) | SS (Brix) | SS/TA | Color (°Hue) |
|---|---|---|---|---|---|
| Bluebelle | 3.31±0.15 b | 0.51±0.06 abc | 15.97±0.10 ab | 6.55±0.96 c | 288.44±0.64$^{ns}$ |
| Bluegem | 3.32±0.03 b | 0.72±0.09 a | 15.51±0.63 abc | 4.67±0.56 c | 286.30±3.65$^{ns}$ |
| Briteblue | 3.23±0.12 b | 0.73±0.09 a | 15.62±0.44 abc | 4.56±0.74 c | 283.96±1.04$^{ns}$ |
| Climax | 3.28±0.04 b | 0.67±0.08 ab | 16.89±0.42 a | 4.95±0.64 c | 293.62±7.16$^{ns}$ |
| Delite | 3.26±0.15 b | 0.70±0.08 a | 14.79±0.21 bcde | 4.73±0.75 c | 289.08±0.53$^{ns}$ |
| Powderblue | 3.27±0.04 b | 0.70±0.13 ab | 15.08±0.32 bcd | 4.82±0.86 c | 289.05±1.49$^{ns}$ |
| Woodard | 3.28±0.10 b | 0.64±0.08 ab | 15.53±0.45 abc | 5.18±0.80 c | 286.89±1.50$^{ns}$ |
| PW1 | 3.76±0.07 a | 0.23±0.05 d | 13.33±0.12 f | 16.83±4.52 a | 290.06±0.84$^{ns}$ |
| PW2 | 3.38±0.06 b | 0.55±0.14 abc | 14.54±1.04 cdef | 6.46±1.49 c | 290.16±24.34$^{ns}$ |
| BB3 | 3.35±0.02 b | 0.29±0.10 cd | 14.07±0.38 def | 12.31±3.49 ab | 291.71±1.25$^{ns}$ |
| BB4 | 3.31±0.08 b | 0.43±0.03 bcd | 14.89±0.40 bcde | 7.64±0.53 bc | 289.92±3.09$^{ns}$ |
| PW5 | 3.25±0.04 b | 0.57±0.03 ab | 14.87±0.62 bcde | 5.75±0.33 c | 284.37±4.65$^{ns}$ |
| BB6 | 3.35±0.09 b | 0.59±0.13 ab | 13.52±0.21 ef | 5.88±1.27 c | 305.41±5.39$^{ns}$ |
| CV (%) | 2.62 | 15.91 | 3.18 | 25.55 | 2.58 |

Results expressed as means of three repetitions ± standard deviation (mg 100g$^{-1}$ wet mass). Equal letters in the same column do not differ by Tukey's test (P ≤ 0.05). CV (%): coefficient of variation. ns: not significant by the F test (P ≤ 0.05) of the analysis of variance.

6.65 µMol Trolox 100g$^{-1}$, giving superiority to the PW1 genotype and the 'Bluegem' cultivar (Table 3). However, when using the ABTS protocol, the antioxidant contents were found in the range between 28.31 to 46.23 µMol Trolox 100g$^{-1}$, with a statistically significant highlight (P ≤ 0.05) for 'Bluegem', 'Delite', 'Woodard' and BB3 genotype. It is also evident in this study that the BB4 genotype had a lower content of antioxidants in the two protocols used (3.27 and 28.31 µMol Trolox 100g$^{-1}$).

*Individual anthocyanins by HPLC-MS*

In the present study, five anthocyanins from three groups of anthocyanidins (cyanidin,

Table 2 - Total phenolic compounds, total flavonoids and total anthocyanins in blueberry fruits from seven commercial cultivars and six genotypes.

