Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v45i1.61765

SCIENCE, FOOD TECHNOLOGY AND FOOD ENGINEERING

Physicochemical characteristics and bioactive compounds of three puçá (*Mouriri pusa* Gardner) varieties, an underexploited fruit from the Brazilian Cerrado

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ABSTRACT. Puçá fruits are native to the Cerrado biome yet little explored, presenting different varieties with distinct fruit peel colors. Although puçá fruits have been known to exhibit a good source of bioactive compounds, the phenolic profile of some varieties remains unknown. Based on this context, our research aimed to evaluate the chemical composition and bioactive compounds and characterize for the first time the phenolic profile in yellow puçá, brown puçá, and black puçá by high-performance liquid chromatography coupled with diode array detection (HPLC–DAD). The three puçá varieties contained considerable quantities of important food constituents, such as high concentrations of vitamin C, carotenoids and phenolic compounds. These fruits are mostly composed of phenolic acids, with *p*-coumaric acid being the major compound in all varieties, while ellagic acid was detected only in the brown puçá. Moreover, (−)-epicatechin was found only in the yellow puçá. This study is the first to report the identification of the phenolic profile in puçá. Moreover, our data indicate that the three fruit varieties present a promising chemical composition, suggesting that they may serve as potential sources of natural antioxidants. In addition, these findings can contribute to the establishment of puçá as a novel ingredient for formulations with functional claims.

Keywords: ascorbic acid; Brazilian fruit; carotenoids; HPLC–DAD; phenolic compounds.

Received on December 3, 2021.
Accepted on May 13, 2022.

Introduction

Mouriri pusa Gardner is a fruit-bearing plant belonging to the *Melastomataceae* family that produces edible fruits much appreciated by traditional communities, popularly known as puçá (Teixeira et al., 2019), which is a native fruit of the Brazilian savanna or Cerrado. The fruit is a globose berry with 1-4 seeds surrounded by fleshy pulp with a sweet taste; it is edible and considered the tastiest among all *Mouriri* species (Lorenzi, 2021). There are three popular varieties of puçá fruit based on peel color (yellow, brown, and black) (Figure 1), and data on its nutritional and functional value are scarce. Scientific literature confirms that the methanolic extract of leaves showed intense gastroprotective action and potential effect in increasing regeneration of damaged gastric mucosa with safety for human use (Andreo et al., 2006; Vasconcelos, Andreo, Vilegas, Hiruma-Lima, & Pellizzon, 2010). Furthermore, another study verified that *M. pusa* had promising immunomodulatory and antiproliferative activities in the treatment of murine breast cancer (Carli et al., 2009).

Like other Cerrado fruits, puçá has a promising composition of bioactive constituents, such as vitamin C, carotenoids and phenolic compounds (Rufino et al., 2010). These last compounds are known to present a heterogeneous group of molecules with a diversity of chemical structures and are one of the main classes of secondary metabolites in plants commonly classified into two major classes: flavonoids and nonflavonoids (Santos-Buelga, González-Paramás, Oludemi, Ayuda-Durán, & González-Manzano, 2019). According to the literature, the frequent intake of these compounds has been associated with a decrease in chronic human diseases, including cardiovascular diseases, type II diabetes, some types of cancers and neurodegenerative disorders (Araujo et al., 2021; Santos-Buelga et al., 2019). To the best of our knowledge, the phenolic profile of puçá fruits remains unknown in the scientific literature.

