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Characterization of araçá fruits (*Psidium cattleianum* Sabine): Phenolic composition, antioxidant activity and inhibition of α-amylase and α-glucosidase

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ABSTRACT

Araçá fruits (*Psidium cattleianum* Sabine) have been reported to have several biological activities, including the inhibition of the digestives enzymes. Nevertheless, the compounds responsible for these activities are not known. This study evaluated the potential of araçá phenolic fractions to inhibit the activity of α-glucosidase and α-amylase and related it to their phenolic composition. Extracts of edible portions (pulp-peel and seeds) of fruits from three araçá genotypes were purified and fractionated. Total phenolic content was determined using a spectrophotometric method, and individual phenolic compounds were quantified using HPLC-MS. Their enzyme inhibition and antioxidant potential were measured using *in vitro* assays. Higher amounts of total phenolic content were observed in pulp-peel than in seeds. These results were partially associated with the slightly higher enzyme inhibition of pulp-peel fractions. In general, araçá phenolic fractions were more active towards α-glucosidase than α-amylase when compared to acarbose, the current drug used to inhibit α-glucosidases. The antioxidant potential was dependent on accession, part of the fruit and fraction. Moreover, compounds present in the crude and purified extracts, and fractions III (quercetin, ellagic acid and *p*-coumaric acid) and IV (cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside) for some samples resulted in significant inhibition of the two enzymes.

1. Introduction

Araçá (*Psidium cattleianum* Sabine), belonging to the Myrtaceae family, is a native fruit with botanical origin in the south of Brazil. Fruits can be consumed *in natura*, or in sweets, e.g., jellies and juices. In addition, to its pleasant and unique flavor, it has high a content of bioactive compounds (Franzon et al., 2009), which may be related to its analgesic effect (Fidyt et al., 2016), antioxidant (Dalla Nora et al., 2014; Medina et al., 2011), antimicrobial (Medina et al., 2011), antiproliferative (Medina et al., 2011), antihyperglycemic (Vinholes et al., 2017) and antidiabetic (de Souza Cardoso et al., 2018) effects.

The biological activities reported for araçá are mainly related to their chemical composition, particularly with the presence of phenolic compounds, which are specialized metabolites with high antioxidant capacity (Verma et al., 2013) and the potential to inhibit digestive enzymes (Vinholes et al., 2017). Different phenolic compounds have been reported in araçá such as ellagic, *p*-coumaric, chlorogenic and gallic acids (Medina et al., 2011; Ribeiro et al., 2014), flavonoids such as quercetin, myricetin, and epicatechin (Medina et al., 2011) and anthocyanins such as cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside (Dalla Nora et al., 2014).

Type 2 diabetes mellitus is characterized by an abnormal increase in blood glucose immediately after meals (postprandial glycemia). Recently, the interest in fruit phenolic compounds capable of inhibiting the digestive enzymes associated with this disease, is growing. The primary role of digestive enzymes is to break the carbohydrates into small units to be absorbed by the organism. α-Amylase, present in saliva and pancreas, is responsible for breaking the starch, given rise to disaccharides that are further hydrolyzed by α-glucosidases, present in the intestine, liberating glucose units to the blood (Ranilla et al., 2010). The targetted inhibition of these enzymes is used to delay the intestinal absorption of glucose, reducing the postprandial blood glucose levels.

Araçá extracts were tested *in vivo* for the control of hyperglycemia, and decreased glycemia and triglycerides levels (de Souza Cardoso

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et al., 2018). A recent study showed the araçá extracts *in vitro* ability to inhibit α-glucosidase, and the results were promising (Vinholes et al., 2017). However, the metabolites responsible for this inhibition and its location in the fruit have not yet been determined. The content changes with different tissues. Compounds such as phenolic compounds are more concentrated in the pulp-peel than in the seed (Contreras-Calderón et al., 2011; Farhadi et al., 2016; Kubola & Siriamornpun, 2011). Another aspect that should be considered is the genetic variability of this fruit, either for different cultivars or for variations within the same cultivar (Vicente et al., 2004).

Although araçá phenolic extracts have been described as inhibitors of carbohydrate hydrolyzing enzymes, the compounds responsible for this inhibition remain unknown. Therefore, different phenolic fractions from the pulp-peel and seed of three araçá genotypes were studied, aiming at identifying and quantifying the phenolic compounds related to the enzymes' inhibitory activities.

