

Phenolic profiling, organic acids and sugars composition of feijoa (Acca sellowiana (O. Berg) Burret) and uvaia (Eugenia pyriformis Cambess) from the southern Brazilian highlands

Bruna Rafaela da Silva Monteiro Wanderley¹[®] Isabel Cristina da Silva Haas¹[®] Fabíola Carina Biluca¹[®] Patricia Brugnerotto¹[®] Trilícia Margarida Gomes¹[®] Ana Carolina Moura de Sena Aquino²[®] Ana Carolina Oliveira Costa¹[®] Vívian Maria Burin¹[®] Renata Dias de Mello Castanho Amboni¹[®] Carlise Beddin Fritzen-Freire^{1*}[®]

¹Departamento de Ciência e Tecnologia de Alimentos, Universidade Federal de Santa Catarina (UFSC), 88034-001, Florianópolis, SC, Brasil. E-mail: carlise.freire@ufsc.br. *Corresponding author.

²Instituto Federal de Santa Catarina (IFSC), Urupema, SC, Brasil.

ABSTRACT: Brazil is a large country with high biodiversity in its different regions. However, species of native fruits widely reported in the southern Brazil have not been properly explored so far, remaining underutilized by the food industry. This study evaluated the polyphenolic profile, as well as the composition of organic acids and sugars of the pulps of the feijoa, and the uvaia from southern brazilian highlands. The uvaia pulp showed the highest total polyphenol content and the highest antioxidant capacity by the methods used. The polyphenol (+)-catechin $(6.54 \text{ mg } 100\text{g}^{-1})$ was the major phenolic compound in uvaia pulp, which has not yet been reported in the literature for fruits of other regions. In addition, the feijoa pulp stood out for the presence of (-)-epicatechin $(18.29 \text{ mg } 100\text{g}^{-1})$. The pulps of native fruits in this study only quantified values for citric and malic acids. Malic acid $(553.00 \text{ mg } 100\text{g}^{-1})$ was the main acid in the uvaia pulp, and citric acid $(455.60 \text{ mg } 100\text{g}^{-1})$ was the main acid in the feijoa pulp. It is possible to note that the feijoa pulp showed the highest total sugar content $(11.14 \text{ g } 100\text{g}^{-1})$ and was the only pulp that contained sucrose. The uvaia pulp, conversely, showed fructose $(3.10 \text{ g } 100\text{g}^{-1})$ as the main sugar. The results obtained in this study contributed to the valuation and conservation of the species investigated, representing a promising alternative for the use of these native fruits in the development of new products.

Key words: Acca sellowiana, Eugenia pyriformis, Brazilian native fruits, chemical characterization.

Perfil fenólico, composição de ácidos orgânicos e de açúcares de feijoa (Acca sellowiana (O. Berg) Burret) e uvaia (Eugenia pyriformis Cambess) do planalto sul brasileiro

RESUMO: O Brasil é um grande país e com elevada biodiversidade em suas diferentes regiões. Entretanto, espécies de frutas nativas amplamente encontradas no sul do Brasil ainda não foram devidamente exploradas, permanecendo desconhecidas e subutilizadas pela indústria de alimentos. Nesse sentido, o objetivo deste estudo foi avaliar o perfil fenólico, bem como a composição de ácidos orgânicos e de açúcares de polpas de feijoa (Acca sellowiana) e de uvaia (Eugenia pyriformis) provenientes do planalto sul brasileiro. A polpa de uvaia apresentou o maior teor de polifenóis totais e a maior capacidade antioxidante pelos métodos utilizados. A (+)-catequina (6.54 mg 100g¹) foi o principal composto fenólico da polpa da uvaia, o que ainda não foi relatado na literatura para frutas de outras regiões. Além disso, a polpa da feijoa se destacou pela presença de (-)-epicatequina (18.29 mg 100g¹). Dentre os ácidos orgânicos avaliados, as polpas de frutas nativas apresentaram valores para os ácidos cítrico e málico, sendo o ácido málico (553.00 mg 100g¹) o principal na polpa de uvaia, e o cítrico (455.60 mg 100g¹) na polpa de feijoa. Em relação aos açúcares, a polpa da feijoa apresentou o maior teor de açúcar total (11.14 g 100g¹) e foi a única polpa que continha sacarose. Já a polpa da uvaia o dus aspresentou a frutose (3.10 g 100g¹) como principal açúcar. Os resultados obidos neste estudo contribuem para a valorização e conservação das espécies investigadas, representando uma alternativa promissora para a utilização dessas frutas nativas na desenvolvimento de novos produtos.