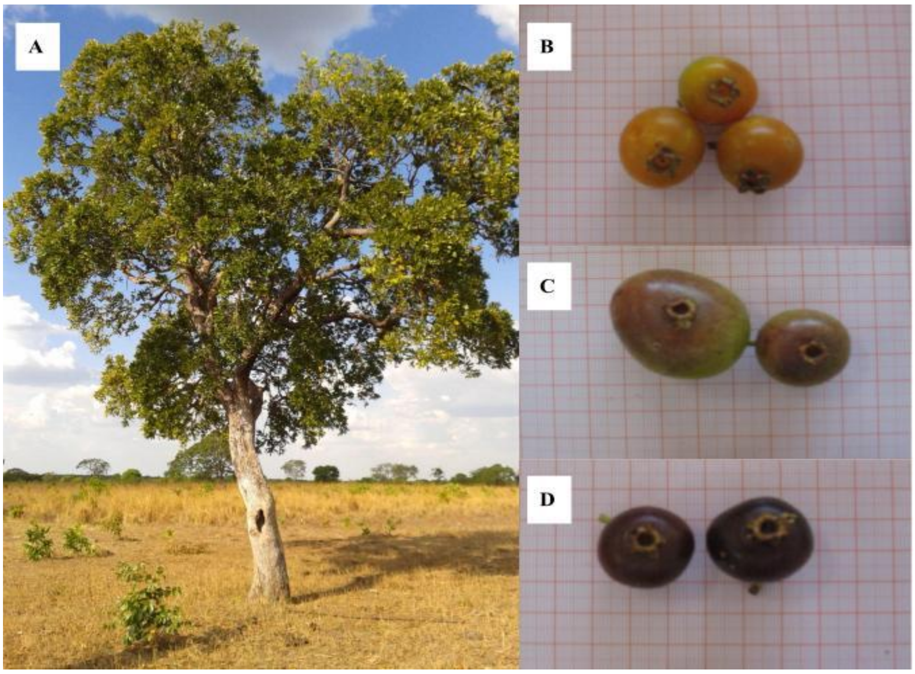

Figure 1. A) *Mouriri pusa* tree, B) yellow puçá, C) brown puçá, and D) black puçá.

Making use of native plants can provide sustainable solutions to diversify food production and improve food security (Schmidt et al., 2020). The market and management of these fruits can also contribute to Brazilian fauna conservation and improve the income of the local family agroindustry (Teixeira et al., 2019). Thus, supporting the puçá fruit production chain is fundamental not only to the sustainable handling of *M. pusa* and the Cerrado biome but also to ensure an important source of income to the local populations. In this sense, it is considered fundamental to increase the possibilities of exploitation and valorization of native fruit species for the development and consumption of food products with claimed health benefits.

Due to its potential of consumption, as well as the scarcity of data on the puçá fruit, our research aimed to evaluate the physicochemical characteristics and bioactive compounds in three puçá varieties, which are fruits highly appreciated and consumed in the Cerrado. This information may encourage fruit consumption and increase the number of scientific studies aiming to disseminate information that stimulates Brazilian biodiversity valuing.

Material and methods

Chemical reagents

The following reagents from Sigma Aldrich (St. Louis, MO) were used: methanol, acetone, glacial acetic acid, Folin–Ciocalteu's phenol reagent, 2,4-dinitrophenylhydrazine and sodium carbonate. Water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). Phenolic standards were supplied as follows: gallic acid, (+)-catechin, chlorogenic acid, caffeic acid, vanillic acid, *p*-coumaric acid, ellagic acid, *trans*-cinnamic acid, (−)-epicatechin and rosmarinic acid (Sigma Aldrich, St. Louis, MO). Ferulic acid, *o*-coumaric acid, and *m*-coumaric acid were purchased from Fluka Chemie (Steinheim, Germany). For the chromatographic analysis, samples and solvents were filtered using 0.45 μm membranes (Millipore Corp., Bedford, MA).

Fruit samples

Three different varieties of puçá fruit (*Mouriri pusa*) were studied. On the basis of the external color, the varieties are called yellow puçá (°h = 37.19; C* = 5.12; L = 31.02), brown puçá (°h = 293.89; C* = 2.93; L = 27.30), and black puçá (°h = 271.31; C* = 4.7; L = 26.17). Fruits were harvested manually from twenty trees between November 2014 and October 2015 in Cerrado regions located in Gurupi, Tocantins State, Brazil (latitude 11°43'30'' S and longitude 49°4'34'' W). Approximately 10 kg of fruit was harvested, and only ripe fruits free of visual defects were selected. They were washed, dried with paper towels, measured, weighed, frozen in liquid nitrogen, packaged in polyethylene pouches to prevent dehydration, and kept at -18 °C in the dark during the 12 h transportation to the Federal University of Lavras, Brazil. The fruits were then stored at -80

°C until sample preparation. Sample preparation was performed quickly to avoid thawing. Only the edible parts of the fruit were used. The fruits were first peeled and then homogenized. After homogenization, the samples were immediately refrozen at -80°C.

