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Bioactive compounds and *in vitro* antioxidant activity of pulps from fruits from the Brazilian atlantic forest

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ABSTRACT. The fruits belonging to the family *Myrtaceae* are known sources of compounds with functional characteristics. Nevertheless, the studies are focused only on some species of this family. In this sense, we aimed to quantify the bioactive compounds present in the pulps of cambuci, feijoa, uvaia and grumixama; to evaluate the *in vitro* antioxidant capacity of each one and to correlate the contribution of these bioactive compounds with the antioxidant activity of each fruit pulp. For this, the compounds ascorbic acid, phenolic compounds, carotenoids, and flavonoids were quantified for the pulps of cambuci, feijoa, uvaia, and grumixama, as well as the *in vitro* antioxidant capacity by the methods DPPH and ABTS. The results were evaluated by multivariate statistical techniques. The pulps present good antioxidant potential, the one from cambuci presented the highest values for antioxidant activity given by the method DPPH (61.86 μg of Trolox g^{-1}), that from uvaia was prominent by the presence of ascorbic acid (85.40 ascorbic acid 100 g^{-1}) and that from feijoa, by the flavonoid contents (62.45 mg quercetin g^{-1}) and phenolic compounds (10.21 mg gallic acid equivalent g^{-1}). A correlation was observed between pulp antioxidant capacity and the contents of ascorbic acid and carotenoids; on the other hand, the phenolic compounds and flavonoids little contributed for the anti-free radical activity of the methods DPPH and ABTS.

Keywords: Cambuci; feijoa; uvaia; grumixama; multivariate analysis.

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Introduction

The biome Atlantic Forest has been constantly losing species because of the process of expansion of the agricultural frontiers, mainly of monocultures. Among these species, there is an abundant natural richness of native fruits, with characteristic flavors that are attractive for industrialization, and food science and technology become fundamental tools to study the potential of the foods derived from this biome, providing a sustainable management of this ecosystem, assisting its preservation and increasing the income alternatives by the use of natural resources.

The greater knowledge of the native fruits to Brazil and of their properties is interesting for new sources of compounds with antioxidant capacity to be introduced, as well as to meet market niches eager for novelties. The protective effect of the bioactive compounds found in the fruits is greatly attributed to the biological properties that promote health, such as the antioxidant capacity of vitamin C (ascorbic acid), of the carotenoids and of phenolic compounds such as the flavonoids (Seifried, Anderson, Fisher, & Milner, 2007). These antioxidants can act as free radical scavengers, peroxide decomposers, singlet oxygen suppressors, enzyme inhibitors, and synergists (Rufino et al., 2010). Nevertheless, the synthesis and accumulation of these compounds in fruits are variable depending on species, variety, management, climate conditions, ripening stage and storage conditions (Veberic, Colaric, & Stampar, 2008).

Family *Myrtaceae* is one of the most important families in terms of species richness from the Atlantic Forest and contains diverse native fruit trees. This family is prominent for presenting species which generate small fruits, being found from Amazonas to the Southern region of Brazil, and among the species there is cambuci (*Campomanesia phaea*), feijoa (*Feijoa sellowiana*), uvaia (*Eugenia pyriformis* Camb.), and grumixama (*Eugenia brasiliensis*). Among them, cambuci is the most researched fruit, and its antioxidant potential has been gaining notoriety. (Donado-Pestana, Belchior, Festuccia, & Genovese, 2015) indicated that cambuci extracts rich in phenolic compounds have potentially beneficial actions *in vivo*, with protection of mice to the inflammation of the adipose tissue. Nonetheless, for the other fruits (uvaia, grumixama, and feijoa), studies evaluating their fruit antioxidant capacity are scarce and the knowledge in this area is incipient.

Therefore, the aim of this study was to identify the bioactive compounds and antioxidant activity in the pulps of uvaia, grumixama, cambuci, and feijoa and to correlate the contribution of vitamin C, phenolic compounds, flavonoids, and carotenoids with the antioxidant activity.

Material and methods

The fruits of cambuci, feijoa, uvaia and grumixama were obtained from a rural producer established in Paraibuna, São Paulo State, Brazil. The fruits were harvested in their characteristic ripening stage, which was determined by their external visual appearance. The harvest was done manually in the first hours of day. The fruits were selected and sanitized in a sodium dichloroisocyanurate dihydrate solution (3% of active chlorine) at 200 mg L^{-1} for 15 minutes.