Palavras-chave: Acca sellowiana, Eugenia pyriformis, frutas nativas brasileiras, caracterização química.

INTRODUCTION

Brazil is considered one of the main centers of genetic diversity of fruits in the world,

this is mainly due to the country's geographical and climatic diversity (PEREIRA et al., 2012; RUFINO et al., 2010). However, countless species of native fruits have been underutilized by the food industry. These

Received 06.11.21 Approved 01.04.22 Returned by the author 04.06.22 CR-2021-0458.R3 Editors: Leandro Souza da Silva D Ivo Oliveira fruits have interesting particularities in relation to their phytochemical composition, which depends directly on the region of cultivation (SCHIASSI et al., 2018).

In Southern Brazil, some native species are adapted to cold climate, with a common dispersion in areas with altitudes higher than 800 meters. The highland region of Santa Catarina state has shown great potential to produce fruits, especially its highaltitude locations (900 to 1,400 meters), where the climate is temperate, with greater solar availability and greater thermal amplitude (MENEGUZZI et al., 2020). Fruits grown in temperate regions have stood out for their high content of bioactive compounds, besides presenting high productivity and good profitability to rural producers (MARSH, 2012).

Among the fruits widely reported in the southern Brazilian highlands, the native fruits of the family Myrtaceae stand out, especially the feijoa (Acca sellowiana) and the uvaia (Eugenia pyriformis) (AMARANTE et al., 2017; SILVA et al., 2019). However, these fruits are almost unknown in the national and international market, and for that reason, the investigation of its chemical composition can help in the valorization of species. Furthermore, in general, native fruits have important nutritional aspects related to their composition of organic acids, sugars, polyphenols, vitamins and minerals. In this regard, the increase in world demand for foods rich in bioactive compounds, with different flavors and aromas, has stimulated researchers of native fruits (CASTELUCCI et al., 2020; SCHIASSI et al., 2018) and; consequently, the consumption of underexploited species, besides promoting the economic development of the producing regions. Therefore, this study evaluated of the polyphenolic profile and the composition of organic acids and sugars of the pulps of the feijoa and the uvaia from highlands of South Brazil.

MATERIALS AND METHODS

Fruit samples

The feijoa fruits (*Acca sellowiana*) of variety Helena were harvested in April 2019 from plantations at the EPAGRI Experimental Station (São Joaquim, SC, Brazil, 28 °16 '40.02 "S, 49 °56 '09.10 "W, 1400 m of altitude), sanitized with sodium hypochlorite (100 mg L⁻¹) and manually pulped. The seeds were removed using an electric pulping machine (Model 56B0478, Eletromotores WEG S.A., Jaraguá do Sul, Brazil). The uvaia fruits (*Eugenia pyriformis*) were collected in February 2019 from their natural habitats (Urupema, SC, Brazil, 28°01'27.5'S

 $49^{\circ}56'23.6''W - 1,248$ m altitude) and sanitized with sodium hypochlorite (100 mg L⁻¹). The pulp was removed using an electric pulping machine (model DP-50, Tomasi, São Paulo, Brazil). All the samples were stored in polyethylene bags and frozen (-18 °C).

Preparation of the extracts for the determination of total and individual phenolic compounds and in vitro antioxidant capacity

The extracts were prepared according to the methodology proposed by DANTAS et al. (2019) with modifications, where 2.5 g of pulp was homogenized with 25 mL of methanol (100%) for 30 minutes in a magnetic stirrer, and finally centrifuged (Hermle Z200A, Wehingen, Germany) at 4,000 rpm for 10 minutes, collecting the supernatant for spectrophotometric analysis. For the quantification of individual phenolic compounds, the supernatant was concentrated in a rotary evaporator (TE-211, Tecnal, Piracicaba, Brazil) at 30 °C and then resuspended in a methanol:water (1:1 v/v) solution for subsequent injection in the liquid chromatograph.

Total phenolic compounds and in vitro antioxidant capacity

The content of total phenolic compounds was determined by the Folin-Ciocalteu method (SINGLETON; ROSSI, 1965) in a UV-VIS spectrophotometer (model U-1800, Hitachi, Japan). Results were expressed in mg gallic acid equivalent (GAE) per 100g of fresh weight (FW) sample. The antioxidant capacity was determined by the ABTS⁺ free radical capture activity method (RE et al., 1999) and by the ferric reducing antioxidant potential (FRAP) assay method (BENZIE; STRAIN, 1996). The results of the ABTS⁺ and FRAP assays were expressed in μ M Trolox equivalent (TE) per gram of sample (FW).