| Cultivars/Genotypes | Total Phenolics (mg 100g$^{-1}$ gallic acid) | Total Flavonoids (mg 100g$^{-1}$ catechin) | Total Anthocyanins (mg 100g$^{-1}$ pelargonidin) |
|---|---|---|---|
| Bluebelle | 5.63±0.21 ab | 8.20±0.77 bc | 6.48±0.31 e |
| Bluegem | 5.58±0.28 ab | 10.06±0.52 a | 7.95±0.88 cde |
| Briteblue | 5.66±0.61 ab | 9.47±0.19 ab | 8.86±0.52 bc |
| Climax | 5.59±0.30 ab | 8.81±0.36 ab | 10.11±0.69 ab |
| Delite | 5.39±0.01 ab | 8.74±0.39 ab | 8.66±1.14 bcd |
| Powderblue | 5.84±0.06 ab | 9.62±0.45 ab | 7.76±0.74 cde |
| Woodard | 5.63±0.07 ab | 9.07±0.61 ab | 8.76±1.00 bc |
| PW1 | 5.87±0.11 a | 10.31±0.59 a | 9.38±0.47 bc |
| PW2 | 5.51±0.09 ab | 10.03±0.47 a | 6.14±0.81 e |
| BB3 | 5.17±0.41 ab | 9.72±0.54 ab | 9.05±0.37 bc |
| BB4 | 5.00±0.39 b | 5.80±0.48 d | 7.38±0.43 cde |
| PW5 | 5.78±0.13 ab | 8.96±0.83 ab | 11.71±0.79 a |
| BB6 | 5.28±0.34 ab | 7.04±0.49 cd | 6.60±0.41 de |
| CV (%) | 5.19 | 6.04 | 8.43 |

Results expressed as means of three repetitions ± standard deviation (mg 100g$^{-1}$ dry mass). Equal letters in the same column do not differ by Tukey's test (P ≤ 0.05). CV (%): coefficient of variation.

Table 3 - Antioxidant activity in blueberry fruits, from seven commercial cultivars and six genotypes.

| Cultivars/Genotypes | DPPH (µMol Trolox 100g$^{-1}$) | ABTS (µMol Trolox 100g$^{-1}$) |
|---|---|---|
| Bluebelle | 4.44±0.05 de | 39.02±0.77 def |
| Bluegem | 6.05±0.18 ab | 46.23±0.63 a |
| Briteblue | 5.67±0.11 bc | 42.14±0.36 bcd |
| Climax | 5.33±0.12 bc | 40.69±0.97 cde |
| Delite | 5.10±0.55 cd | 43.27±0.93 abc |
| Powderblue | 5.49±0.35 bc | 38.15±0.73 ef |
| Woodard | 5.18±0.23 cd | 43.35±0.33 abc |
| PW1 | 6.65±0.27 a | 42.14±0.80 bcd |
| PW2 | 5.69±0.03 bc | 35.74±1.59 f |
| BB3 | 5.05±0.23 cde | 44.65±1.41 ab |
| BB4 | 3.27±0.21 f | 28.31±2.95 g |
| PW5 | 5.52±0.40 bc | 41.58±1.03 bcde |
| BB6 | 4.23±0.39 e | 36.44±0.39 f |
| CV (%) | 5.38 | 2.98 |

Results expressed as means of three repetitions ± standard deviation (mg 100g$^{-1}$ dry mass). Equal letters in the same column do not differ by Tukey's test ($P \leq 0.05$). CV (%): coefficient of variation.

malvidin and delphinidin) were identified (Table 4). When quantifying the malvidin-3-O-glycoside content, the results differed between 18.33 to 63.00 mg 100g$^{-1}$ of pelargonidin, where 'Bluebelle' and 'Bluegem' had a statistically higher value ($P \leq 0.05$) and 'Climax' with lower value. In relation to malvidin-3-O-galactoside, there was no significant difference between cultivars, suggesting that they had a high content of this anthocyanidin.

Likewise, for cyanidin-3-O-glycoside, the values ranged from 12.00 to 22.33 mg 100g$^{-1}$ of malvidin, evidencing 'Briteblue', 'Climax' and 'Delite' with significantly higher values ($P \leq 0.05$) in relation to the other cultivars. Simultaneously, when analyzing the content of cyanidin-3-O-galactoside, the values differed between 13.67 to 71.67 100g$^{-1}$ of malvidin, where 'Briteblue' and 'Climax' stood out with the highest values.

However, for delphinidin-3-O-galactoside, the values obtained are between 9.33 and 34.67 mg 100g$^{-1}$ of malvidin, inferring that 'Briteblue' and 'Climax' presented a high content of this anthocyanidin.

## DISCUSSION

As previously verified, the PW1 genotype stood out in the pH and acidity parameters and the attributed results occurred as expected, as it has unique characteristics, and the high pH value reported, as well as the lower acidity, is justified by the genetic differences existing in this plant, which interfere with the quality parameters of the fruits (SPINARDI et al., 2019).