Each fruit was individually pulped, in an industrial blender with a stainless-steel cup (Spolu-Benesse from Brazil, Itajobi, São Paulo State) with power of 700 W and rotation of 18,000 rpm, for 3 minutes. In this process, water was added at the proportion of two parts of fruit to one of water (2:1) to obtain a better homogenization of the parts. The pulps were stored in plastic polyethylene bags with capacity for 100 mL and stored at 5°C in cold chamber until the moment of the analyses.

The evaluation of ascorbic acid was performed by the method of Tillmans, which is based on the reduction of the 2,6-diclophenol-indophenol sodium (DFNa) by the ascorbic acid, with the results expressed in mg of ascorbic acid 100 g^{-1} of fresh pulp (Strohecker & Henning, 1967). The Carotenoids were quantified according to the spectrophotometric methodology of Lichtenthaler (1987), with the results expressed in µg of carotenoids g^{-1} of fresh pulp.

The content of Total Phenolic Compounds was determined by the spectrophotometric method of Folin-Ciocalteau using gallic acid as reference standard (Singleton, 1985). The results were expressed in mg gallic acid equivalent g^{-1} of fresh pulp. The Total Flavonoids were determined by Park, Koo, Sato, and Contado (1995). An analytical curve was constructed with increasing concentrations of quercetin prepared in ethanolic solution. The results were expressed in mg quercetin g^{-1} of fresh pulp.

To perform the analyses of antioxidant activity by the methods of ABTS and DPPH radical scavenging, ethanolic extracts of the fruit pulps were performed. For this, 10 g of pulp were diluted in 20 mL of 80% ethanol, homogenized and centrifuged at 8228 rcf, 4°C, for 15 minutes. The supernatant obtained corresponded to the ethanolic extract that was used in the analyses.

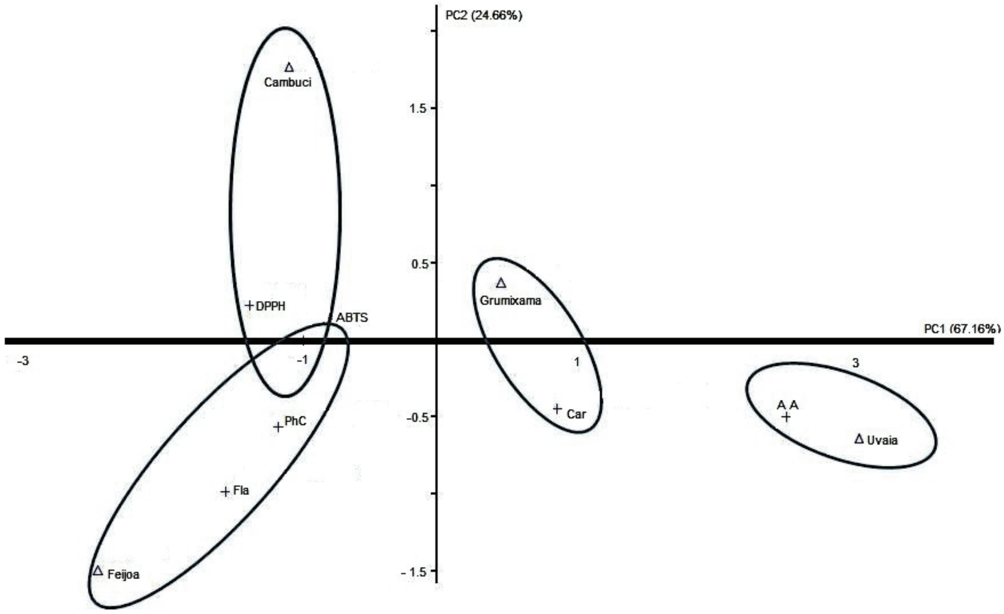
The evaluation of the scavenging activity of radical DPPH was performed by Brand-Williams, Cuvelier, and Berset (1995). The ethanolic extracts of the fruit pulps were diluted and an aliquot of 0.5 mL was added to 3.0 mL of 80% ethanol and 0.3 mL of radical DPPH in ethanol (0.2 mg mL^{-1}). The reduction of radical DPPH was evaluated by the absorbance in spectrophotometer at 515 nm after 50 minutes of reaction. A curve was performed containing increasing concentrations of the reagent Trolox and the results were expressed in µg of Trolox g^{-1} of fresh pulp.