Chemical analysis

The values of the titratable acid (TA), soluble solids (SS), pH, total sugar (TS) and ratio (SS/TA) were determined (AOAC, 1998). The results of TA and TS are expressed as mg 100 g^{-1} of fresh weight, and SS is expressed as °Brix.

The vitamin C content of each fruit pulp was determined by a colorimetric method with 2,4-dinitrophenylhydrazine (2,4-DNPH) according to Strohecker, Zaragoza, and Henning (1967). The samples were analyzed in a spectrophotometer (Beckman, model 640 B) at an absorbance of 520 nm. The results are expressed as mg ascorbic acid 100 g^{-1} of fresh weight.

Carotenoids were extracted and quantified according to the method proposed by Rodriguez-Amaya (2001). The samples were analyzed in a spectrophotometer (Beckman, model 640 B) at an absorbance of 450 nm, and the results were expressed in mg of β-carotene 100 g^{-1} fresh weight.

The total phenolic content of the fruits was determined as described by Waterhouse (2002) using Folin–Ciocalteau reagent. The absorbance of the mixture was measured at a wavelength of 765 nm. Gallic acid was used as a reference standard (1.25 – 40 μg mL^{-1}), and the results were expressed as milligrams of gallic acid equivalents (mg GAE) 100 g^{-1} fresh weight.

Analysis of individual phenolic compounds was carried out according to the method described by Ramaiya, Bujang, Zakaria, King, and Sahrir (2013). Chromatographic analyses were performed using a Shimadzu chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with four high-pressure pumps (model LC-20AT), a diode array detector (model SPD-M20A), degasser (model DGU-20A5), CBM-20A interface, CTO-20AC oven, and autosampler (model SIL-20A). Separations were performed using an Ascentis C18 5 μm (250 × 4 mm) column connected to a guard column (Ascentis C18, 4.0 × 10 mm, 5 μm). Briefly, puçá fruits (5 g) were homogenized with 40 mL of solution containing 70% methanol–water (v/v). The samples were vortexed and placed in an ultrasonic bath at room temperature for 60 min. The extracts were obtained after centrifugation at 1400 × g for 15 min. and then filtered through a paper filter (pore diameter 14 μm). Prior to injection, the samples were filtered through a 0.45 μm pore size membrane filter (Millipore Corp., Bedford, MA). The mobile phase consisted of 2% (v/v) acetic acid in water (mobile phase A) and methanol/water/acetic acid, 70:28:2, respectively (mobile phase B) at a flow rate of 1.0 mL $min.^{-1}$ with a 65 min. run time and a gradient elution program by the following linear gradient steps: start condition 100% A in 0-5 min., then 70% A in 5-25 min., 60% A in 25-43 min., 55% A in 43-50 min., 0% A in 50-55 min. and 100% A in 55-65. The injection volume was 20 μL. Analyses were performed at 15 °C. The phenolic compounds were detected at 280 nm. The phenolic compounds were identified by comparison of their retention times, UV–vis absorption spectra, and authentic standards (gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, ferulic acid, *trans*-cinnamic acid, vanillic acid, ellagic acid, rosmarinic acid, *m*-coumaric acid, *p*-coumaric acid, and *o*-coumaric acid).

Statistical analysis

All results of the chemical analyses (n = 3) are presented as the means ± SD (standard deviation) of replications. The results of physicochemical characteristics were analyzed by analysis of variance (ANOVA), and the means with a significant difference were compared by Tukey's test at 5% probability. Sisvar® software was used for the statistical analysis of the data.