2. Materials and methods

2.1. Standards and reagents

Reagents were purchased from different suppliers. Radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) D9132, Trolox, potassium phosphate buffer (pH 7.0), α-glucosidase (type I from baker's yeast) G5003, 4-nitrophenyl-α-D-glucopyranoside (PNP-G) N1377, α-amylase (porcine pancreas) A6255, Folin-Ciocalteu reagent V0S0427, chlorogenic acid (CA) C3878, quercetin Q4951, catechin, myricetin, kaempferol, vanillic acid, ellagic acid, syringic acid, *p*-coumaric acid, 4-hydroxybenzoic acid, pelargonidin and sodium carbonate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (95%), acetone, methanol, acetonitrile, ethyl acetate and hydrochloric acid were purchased from VETEC (Duque de Caxias, RJ, Brazil) and potassium iodide and iodine were purchased from Synth (Diadema, SP, Brazil). Acarbose (Glucobay®) was from Bayer Pharma AG (Leverkusen, Germany), Certified soluble potato starch p.a. was from Synth (Diadema, SP, Brazil).

2.2. Samples

Three genotypes of araçá (*Psidium cattleianum* Sabine) belonging to the plant breeding program and the Active Germplasm Bank of native fruits at Embrapa Clima Temperado, 31°40′47"S, 52°26′24"W, RS, Brazil, were used (red genotypes accessions (AC): AC-44 and AC-87; yellow genotype cultivar: Bicudo). Fruits from 3 plants of each accession and cultivar were harvested in 2016, between March and April, at a full-ripe stage, to obtain a representative sample. Those fruits selected were free of injuries and visible infections and were uniform in color and size. After harvesting, samples were transported within 30 min to the laboratory in boxes at 25 °C and the pulp-peel and seeds were separated manually, and frozen at −20 °C. The samples were freeze-dried (L101, Liobrás, São Carlos, SP, Brazil), ground to a fine powder (particles diameter expected to be < 5 μm) under liquid nitrogen in a laboratory ball mill (MA 350, Marconi, Piracicaba, SP, Brazil), transferred to Falcon tubes (Global Trade Technology, Monte Alto, SP, Brazil) and immediately stored at - 80 °C (Indrel IULT 335D deep freezer, Londrina, Paraná, Brazil) for a maximum of 3 wk.

2.3. Extraction and fractionation

Araçá fruit lyophilized samples (250 mg) were mixed with methanol (1:40, w/v) and stirred (Vortex, Phoenix AP56, Araraquara, São Paulo, Brasil) for 5 min. The homogenates were filtered using a paper filter (Whatman n°. 4 - Merck KGaA, Darmstadt, Germany), the precipitate was discarded, and the resulting filtrate was designated as the crude extract. The purified extract was obtained by applying the crude extract (pH adjusted to 7.0) to a pre-conditioned C18 SPE cartridge 10 g sorbent/cartridge, particle size 55–105 μm, pore size 125 Å (Waters Corp., Milford, MA, USA) (50 ml of methanol followed by 50 ml of water) under low vacuum (up to 12 mm Hg) using an SPE extractor (Waters Corp.), cleaning-up the loaded sample with water (pH 7.0) to increase the concentration of phenolic compounds and remove any potential interfering compounds such as sugars and other polar compounds, and eluting the sample retained in the column with methanol (95%, pH 7.0) (de Oliveira Raphaelli et al., 2019).

The fractionation was carried out using two SPE cartridges. The first pre-conditioned SPE cartridge (50 ml of methanol followed by 50 ml of distilled water) was loaded with crude extract (diluted in water at a 1:5 ratio and pH adjusted to 7.0) using the same conditions described in the purification process, and the residue obtained was collected and stored at −20 °C, protected from light for a maximum of 7 days. The different fractions were obtained from the same cartridge and eluted sequentially as follows: fraction II (F-II), with 50 ml 16% acetonitrile pH 2.0; fraction III (F-III), with 50 ml 95% ethyl acetate; and fraction IV (F-IV) with 50 ml 95% methanol. The second cartridge was conditioned with 50 ml 95% methanol pH 2.0, followed by 50 ml distilled water pH 2.0. The residue collected from the first cartridge was adjusted with HCl to pH 2.0 using a pH meter (827 pH lab, Metrohm, Perdizes, SP, Brazil) and applied to the second cartridge using the same conditions described in the purification process. The fraction I (F–I) was obtained by elution with 50 ml 95% methanol. The crude and purified extracts and all fractions were evaporated under vacuum for 90 min at 40 °C, lyophilized, protected from light, and stored at - 20 °C for a maximum of 3 wk.

2.4. Chemical composition

2.4.1. Total phenolic content (TPC)

TPC was measured using the Folin-Ciocalteu method adapted from Swain and Hillis (1959). Briefly, an aliquot of 250 μl of sample or methanol control were combined with 250 μl of 0.25 M Folin-Ciocalteu reagent. After 3 min, 500 μl 1 M Na_2CO_3 was added, and the mixtures were incubated for 2 h at room temperature (23–24 °C). After 3 min, the absorbance was measured at 725 nm using a spectrophotometer (Genesys, Thermo, Brooklyn, NY, USA). The results were expressed as g of CA equivalents (E)/100 g dry basis (db) using a standard curve of CA (0.0 mg/ml - 0.5 mg/ml).