The antioxidant capacity by the ABTS method was performed by Rufino et al. (2010), with modifications. Initially, the radical ABTS was obtained by the reaction of 140 nM of potassium persulfate with 7 mM of ABTS and stored in the dark at room temperature for 16 hours. Once the radical was formed, it was diluted with ethanol until the absorbance value of 0.700 ± 0.050 was obtained in wavelength of 734 nm in a spectrophotometer. A total of 30 µL of each pulp ethanolic extract were transferred to test tubes and 3.0 mL of the radical ABTS were added, with the absorbance read at 734 nm, after 6 minutes of reaction, using ethanol as blank. For the production of the curve, the reagent Trolox was used. The results were expressed in µmol trolox g^{-1} of fresh pulp.

To determine the contribution of ascorbic acid, total carotenoids, phenolic compounds, and total flavonoids with the antioxidant activity by the radicals DPPH and ABTS of the samples studied, the Pearson correlation was performed using the statistical program PC-ORD. The data set was also subjected to the Multivariate statistical treatment and the Principal Component Analysis was performed with graphic representation of the results.

Results and discussion

In the principal component analysis involving the fruit pulps and the bioactive compounds and antioxidant activity, it was possible to extract two principal components that explained 91.82% of the total variance. The first principal component (PC1) explained 67.16% of the statistical variance and was positively correlated with the variables total carotenoids and ascorbic acid and negatively with the antioxidant activity by the method ABTS. The second principal component (PC2) explained 24.66% of the statistical variance and was positively correlated with the variables Total Phenolic Compounds and Total Flavonoids (Figure 1).



PC= Principal component; AA = Ascorbic acid; PhC = Phenolic compounds; Fla = Total flavonoids; Car = Total carotenoids, DPPH = scavenging activity of the radical DPPH; ABTS = antioxidant activity by the method ABTS.

Figure 1. Graphic representation of the Principal Components relative to the bioactive compounds and antioxidant capacity evaluated in the pulp samples of cambuci, feijoa, uvaia, and grumixama.

The interrelation among the parameters studied divided the compounds carotenoids and ascorbic acid on the right side of the graph and phenolic compounds, flavonoids, and *in vitro* antioxidant capacity on the left side of the graph, showing that the samples which contain considerable contents of phenolic compounds and flavonoids present a higher total *in vitro* antioxidant capacity; conversely, the pulps with higher contents of vitamin C and carotenoids have a lower antioxidant capacity. It was possible to divide the pulps according to the bioactive compounds studied and the antioxidant capacity that best characterizes them, with feijoa pulp characterized by the flavonoid contents, phenolic compounds and antioxidant activity, grumixama pulp by the carotenoid contents and uvaia pulp by the ascorbic acid. On the other hand, cambuci pulp was characterized by the *in vitro* antioxidant activity (DPPH and ABTS), being possible, from this, to choose the fruit that best matches the intended purposes, or the kind of bioactive compound that one wants to consume or use in formulations.

Uvaia pulp was characterized in PCA by a considerable amount of ascorbic acid (85.40 mg of ascorbic acid 100 g^{-1}) (Figure 1, Table 1) which is even above the values of ascorbic acid observed in other studies with the same species (Rufino et al., 2010; Zillo, Silva, Zanatta, Carmo, & Spoto, 2013). Nevertheless, cambuci pulps (3.16 mg of ascorbic acid 100 g^{-1}), feijoa (3.16 mg of ascorbic acid 100 g^{-1}), and grumixama (8.72 mg of ascorbic acid 100 g^{-1}) are characterized by the low content of this compound. These results are inferior to those found for feijoa derived from the State of Santa Catarina (Brazil) that presented 55.2 mg of ascorbic acid 100 g^{-1} (do Amarante, de Souza, Beninca, & Steffens, 2017a). Regarding cambuci, Azevedo, Silva, Jacomino, and Genovese (2017), studying the ascorbic acid content of these fruit, cultivated in four cities of the State of São Paulo (Brazil), did not identify the presence of this compound in the samples. This result is consistent with the low contents of ascorbic acid in the cambuci pulps verified in this study. Still, the values obtained in the pulps of cambuci, feijoa, and grumixama were close to those found for passion fruit pulp

(Genovese, Pinto, De Souza Schmidt Goncalves, & Lajolo, 2008) and higher than in the pulps of some tropical fruits, such as bacuri, buriti, and murici (Barreto, Benassi, & Mercadante, 2009).