Results and discussion

Table 1 presents the physicochemical characteristics of black puçá, brown puçá and yellow puçá fruits. Except for pH, significant differences ($p < 0.05$) were detected in all measured parameters. The levels of acidity ranged from 0.38 g of citric acid 100 g^{-1} in the brown puçá to 0.60 g of citric acid 100 g^{-1} in the black puçá. Moreover, all evaluated fruits showed high contents of soluble solids and total sugars, although black puçá had the lowest levels (27.49 °Brix and 22.67%, respectively) among the analyzed fruits. Nonetheless, the brown puçá and yellow puçá stand out for presenting the highest levels of soluble solids and sugars and the lowest levels of acidity; consequently, they have the highest ratios of soluble solids/acid. The contents of soluble solids, normally related to the levels of sugars and organic acids, are an important feature of products that are sold fresh as consumers prefer sweeter fruits (Magwaza & Opara, 2015). The soluble solids levels in puça varieties are much higher than those reported in the literature for blackberry (6.67 °Brix) (Schulz et al., 2019), jussara (3 °Brix), and jabuticaba (12 °Brix) (Inada et al., 2015).

Table 1. pH, titratable acidity, soluble solids, total sugar, ratio of soluble solids/titratable acidity, ascorbic acid, carotenoids and total phenolics of yellow puçá, brown puçá and black puçá fruit.

	Fruits		
	Yellow puçá	Brown puçá	Black puçá
pH	4.93 ± 0.10[a]	4.95 ± 0.04[a]	4.90 ± 0.19[a]
Titratable acidity (g citric acid 100 g^{-1})	0.39 ± 0.04[b]	0.38 ± 0.04[b]	0.60 ± 0.09[a]
Soluble solids (°Brix)	31.00 ± 1.73[a]	33.00 ± 1.00[a]	27.49 ± 0.63[b]
Total sugar (%)	31.05 ± 0.75[a]	26.58 ± 1.72[b]	22.67 ± 1.38[c]
Ratio	79.86 ± 8.92[a]	84.98 ± 8.20[a]	46.37 ± 5.75[b]
Ascorbic acid (mg 100 g^{-1} f.w.)	69.58 ± 1.31[b]	77.28 ± 1.75[a]	76.15 ± 1.50[a]
Carotenoids (mg of β-carotene 100 g^{-1} f.w	45.45 ± 2.91[b]	80.79 ± 0.14[a]	21.76 ± 0.58[c]
Total phenolics (mg GAE 100 g^{-1} f.w.)	819.01 ± 22.87[b]	1,423.34 ± 74.30[a]	808.26 ± 27.63[b]

Mean value ± standard deviation of fruit pulp weight; n = 3. GAE (gallic acid equivalents), f. w. (fresh weight). Means followed by the different lowercase letters in the same line differ ($p < 0.05$) by Tukey's test.

Among the different varieties analyzed, brown puçá presented the highest total carotenoid content, followed by yellow puçá (Table 1). The total carotenoid content in black puçá (21.76 mg of β-carotene 100 g^{-1} f.w.) was higher than that found in black puçá by Rufino et al. (2010) (4.2 mg 100 g^{-1} f.w.) and Borges et al. (2022) (1.5 mg 100 g^{-1} f.w.). Puçá fruit may be considered an excellent source of carotenoids, exhibiting values higher than araçá, buriti, cagaita, yellow mombin, mangaba and marolo (0.43, 4.65, 0.96, 0.42, 0.86 and 0.52 mg β-carotene 100 g^{-1} f.w., respectively) (Schiassi, Souza, Lago, Campos, & Queiroz, 2018), species also found in savannah-like vegetation in Brazil. The quantification of carotenoids has become an important alternative, since these natural food pigments have antioxidant properties, especially pro-vitamin A activities (Rodriguez-Amaya, 2019).

The ascorbic acid content in black puçá and brown puçá was higher than that in yellow puçá. Our ascorbic acid results for black puçá were almost three times higher than those shown by Rufino et al. (2010) (28.9 mg 100 g^{-1} f.w) and Borges et al. (2022) (2.9 mg 100 g^{-1} f.w.) in the same variety. According to the classification by Ramful, Tarnus, Aruoma, Bourdon, and Bahorun (2011), all fruits analyzed may be qualified as fruits with high ascorbic acid content (>50 mg 100 g^{-1}). Considering that the reference daily intake (RDI) of ascorbic acid for adults and children aged 4 years is 90 mg (FDA, 2020), the consumption of approximately 100 g of puçá fruit is enough to satisfy this recommendation. Puçá fruits also exhibited higher ascorbic acid content than other Cerrado fruits, such as araçá (40.75 mg 100 g^{-1} f.w.), cagaita (31.95 mg 100 g^{-1} f.w.), and marolo (46.00 mg 100 g^{-1} f.w.) (Schiassi et al., 2018).