2.4.2. Phenolic compounds characterization using HPLC-Q-TOF-MS

2.4.2.1. Instrumentation and conditions. Individual phenolic compounds were measured in all phenolic fractions and also in the purified and crude extracts. The identification and quantification of the compounds using LC-MS were carried out in a Luna C18 column in a LC (UFLC, Shimadzu Co., Kyoto, Japan), coupled to a high-resolution quadrupole-time-of-flight mass spectrometer (Maxis Impact, Bruker Daltonics, Bremen, Germany), using the conditions reported by Hoffmann et al. (2018). The flow was 0.2 ml/min, column temperature 40 °C and the mobile phases were water acidified with 0.1% formic acid (Merck KGaA) (eluent A) and acetonitrile with 0.1% formic acid (eluent B). The gradient program was as follows: 10% B and increased linearly to 90% B at 18 min and was maintained for 3 min at 90% B; returned to 10% B in 2 min and was maintained at 10% B for 7 min. The injection volume was 10 μl.

Mass spectra (MS) were acquired over a mass range of *m/z* 50 to 1200. MS parameters were as follows: ESI was operated in negative mode for fractions I and III and for purified and crude extracts, and in positive mode for fractions II and IV, source temperature of 180 °C, capillary voltage of 4 kV, nebulizer gas, nitrogen, 2 bar; dry gas, nitrogen, 8 l/min, RF of collision 150 Vpp; transfer time 70 μs and prepulse storage of 5 μs. The equipment was calibrated with 10 mM sodium formate (Merck KGaA), covering the entire acquisition range (*m/z* 50 to 1200). Automatic MS/MS experiments were done by adjusting collision energy values: *m/z* 100, 15 eV; *m/z* 500, 35 eV; *m/z* 1000, 50 eV, using nitrogen as the collision gas.

Phenolic compounds were identified by comparison with the retention times, accurate mass error < 5 ppm (mass error between experimental $[M-H]^-$ and theoretical $[M-H]^-$), isotope distribution similarity values (mSigma) < 50 and with data of compounds reported in the literature for araçá fruits (Medina et al., 2011; Ribeiro et al., 2014; Silva et al., 2014). Quantification was done using standard curves of catechin, myricetin, kaempferol, quercetin, vanillic acid, ellagic acid, syringic acid, *p*-coumaric acid and 4-hydroxybenzoic acid. For cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside quantification was done using the pelargonidin calibration curve. Results were expressed as μg/g for crude extracts and μg/mg for purified extracts and phenolic fractions.

2.5. Araçá extracts antioxidant and inhibitory α-amylase and α-glucosidase enzymes potential

The araçá extracts ability to inhibit the α-amylase and α-glucosidase activities and their antioxidant activity were measured using spectrophotometric methods.

2.5.1. Antioxidant activity

Activity was determined using the method of Thaipong et al. (2006) using the stable radical $DPPH^{\bullet}$. Briefly, 200 μl of each extract or methanol (control) was added to 2800 μl 0.10 mM $DPPH^{\bullet}$ methanolic solution. The reaction was incubated in the dark for 24 h at room temperature, and the absorbance of the samples was measured at 515 nm. Trolox (T) was used as a reference for the calibration curve and results were expressed as mg TE/g of sample db.

2.5.2. α-Amylase inhibition

Inhibition was measured using the procedure reported by Phan et al. (2013) and Satoh et al. (2015) with some adaptations. The reaction mixture was composed of 60 μl of sample, 200 μl of phosphate buffer 0.05 M (pH 7.0) and 50 μl 6 U/ml α-amylase solution; the mixture was incubated for 5 min at 37 °C. The reaction was started with the addition of 250 μl of soluble starch as a substrate, followed by incubation for 15 min at 37 °C. The reaction was stopped by the addition of 50 μl 1.0 M hydrochloric acid. For color formation, 100 μl 5 mM iodine solution +5 mM potassium iodide was added. The absorbance was measured on 96-well polystyrene microplates (Spectra Max 190, Molecular Devices, San Jose, CA, USA) at 690 nm. IC_{50} values were calculated using at least 5 concentrations for each extract (serial dilution). The percent inhibition (*I%*) for the α-amylase enzyme inhibition assay was calculated using equation (1).

$$I\% = \frac{(A_{control\ -}\ A_{test1}) - (A_{sample-}\ A_{test2})}{(A_{control}\ -\ A_{background1})} \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of 100% of enzyme activity (+enzyme - inhibitor); A_{sample} is the absorbance of the sample (+enzyme + inhibitor); A_{test1} is the absorption of starch due to reducing sugars (-enzyme - inhibitor), and A_{test2} is the inhibitor + the starch (- enzyme + inhibitor). Acarbose was used as a positive control.