Table 1. Contents of ascorbic acid, phenolic compounds, flavonoids, carotenoids and antioxidant activity of the pulps of cambuci, feijoa, uvaia, and grumixama (mean values, n = 9, means ± standard deviation).

Analyses	Pulps			
	Cambuci	Feijoa	Uvaia	Grumixama
Ascorbic acid (mg ascorbic acid 100 g^{-1})	3.16 ± 0.20	3.16 ± 0.20	85.40 ± 2.21	8.72 ± 1.12
Phenolic compounds (mg gallic acid mL^{-1})	3.43 ± 0.50	10.21 ± 1.20	2.39 ± 0.15	1.83 ± 0.60
Total flavonoids (mg of quercetin 100 g^{-1})	1.40 ± 0.45	62.45 ± 9.94	3.89 ± 0.43	19.72 ± 1.12
Total carotenoids (μg of β-carotene g^{-1})	2.99 ± 0.81	12.85 ± 2.92	24.70 ± 1.65	13.87 ± 2.62
DPPH (μg trolox g^{-1})	61.86 ± 2.00	53.74 ± 2.00	5.72 ± 0.74	8.44 ± 1.17
ABTS (μmol trolox g^{-1})	32.06 ± 3.43	42.87 ± 4.94	6.07 ± 0.29	29.41 ± 2.96

N= number of repetitions; DPPH = scavenging activity of the radical DPPH; ABTS = antioxidant activity by the method ABTS.

Many studies reported that the main sources of ascorbic acid in the human diet are the citric fruits, which may present contents superior to 100 mg 100 g^{-1} (fresh base) (Assefa, Saini, & Keum, 2017). In a study with acerola, guava and strawberry, which are admittedly a source of ascorbic acid, 778.10 mg of ascorbic acid 100 g^{-1} were found in acerola pulp, 69.70 mg 100 g^{-1} in guava pulp and 44.40 mg 100 g^{-1} in strawberry pulp (Freire, Abreu, Rocha, Corrêa, & Marques, 2013). The uvaia pulp analyzed in this study presented superior values of ascorbic acid than those found in guava and strawberry pulps, potentially representing an excellent source of this compound in the diet.

The ascorbic acid amounts are affected by several environmental factors, and during pulp processing and acquisition, vitamin C can be degraded because of the time spent between processing and cooling or air incorporation during processing steps, which favors the reactions of aerobic degradation by oxidation; or even thermal degradation (Damiani et al., 2011).

Regarding the contents of phenolic compounds, feijoa pulp presented the highest means (10.21 mg gallic acid mL^{-1}) (Table 1). This value is below those found for total phenolic compounds in feijoa genotypes (88.3 mg gallic acid 100 g^{-1} fresh pulp) (do Amarante, de Souza, Beninca, & Steffens, 2017b) and in 12 feijoa cultivars (Pasquariello et al., 2015). In the pulps of cambuci, uvaia, and grumixama, the quantities 3.43, 2.39, and 1.83 mg gallic acid mL^{-1}, respectively, were detected. The amount of total phenolic compounds verified in cambuci pulp are in accordance with those indicated by Donado-Pestana et al. (2015), who found 4.01 mg gallic acid mL^{-1}, and with those of Azevedo et al. (2017), who found values between 35.74 and 51.32 g of catechin equivalent kg^{-1} of this fruit. Mezadri, Villano, Fernandez-Pachon, Garcia-Parrilla, and Troncoso (2008), studying acerola pulp, found values of total phenolic compounds varying from 8.05 to 11.50 mg g^{-1} of fresh pulp, and considered this pulp as presenting a high content of phytochemicals, which are responsible for the antioxidant activity.