Evaluation of individual polyphenols

The determination individual of polyphenols was performed as proposed by BURIN et al. (2014), with modifications, using a liquid chromatography system (Shimadzu, Kyoto, Japan) equipped with a vacuum degasser (DGU-20A), a quaternary pump system (LC-20AT) and a diode array detector (SPD-M20A). The analytical separation was performed on a CLC-ODS C18 reverse phase column $(4.6 \text{ mm} \times 250 \text{ mm}, 5 \mu\text{m})$ (Shimadzu, Kyoto, Japan). For the analysis of hydroxybenzoic acids, the mobile phase consisted of ultrapure water:acetic acid (98:2 v/v) (solvent A) and acetonitrile:solvent A (80:20 v/v) (solvent B). The flow rate used was 1.0 mL/ min, with detection at 280 nm. For the quantification

of hydroxycinnamic acids, flavonols, flavanols and *trans*-resveratrol, the mobile phase consisted of ultrapure water: acetic acid (98:2 v/v) (solvent A) and ultrapure water: acetic acid:acetonitrile (58:2:40 v/v/v) (solvent B), with flow of 0.9 mL/min and detection at 320 nm, 360 nm, 280 nm and 306 nm, respectively. The identification of the phenolic compounds was performed considering the combined information from chromatographic parameters of the analyte peaks (retention time, elution order, λ max) and comparison with their respective calibration curves of standard solutions. Results were expressed in mg of the phenolic compound identified per 100 g of sample (FW).

Determination of aliphatic organic acids and sugars

The pulp samples were independently homogenized, and 1 g of each sample was diluted in 5 ml of ultrapure water Milli-Q[®] (Millipore, Bedford, MA, USA). After that, the samples were submitted to an ultrasonic bath (Unique 1400A, São Paulo, Brazil) for approximately 10 min. The samples were centrifuged (MiniSpin® plus) at 9,861 g for 10 min and the supernatant were analyzed. After centrifugation, aliquots of the supernatant were transferred to eppendorf tubes and appropriate dilutions were performed for each sample. The determination of aliphatic organic acids was performed following the procedure described by BRUGNEROTTO et al. (2019) and the determination of sugars was carried out according to RIZELIO et al. (2012). Both analyses were conducted using a capillary electrophoresis system (model 7100, Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector and the HP ChemStation® software (rev A.06.01) for the acquisition and treatment of the data. The results of aliphatic organic acids were expressed in mg 100 g⁻¹ and the results of sugars were expressed in g 100 g⁻¹ (FW).

Statistical analysis

All the analyses were performed in triplicate and the results were expressed as mean \pm standard deviation (n=3). The analysis of variance (ANOVA) and Tukey's test (P < 0.05) were performed using the STATISTICA[®] software version 10.0 (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The total phenolic compounds (TPC) and the *in vitro* antioxidant capacity are shown in figure 1, with differences (P < 0.05) being noted among the samples for the parameters evaluated. The content of phenolic compounds in the feijoa pulp $(30.39 \pm 1.21 \text{ mg GAE } 100\text{g}^{-1})$ was lower than that reported by AMARANTE et al. (2017) for feijoa pulp of the variety Helena (75.7 mg GAE 100g^{-1}), using different extracting agents. The uvaia pulp showed a TPC value of $70.82 \pm 3.72 \text{ mg GAE } 100\text{g}^{-1}$. SGANZERLA et al. (2018) reported values ranging from 34.70 to 189.41 mg GAE 100g^{-1} for uvaia pulp.

Regarding the results of antioxidant capacity among the pulps investigated, the uvaia pulp stood out for showing greater antioxidant potential (P < 0.05), regardless of the method used (ABTS⁺, 8.96 \pm $0.28 \mu M TE g^{-1}$ or FRAP, $2.14 \pm 0.08 \mu M TE g^{-1}$). The values obtained in this study were more expressive than the results obtained on the uvaia fruits harvested in the southeast region of Brazil (CASTELUCCI et al., 2020). The feijoa pulp showed the lowest (P < 0.05) antioxidant capacity values (ABTS⁺, 4.53 \pm 0.11 μ M TE g⁻¹ or FRAP, $1.37 \pm 0.03 \mu$ M TE g⁻¹). According to CASTELUCCI et al. (2020), this may be related to the processing stage of the feijoa fruit, where it is possible to note that the degradation of the bioactive compounds occurs due to the oxidative process promoted by enzymatic reactions, which influences directly on the antioxidant activity of this fruit.