However, for soluble solids, it was seen that the commercial cultivar Climax verified a high content of this constituent. Thus, when comparing this result with other studies, it is observed that it similarly gained prominence in relation to soluble solids in relation to other cultivars belonging to the rabbiteye group (PERTUZATTI, 2009; RADÜNZ et al., 2014). Possibly, the high content reported by 'Climax' can be justified by the climatic conditions of the city of Pelotas (RS), as during the 2019/20 cycle there was low rainfall, according to data presented by Embrapa (2019), and with that, higher concentration of soluble solids may occur in the berries.

As for the SS/TA ratio, it was observed that the PW1 genotype conferred a good relationship between the two constituents, which is essential, as it represents the balance between sugars and acids, in addition to being related to the maturation index and being fundamental for the contribution of fruit flavor (ALMUTAIRI et al., 2017). The physicochemical parameters presented are ideal for small fruits, as the blueberries must have a SS content greater than 10 °Brix, pH between 2.25 and 4.25, TA between 0.3 and 1.3 mg 100g$^{-1}$ and the SS/TA ratio between 10 and 33 (SAFTNER et al., 2008), and these results were obtained in the vast majority of samples in the present study, thus suggesting that the evaluated fruits have good quality.

In the coloration, no differences were observed between the samples, however, it is essential to emphasize that the color is a fundamental physical characteristic, as it is responsible for the classification of the fruit maturation stage, which is an important characteristic for commercialization, in addition to being directly related to the content of anthocyanins (LOBOS et al., 2018).

With regard to total phenolic compounds, superiority was observed in most genotypes and cultivars and, based on this, other authors, when working with selections and cultivars of blueberry from the rabbiteye group, reported similar values for total phenolics (VIZZOTTO et al., 2013). However, it was observed in other studies, with cultivars belonging to the highbush group, a lower content of phytochemicals compared to the rabbiteye group (AKŠIĆ et al., 2019; GÜNDÜZ et al., 2015) and therefore, the data presented above are fundamental for

Table 4 - Quantification of individual anthocyanins (malvidin-3-O-glycoside; malvidin-3-O-galactoside; cyanidin-3-O-glycoside, cyanidin-3-O-galactoside; delphinidin-3-O-galactoside) in blueberry fruits, from seven commercial cultivars.

| Cultivars | Malvidin-3-O-glycoside | Malvidin-3-O-galactoside | Cyanidin-3-O-glycodide | Cyanidin-3-O-galactoside | Delphinidin-3-O-galactoside |
|---|---|---|---|---|---|
| | ----------(mg 100 $g^{-1}$ malvidin)---------- | | | | |
| Bluebelle | 61.33±5.03 a | 103.33±11.71$^{ns}$ | 16.33±1.15 bc | 13.67±1.53 c | 10.33±1.53 b |
| Bluegem | 63.00±4.36 a | 108.00±4.58$^{ns}$ | 15.00±1.00 bc | 18.33±3.05 bc | 11.67±1.15 b |
| Briteblue | 20.33±2.08 c | 111.33±8.5$^{ns}$ | 22.33±3.05 a | 71.00±2.64 a | 34.67±2.08 a |
| Climax | 18.33±2.52 c | 91.00±6.08$^{ns}$ | 21.33±2.08 a | 71.67±2.52 a | 31.33±2.08 a |
| Delite | 51.33±2.08 b | 90.33±6.66$^{ns}$ | 19.67±1.15 ab | 22.67±1.53 b | 10.00±1.00 b |
| Powderblue | 49.00±3.00 b | 94.67±6.66$^{ns}$ | 12.00±1.00 c | 21.67±2.31 b | 12.33±3.05 b |
| Woodard | 47.00±1.00 b | 103.67±6.66$^{ns}$ | 15.00±2.00 bc | 20.67±1.53 b | 9.33±2.52 b |
| CV (%) | 7.09 | 7.55 | 10.28 | 6.53 | 11.91 |

Results expressed as means of three repetitions ± standard deviation (mg 100$g^{-1}$ dry mass). Equal letters in the same column do not differ by Tukey's test ($P \leq 0.05$). CV (%): coefficient of variation. ns: not significant by the F test ($P \leq 0.05$) of the analysis of variance.

the blueberry producers in the southern region of Brazil, as the rabbiteye group cultivars adapt well in regions with few hours of cold, and as seen, their fruits have superior content in terms of phenolic compounds.