Flavonoids are part of the major group of phenolic compounds, being fruits and vegetables the main sources of this compound. Regarding flavonoid contents, feijoa presented the highest means (62.45 mg of quercetin 100 g^{-1}), followed by the pulp of grumixama (19.72 mg of quercetin 100 g^{-1}), uvaia (3.89 mg of quercetin 100 g^{-1}) and cambuci (1.40 mg of quercetin 100 g^{-1}). Similarly, an elevated number of flavonoids in feijoa was revealed by Aoyama, Sakagami, and Hatano (2018), who also reported the isolation of 21 phenolics in the fruit. Flavonoids are relatively stable compounds, since they resist oxidation, high temperatures and moderate acidity variations (Machado, Nagem, Peters, Fonseca, & Oliveira, 2008). Feijoa pulp presented superior flavonoid amounts to that observed in a study with acerola, in which the flavonoid contents varied from 9.31 to 20.22 mg of quercetin 100 g^{-1} (Lima, Mélo, Lima, & Nascimento, 2000). Thus, these results indicate that feijoa pulp can be regarded as a considerable source of this compound.

The values of carotenoids found for the pulps of cambuci, feijoa, uvaia, and grumixama were 2.99, 12.85, 24.70, and 13.87 μg of β-carotene g^{-1}, respectively. The amount of carotenoids observed in the pulps of uvaia and of grumixama is above that verified by the researchers da Silva, Rodrigues, Mercadante, and de Rosso (2014), who confirmed high carotenoid amounts in the pulps of uvaia (13.06 μg g^{-1} of fresh pulp) and grumixama (5.15 μg g^{-1} of fresh pulp). Carotenoids comprise a group of pigments that develop an important role in human health and prevent a series of diseases. According to Rodriguez-Amaya (1999), only the foods that contain more than 20 μg g^{-1} of carotenoids are considered relevant for health. The uvaia pulp analyzed presented values above 20 μg g^{-1} of carotenoids; therefore, it can be indicated as a potential source of this compound. Still comparing uvaia pulp with other fruit pulps, its values for carotenoids are higher in relation to the pulp of acerola (7.1 μg g^{-1}) (Agostini-Costa, Abreu, & Rossetti, 2003) and of whole açaí pulp (0.21 to 3.84 mg of β-carotene 100 g^{-1}) (Santos et al., 2008).

During fruit processing, the carotenoids are degraded and this decomposition occurs mainly by oxidative enzymatic reaction, by photo and auto-oxidation among other factors, such as exposure to light and oxygen, type of food matrix, presence of enzymes, water availability and presence of antioxidants, which might influence the oxidative process (Rodriguez-Amaya, 1999).

Regarding the *in vitro* antioxidant activity by the method DPPH, the pulps of cambuci and feijoa were characterized by an elevated antioxidant activity, presenting the values of 61.86 and 53.74 μmol trolox g^{-1} of fresh pulp, respectively (Table 1). Azevedo et al. (2017) also evidenced a very high antioxidant capacity by the method DPPH of ripe cambuci from four cities of the State of São Paulo (Brazil). In a study with several Brazilian fruits, Abe, Lajolo, and Genovese (2012) found values for the free radical scavenging capacity varying between 5.6 and 141 mmol Trolox kg^{-1} (fresh fruit). Among these fruits, grumixama (64 mmol Trolox kg^{-1}), together with camu-camu (141 mmol Trolox kg^{-1}) and jaboticaba (62 mmol Trolox kg^{-1}) are the ones that presented the highest values.

The antioxidant activity of radical ABTS of the pulp samples of feijoa, cambuci, grumixama and uvaia were of 42.87, 32.06, 29.41, and 6.07 μmol Trolox g^{-1} of fresh pulp, respectively (Table 1). The value referring to the *in vitro* antioxidant activity (ABTS method) observed in this work for uvaia is inferior to the exposed by Rufino et al. (2010) (18.0 μmol Trolox g^{-1} of fresh pulp). Nevertheless, the feijoa pulp in this study presented a value approximately five times higher than that obtained for this fruit by do Amarante et al. (2017b). This difference can be due to the different places of cultivation and pulp processing, as well as extract preparation for the analysis.

Furthermore, these values are also superior to those found for already consolidated fruits, such as mango (9.42 μmol Trolox g^{-1}), orange (4.47 μmol Trolox g^{-1}) and nectarine (1.84 μmol Trolox g^{-1}) (Chen et al., 2014). Canuto, Xavier, Neves, and Benassi (2010), studying 15 different pulps of fruits from the Amazon rainforest in relation to their anti-free radical activity (ABTS), observed that the pulps of açaí and acerola were the ones that presented the highest values (12.1 and 10.0 μmol Trolox L^{-1}, respectively), and for the pulps of araçá-boi, which also belongs to the family *Myrtaceae*, the anti-free radical activity found was of 3.0 μmol Trolox L^{-1}.