The brown puçá fruits showed the highest amount of total phenolics, followed by yellow puçá and black puçá (Table 1). Our study has clearly shown that these fruits can be categorized as having a high concentration of phenols, following the polyphenol classification (> 500 mg GAE 100 g^{-1}) proposed by Vasco, Ruales, and Kamal-Eldin (2008). The phenolic content reported by Rufino et al. (2010) was slightly higher than those found in our study (808.26 mg GAE 100 g^{-1}).

This difference observed in the chemical composition (physicochemical characteristics and bioactive compounds) of puçá fruit in our study in comparison to the data from the literature is justified by the influence of several factors, such as the time of harvest, maturity, variety, edaphoclimatic conditions, postharvest handling, processing and storage. The phenolic content of puçá fruits was higher than those reported for other traditional and exotic Brazilian fruit pulps, such as açaí (708.22 mg GAE 100 g^{-1} f.w.), jabuticaba (626.57 mg GAE 100 g^{-1} f.w.), blackberry (198.21 mg GAE 100 g^{-1} f.w.), and uvaia (132.48 mg GAE 100 g^{-1} f.w.) (Stafussa et al., 2018).

The identification of phenolic compounds in three puçá fruit varieties was carried out by comparing the retention times and UV spectra of authentic standards whenever available. Figure 2 presents the base-peak chromatograms for yellow, brown and black puçá.

Table 2 presents the data of the eight phenolic compounds identified in the samples. The phenolic acids gallic, vanillic and *p*-coumaric were found in all fruits evaluated. Chlorogenic, caffeic, ferulic, *o*-coumaric and *m*-coumaric acids were not detected in any of the samples. *Trans*-cinnamic acid was detected in the brown and black puçá varieties but not in the yellow puçá. This is the first study reporting the identification of phenolic compounds in puçá fruits.