The phenolic profiles of the feijoa, and the uvaia pulps are shown in table 1. It is possible to note that there was a significant difference (P < 0.05) in the phenolic composition of the fruits investigated. The main compound quantified in the feijoa pulp was (-)-epicatechin, followed by ellagic acid, (+)-catechin, kaempferol, and caffeic acid. PENG et al. (2020) evaluated feijoa juice from New Zealand and identified procyanidin B1 and (+)-catechin as the major phenolic compounds; however, these authors did not detect kaempferol or caffeic acid. It is known that kaempferol is widely reported in fruits and vegetables and that its consumption promotes several benefits to human health, such as reduction of chronic diseases, through its antioxidant activity against free radicals (CHEN & CHEN, 2013). In turn, caffeic acid is found in several plants, and is recognized for its antioxidant activity and for preventing cardiovascular diseases (KFOURY et al., 2019).

The major phenolic compounds detected in the uvaia pulp were (+)-catechin, kaempferol, (-)-epicatechin, gallic acid, ferulic, ellagic, syringic and vanillic. HAMINIUK et al. (2014) reported that the major phenolic compounds in pulps of different uvaia varieties grown in the southeastern region of Brazil were gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, rutin, myricetin, quercitin, and kaempferol. It is worth noting that the



content of kaempferol detected in this present study was higher than that reported by these authors. The polyphenol (+)-catechin was the major phenolic compound in uvaia pulp, which has not yet been reported in the literature (HAMINIUK et al., 2014; KARWOWSKI et al., 2013). Catechins belong to the flavonoid class and *in vitro* studies report the beneficial potential of catechins in relation to human health, mainly for the control of degenerative and cardiac diseases (KARWOWSKI et al., 2013).

The results obtained for the aliphatic organic acids investigated in the fruit pulps of this study are shown in table 2. Of the 14 organic acids investigated (maleic, malonic, fumaric, tartaric, formic, citric, malic, glycolic, lactic, gluconic, glutaric, succinic, acetic, and propionic acids), only citric acid and malic acid were detected, and citric acid was the major factor (P < 0.05) in the feijoa pulp, while malic acid was predominant (P < 0.05) in the uvaia pulp. SILVA et al. (2019) also noted significant levels of citric acid and malic acid in pulps of different uvaia varieties (mean values of 39.46 mg $100g^{-1}$ and 34.70 mg $100g^{-1}$, respectively). However, these authors observed the succinic acid being the predominant acid in most of the varieties investigated from southeastern region of Brazil. The values obtained for malic acid may be related to the greater availability of solar incidence in high-altitude regions

Phenolic compounds	Elution order*	RT (min)	λ (nm)	Feijoa	Uvaia	
Hidroxybenzoic acids						
Gallic acid	1	9.58 ± 0.11	280	<0.08 †	4.26 ± 0.14	
Protocatechuic	2	14.70 ± 0.09	280	${<}0.07^{\dagger}$	${<}0.07^{\dagger}$	
Vanillic	7	23.33 ± 0.20	280	< 0.11*	0.54 ± 0.02	
Syringic	8	24.26 ± 0.16	280	$0.27\pm0.02^{\text{b}}$	$1.51\pm0.03^{\rm a}$	
Ellagic	3	19.50 ± 0.04	280	$3.77\pm0.11^{\rm a}$	$1.63\pm0.11^{\text{b}}$	
Hydroxycinnamic acids						
Trans-caftaric	5	20.42 ± 0.25	320	0.14 ± 0.01	${<}0.07^{\dagger}$	
Caffeic	10	30.30 ± 0.29	320	1.32 ± 0.13	$< 1.01^{\dagger}$	
<i>p</i> -coumaric	11	35.81 ± 0.28	320	< 0.42*	< 0.42*	
Ferulic	12	39.70 ± 0.42	320	$0.41\pm0.01^{\text{b}}$	$2.03\pm0.05^{\rm a}$	
FlavanolFlavanol						
(+)-Catechin	6	22.17 ± 0.12	280	$3.62\pm0.12^{\text{b}}$	$6.54\pm0.39^{\rm a}$	
(-)-Epicatechin	9	27.39 ± 0.19	280	$18.29\pm0.86^{\rm a}$	$4.43\pm0.27^{\rm b}$	
FlavonolFlavonol						
Myricetin	13	49.65 ± 0.21	360	< 0.11*	$< 0.11^{\dagger}$	
Quercetin	15	55.96 ± 0.05	360	$0.98\pm0.03^{\rm a}$	$0.38\pm0.00^{\rm b}$	
Kaempferol	16	66.55 ± 0.19	360	$2.87\pm0.03^{\text{b}}$	$5.63\pm0.22^{\rm a}$	
Stilbene						
Trans-resveratrol	14	50.76 ± 0.18	306	< 0.11*	< 0.11*	
Tyrosol	4	19.99 ± 0.02	280	$< 0.11^{\dagger}$	$< 0.11^{+}$	