However, fluctuations can occur in both phenolic compounds and flavonoids, mainly due to structural chemical divergences directly related to the concentration of these compounds mentioned above, specifically in the configuration and number of hydroxyls present, and thus, there is a variability in their content (CAO et al., 1997). The observed differences are also directly related to some agronomic factors, including soil and management conditions, such as fertilization, solar position, pruning and irrigation of plants in the planting area (CORREIA et al., 2016; SKROVANKOVA et al., 2015).

By quantifying the total anthocyanins, it was possible to obtain a high amount of this compound both in the cultivars and in the evaluated genotypes, as expected, as they are the main polyphenols found in blueberries and possibly this group of phytochemicals is responsible for providing numerous benefits to human health (DEL RIO et al., 2010; KAUME et al., 2012; STEVENSON & SCALZO, 2012).The prominence obtained in the PW5 genotype possibly occurred due to its possible parent being 'Climax', which also conferred a high content of anthocyanins and also because it is a selection with unique genetic characteristics and, therefore, can favor the accumulation of this pigment (SPINARDI et al., 2019; WANG et al., 2012b).

Thus, the high antioxidant potential verified by the blueberries in this study is mainly due to the wide variety of phenolic compounds in their composition, which are considered excellent *in vitro* antioxidants, responsible for several beneficial potentials to human health due to their antioxidant capacity (PERTUZATTI et al., 2016; STEVENSON & SCALZO, 2012) and, as seen above, the genotypes and cultivars evaluated presented a high amount of total phenolics, essentially the PW1 genotype and, for this reason, it may have possibly stood out. However, some authors point out that the antioxidant capacity of blueberries can be altered by a number of factors, including genotype, growing region, maturity stage and post-harvest storage conditions (CONNOR et al., 2002; PRIOR et al., 1998; SPINARDI et al., 2019; ZHANG et al., 2020).

Regarding the quantification of total anthocyanins, it was possible to identify cyanidin, malvidin and delphinidin, which stand out for being the main anthocyanins reported in rabbiteye and high bush cultivars (LOHACHOOMPOL et al., 2008; SUN et al., 2012; YOUSEF et al., 2013). In this sense, malvid in was reported in the blueberry fruits of the present study, followed by cyanidin and delphinidin with greater predominance, also verified by another study, which suggested that in cultivars from the rabbiteye group there was a prevalence of non-methoxylated B-ring anthocyanidins, such as delphinidin, cyanidin and malvidin (PERTUZATTI et al., 2016).

According to some authors, the anthocyanidins cyanidin, malvidin and delphinidin stand out in cultivars belonging to the rabbiteye group (LOHACHOOMPOL et al., 2008; SUN et al., 2012; YOUSEF et al., 2013). Thus, in the present study, through the quantification of individual anthocins, malvidin was reported with a predominance in the blueberry fruits,

followed by cyanidin and delphinidin, corroborating the information contained in the literature.

However, the results obtained by the cultivars show variations in the amount of anthocyanins due to existing genetic differences, as well as the degree of maturation and size of the fruits, as observed by numerous studies (GAO & MAZZA, 1994; MOYER et al., 2002; SCALZO et al., 2013; WANG et al., 2012a; YOUSEF et al., 2013).

## CONCLUSION

Blueberry genotypes and cultivars belonging to the rabbiteye group with high functional potential were identified, with the genotypes PW1, PW2, PW5, BB3 and the cultivars Bluegem, Briteblue, Climax, Delite standing out, which conferred high phenolic composition and antioxidant activity, possibly due to unique genetic characteristics present in their structures.

Thus, the genotypes are considered as promising for the food industry and also for fresh consumption as commercial cultivars already consolidated in the fruit market, given the phytochemical richness present in this small fruit.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Universidade Federal de Pelotas (UFPel) and the Faculdade de Nutrição (FN) for the opportunity to carry out the academic master's and, especially, the Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos (PPGCTA) of the Faculdade de Agronomia Eliseu Maciel (FAEM), which made it feasible to carry out this study. Finally, the authors are grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting scholarships (code 001).

## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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