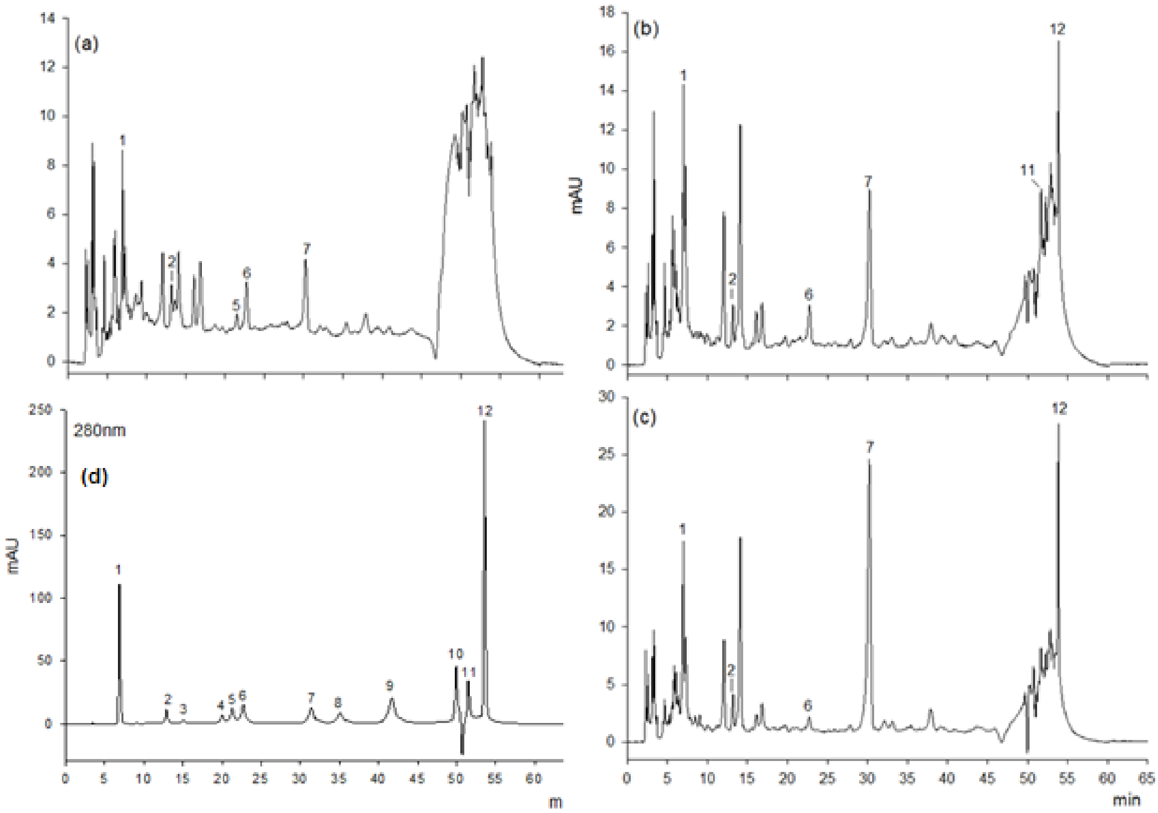

Figure 2. Base-peak chromatograms (BPC) for the three puçá varieties obtained by HPLC-DAD. (a) Yellow puçá, (b) brown puçá, (c) black puçá and (d) authentic standards. Peak identification: 1= gallic acid (6.8 min.); 2= catechin (12.8 min.); 3= chlorogenic acid (15.4 min.); 4= caffeic acid; 5= (-)-epicatechin (21.3 min.); 6= vanillic acid (22.7 min.); 7= *p*-coumaric acid (31.4 min.); 8= ferulic acid (35.2 min.); 9= *m*-coumaric (42.3 min.); 10= *o*-coumaric acid (50.0 min.); 11= rosmarinic acid/ellagic acid (51.49 min.); 12= *trans*-cinnamic acid (53.5 min.).

Table 2. Quantification of phenolics in aqueous-organic extracts of puçá fruits based on mg 100 g^{-1} fresh matter.

Compound	UV λ_{max} (nm)	Chemical formula	Yellow puçá	Brown puçá	Black puçá
Gallic acid	280	$C_7H_6O_5$	0.38 ± 0.08	0.72 ± 0.06	0.82 ± 0.06
Catechin	280	$C_{15}H_{14}O_6$	1.62 ± 0.14	1.85 ± 0.12	2.39 ± 0.26
Vanillic acid	280	$C_8H_8O_4$	0.84 ± 0.06	0.80 ± 0.07	0.75 ± 0.06
Rosmarinic acid	280	$C_{18}H_{16}O_8$	nd	11.55 ± 0.73	nd
(−)-epicatechin	280	$C_{15}H_{14}O_6$	3.77 ± 0.34	nd	nd
p-coumaric acid	280	$C_9H_8O_3$	4.42 ± 0.61	4.76 ± 0.38	11.61 ± 0.99
Ellagic acid	280	$C_{14}H_6O_8$	nd	11.18 ± 1.62	nd
trans-cinnamic acid	280	$C_9H_8O_2$	Nd	0.49 ± 0.07	0.67 ± 0.04

Mean value ± standard deviation of fruit pulp weight; n = 3. nd: not detected.

Phenolic acids were the most abundant class of phenolic compounds that were detected in the present study. These compounds are characterized by a benzene ring, a carboxylic acid grouping, and one or more hydroxyl and/or methoxyl groups, which are responsible for their antioxidant properties and are therefore indicated for health protective effects such as antimicrobial, neuroprotective, anticancer, anti-inflammatory, anti-mutagenic, etc. (Kumar & Goel, 2019).