Table 1 - Concentration of phenolic compounds (mg 100g⁻¹) from the feijoa pulp (Acca sellowiana) and uvaia pulp (Eugenia pyriformis).

Results expressed in fresh weight (FW) as mean \pm standard deviation (n=3). Within a row, different lowercase letters indicate significant differences between samples (P < 0.05).

[†]Limit of quantification.

RT = retention time.

 λ = wavelength.

* Elution order for all phenolic compounds considering the quantification methods.

(MENEGUZZI et al., 2020), which may influence on the increase in malate ions in fruits (JÚNIOR et al., 2014). The high levels of organic acids noted in this study can be justified by the climate of the highaltitude regions of southern Brazil, which present average temperatures of around 20 °C in comparison with regions of higher temperatures, where the fruits use greater amounts of organic acids as substrates for cellular respiration during the maturation process (MENEGUZZI et al., 2020).

Regarding the results of sugars (Table 2), the uvaia pulp showed fructose as the main sugar. As for the feijoa pulp, which was the pulp that showed the highest total sugar content (11.14 g 100g⁻¹), the main sugar was sucrose, representing 58.71% of the sugar composition. The uvaia pulp showed lower sugar values than those reported by SILVA et al. (2019) (average value of 14.89 g 100g⁻¹) for the uvaia varieties. JAWAD et al. (2020) stated that the variation in the sugar levels, as well as in organic acids in fruits of the same species, may be related to the stages of fruit ripening, environmental conditions, and phytohormones.

CONCLUSION

Results of this study revealed that the pulps of feijoa, and uvaia show distinct polyphenol profiles, organic acids, and sugars composition. The uvaia pulp stood out due to the presence of (+)-catechin, which has not yet been reported in the literature for this fruit. Conversely, the (-)-epicatechin was the majority polyphenol in the feijoa pulp. In addition, high concentrations of sucrose and citric acid were observed in the feijoa pulp. From this study it was possible to conclude that the pulps analyzed show a promising

Table 2 - Concentration of aliphatic organic acids (mg 100g⁻¹) and sugars (g 100g⁻¹) from the feijoa pulp (*Acca sellowiana*) and uvaia pulp (*Eugenia pyriformis*).

Compounds	Feijoa	Uvaia			
Aliphatic organic acids					
Citric acid	$455.6 \pm 5.57^{\mathrm{a}}$	$9.43\pm0.35^{\text{b}}$			
Malic acid	$65.36\pm3.18^{\mathrm{b}}$	$553.00 \pm 10.34^{\rm a}$			
Sugars					
Glucose	$2.10\pm0.06^{\rm a}$	$1.70\pm0.01^{\rm b}$			
Fructose	2.50 ± 0.05^{b}	$3.10\pm0.01^{\rm a}$			
Sucrose	6.54 ± 0.01	$< 0.07^{\dagger}$			

Results expressed in fresh weight (FW) as mean \pm standard deviation (n=3). Within a row, different lowercase letters indicate significant differences between samples (P < 0.05) [†] Limit of quantification.

potential for its use in the development of new products, aiming the valorization and conservation of these species, in addition to providing new commercialization alternatives to small rural producers.

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AUTHOR'S CONTRIBUTIONS

Conceptualization: BRSMW, CBFF and RDMCA; Methodology: BRSMW, ICSH, VMB, ACOC and ACMSA; Formal analysis and investigation: BRSMW, FCB, PB and TMG; Writing—original draft preparation: BRSMW; Writing—review and editing: BRSMW, CBFF, RDMCA; ICSH, VMB, ACOC and ACMSA; Supervision: CBFF. All authors critically revised the manuscript and approved the final version.

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.

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