There is no official method for the determination of the antioxidant activity in foods from vegetable origin, having in mind the various antioxidant mechanisms that can occur, as well as the diversity of bioactive compounds. Each method has its distinct principle that uses free radicals or diverse standards, and the studies that aim at evaluating antioxidant properties of vegetable extracts use more than one methodology to infer, with greater security, if the extracts analyzed can also present some activity in combating the free radicals formed inside the human organism (Sousa, Vieira, & Lima, 2011).

Comparing the two methods used in the determination of the total *in vitro* antioxidant activity of the pulps, it was observed that the values found by the method DPPH are superior to those found by the method ABTS. Vedana, Ziemer, Miguel, Portella, and Candido (2008), analyzing different residues of tropical pulps, also observed that the values of total antioxidant capacity of the compound equivalent to Trolox (TEAC) obtained by the methodology of the DPPH were superior to those detected by the methodology of ABTS. Soares, Welter, Kuskoski, Gonzaga, and Fett (2008) also found this correlation analyzing the antioxidant activity of skins of Niágara and Isabel grapes, since they observed the highest values in all results for DPPH in relation to those obtained with ABTS. Analyzing the results obtained by Chen et al. (2014), who evaluated 33 different fruits, the values found are lower for ABTS if compared to DPPH. Thus, the different contents of antioxidant activity found by the two methodologies might be the reflection of a different interaction, or affinity, of the compounds present in the pulp with the free radical scavengers.

Correlation analysis

The correlation coefficients with indexes higher than 0.70 were considered substantial according to Sounis (1985). The ascorbic acid contents presented a high negative correlation with the antioxidant activity by the method ABTS, in the same way that the carotenoid contents presented a strong negative correlation with the *in vitro* antioxidant activity given by the methods DPPH and ABTS (Table 2). This behavior can be observed mainly in the pulps of cambuci and feijoa which, despite having presented the highest means of antioxidant activity, obtained the lowest values of ascorbic acid and of carotenoids.

Some authors also reported a negative influence of the ascorbic acid with antioxidant activity in strawberries and raspberries (r = -0.80) (Kalt, Forney, Martin & Prior, 1999). Other studies found high correlations, however positive ($r^2 \geqslant 0.7$) among ascorbic acid, phenolic compounds and antioxidant activity determined by the ABTS method (Rufino et al., 2010; Chirinos, Galarza, Betalleluz-Pallardel, Pedreschi & Campos, 2010).

Differently from the observed in this work, Santos et al. (2010) did not detect a correlation of the TEAC antioxidant activity with ascorbic acid (r = -0.36; $p < 0.05$) and carotenoids (r = 0.06; $p < 0.05$), in which only the phenolic compounds presented a significant positive correlation (r = 0.79; $p < 0.05$), in whole cupuaçu pulps.

Table 2. Pearson Correlation among the variables; Ascorbic acid, phenolic compounds, flavonoids and carotenoids with the antioxidant activity by the methods DPPH and ABTS of the pulps of cambuci, feijoa, uvaia, and grumixama.

Variables	Correlation coefficient
Ascorbic acid x DPPH	-0.65
Ascorbic acid x ABTS	-0.94
Phenolic compounds x DPPH	0.61
Phenolic compounds x ABTS	0.68
Total flavonoids x DPPH	0.32
Total flavonoids x ABTS	0.69
Total carotenoids x DPPH	-0.80
Total carotenoids x ABTS	-0.72

DPPH = scavenging activity of the radical DPPH; ABTS = antioxidant activity by the method ABTS.

Conclusion

The fruit pulps have expressive amounts of compounds with antioxidant activity. Among them, uvaia pulp was prominent by the high contents of ascorbic acid, feijoa by the elevated contents of flavonoids and phenolic compounds and cambuci by the antioxidant activity by the DPPH method, suggesting that their ingestion positively contributes to human health. On the contrary, grumixama pulp evidenced medium values of the bioactive compounds and of *in vitro* antioxidant activity. A negative correlation was observed between the antioxidant capacity of the pulps and the contents of vitamin C and carotenoids; conversely, the phenolic compounds and flavonoids little contributed to the anti-free radical activity of the methods DPPH and ABTS.

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