The predominant phenolic compounds in brown puçá were ellagic acid and rosmarinic acid (Table 2). Neves et al. (2021) reported 0.101 ± 0.040 – 0.374 ± 0.060 mg 100 g^{-1} fresh matter of ellagic acid in jabuticaba, which is much lower than those found in brown puçá in the present work. Previous studies have shown that ellagic acid confers various biological activities, such as nephroprotective effects (Bhattacharjee, Kulkarni, Chakraborty, Habbu, & Ray, 2021), antiatherosclerotic mechanisms (Mele et al., 2016), and antitumor, antimetastatic and antiangiogenic activities (Ceci et al., 2018). Moreover, other major sources of ellagic acid include cloudberries, pomegranates, nuts, herbs, and roots (Lorenzo et al., 2019). A multitude of biological activities have also been described for rosmarinic acid, involving neuroprotective, hepatoprotective, anti-inflammatory, and anticancer properties (Marchev et al., 2021).

Yellow and black puçá fruit present as the major phenolic constituent *p*-coumaric acid (Table 2), which is the most abundant isomer of hydroxycinnamic acid in nature, and it is widely distributed in free or conjugated forms in fruits and vegetables, including apples, pears, beans, potatoes, and tomatoes (Cha, Lee, Lee, & Park, 2018). The values found in the present study are higher than those reported by You et al. (2011) in selected cultivars of blueberries (0.49 ± 0.03 – 3.03 ± 0.05 mg 100 g^{-1} fresh matter). In a recent study, Karakurt, AbuŞoĞlua, and Arituluk (2020) showed that *p*-coumaric acid inhibits the proliferation of the human colon cancer cell lines DLD-1 and HT-29. In addition, *p*-coumaric acid was reported to suppress hepatic apoptosis via reactive oxygen species (ROS)-mediated DNA damage responses in a mouse model (Cha et al., 2018).

The flavonoid catechin was found in the three puçá varieties, whereas (−)-epicatechin was found only in the yellow puçá (Table 2). Neves et al. (2021) found 0.000 ± 0.000 – 1.750 ± 0.282 mg 100 g^{-1} fresh matter of catechin in jabuticaba pulp, lower values than those reported in this study. In another study, Rojas-Ocampo et al. (2021) did not detect catechin and (-)-epicatechin in blackberry from the Amazonas region. These compounds are known as high defense factors against oxidative damage in tissues (Anitha, Krishnan, Senthilkumar, & Sasirekha, 2021), which justifies their anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. The correlation between bioactivity and chemical characteristics demonstrates that catechin and epicatechin induce apoptosis and cell death and play an important role in inhibiting prostate and breast cancer cell proliferation (Thomas & Dong, 2021). Additionally, phytochemical studies with the methanolic extract of *Mouriri pusa* leaves revealed the existence of flavonoids such as (−)-epicatechin and (+)-catechin (Andreo et al., 2006), as we found in the fruits evaluated.

Bioactive compounds present in puçá fruit, such as ascorbic acid, carotenoids and phenolics, can interact synergistically with each other, resulting in higher antioxidant activity of fruit than the sum of activities of its individual components. In addition, these fruits appear to be an option to increase the Brazilian agricultural matrix, since they possess considerable quantities of nutraceuticals with the potential to be investigated to produce valuable ingredients of impact in the prevention of countless diseases.

Conclusion

The native Brazilian fruits yellow puçá, brown puçá, and black puçá contained considerable quantities of important functional compounds, such as high concentrations of vitamin C, carotenoids and eight individual phenolic compounds identified. Phenolic acids were the main groups of phenolic compounds found in the fruits. (−)-Epicatechin was found only in the yellow puçá. The results showed the potential of three puçá varieties for the production of functional ingredients and nutraceuticals, especially for the prevention and treatment of chronic degenerative diseases induced primarily by oxidative stress. To our knowledge, this is the first time that phenolic profiles in puçá fruits have been measured and highlights these fruits as promising sources of natural antioxidants. However, further studies are required on puçá, such as evaluating the presence of more phenolic compounds, carotenoid profile and potential beneficial health effects.

Acknowledgements

The authors are grateful to the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - Brazil (CAPES) – Finance Code 001, to the *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG) and to the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq).

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