2.5.3. α-Glucosidase inhibition

It was measured using the procedure described by Vinholes et al. (2011) with some adaptations. Briefly, 10 μl of the extract was added to 50 μl 3.25 mM *p*-nitrophenyl-α-D-glucopyranoside substrate (diluted in phosphate buffer pH 7.0). The reaction was started by adding 50 μl 72 mU/ml enzyme solution (diluted in phosphate buffer, pH 7.0); the mixture was incubated at 37 °C for 10 min, and the absorbance at 405 nm was measured immediately. IC_{50} values were calculated using at least 5 concentrations for each extract (serial dilution). The percentage of inhibition (*I%*) for α-glucosidase and the antioxidant activity was calculated using equation (2):

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the extract), and A_{sample} is the absorbance of the extract tested in the reaction mixture. Acarbose was used as a positive control.

2.6. Statistical analysis

The concentration of extract or fraction necessary to inhibit 50% of enzymes activity (IC_{50}) was obtained by non-linear regression using the log(inhibitor) *vs* response least squares fit equation for interpolation purposes using the software package GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). The inhibitor concentrations (μg/ml) were transformed to log concentrations (μg/ml). The log(inhibitor) *vs* response curves are shown in supplementary Fig. S-1 and S-2. IC_{50} values are expressed as the mean ± standard deviation of analyses results done at least in triplicate. TPC and total antioxidant activity were subjected to one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons of means (at the 5% confidence level). The comparison of means was done for each genotype considering the extracts and fractions for the same part of the fruit (pulp-peel or seed).

3. Results and discussion

3.1. TPC

There is growing evidence that the consumption of phenolic compounds in foods can reduce the risk of health disorders due to their antioxidant activity (Shahidi & Ambigaipalan, 2015). The TPC of a matrix is closely related to its antioxidant properties.

TPC is shown for the crude and purified extracts and, for each fraction of the araçá fruit genotypes in Table 1. The TPC of the pulp-peel crude extracts were ~27% higher than in the crude extract of seeds. The genotype was not a factor that influenced the phenolic concentration.

The purified pulp-peel extract of all genotypes showed higher TPC amounts compared to the other phenolic fractions and the crude extracts. The TPC of the purified pulp-peel extract were 80–90% higher than the purified seed extract. A similar trend was observed for F-II of pulp-peel with TPC values 58–76% higher than TPC of F-II of seed. TPC of F-III for AC-44 and AC-87 seed extracts was 60–72% higher than TPC of F-III of pulp-peel, while for Bicudo the F-III pulp-peel extract was 53% higher than its F-III seed extract. Fractions I and IV showed low values of TPC. TPC in the crude extracts were similar to those reported by Vinholes et al. (2017). These authors observed TPC contents of 0.60 ± 0.02 g CAE/100 g of fresh fruit for the yellow araçá and 0.61 ± 0.02 g CAE/100 g of fresh fruit for the red araçá (Vinholes et al., 2017).

3.2. Phenolic compounds characterization using HPLC-Q-TOF-MS

Phenolic compounds identification and quantification were done using HPLC-Q-TOF-MS. Compounds identified included cyanidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, quercetin, kaempferol, catechin, myricetin, and ellagic, 4-hydroxybenzoic, *p*-coumaric, syringic and vanillic acids (Table 2).

Results of phenolic compounds quantification are shown in Table 3. Araçá pulp-peel crude extracts showed a similar phenolic profile, except cyanidin-3-*O*-glucoside found in high amounts in red genotypes and absent in the yellow one. Araçá seed crude extracts followed the same trend; however catechin was the major compound present in Bicudo, while quercetin and ellagic acid were the major ones in the red genotypes.

The phenolic compounds cyanidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, vanillic, ellagic, syringic and *p*-coumaric acids were

Table 1
Total phenolic compounds, total antioxidant activity and IC_{50} values for α-amylase and α-glucosidase for the crude and purified extracts and phenolic fractions obtained from the different parts of the araçá fruit for the Bicudo, AC44 and AC87 genotypes.

Samples		Part of the fruit, extracts and fractions				
	Assays	Pulp-peel				
		Crude	Purified	F–I	F-II	F-III
Bicudo (yellow)	[1]Total phenolic content*	1.95 ± 0.02c	8.1 ± 0.2a	0.13 ± 0.00e	1.4 ± 0.4d	2.7 ± 0.2b
	[2]Total antioxidant activity	1.3 ± 0.1a	0.82 ± 0.10b	0.25 ± 0.03d	0.70 ± 0.02bc	0.57 ± 0.02c
	[3]α-Amylase IC_{50} (μg/ml)	2.4 ± 0.2 (x10^3)	290 ± 190	300 ± 70	1.7 ± 0.001 (x10^3)	13 ± 7
	[3] α-Glucosidase IC_{50} (μg/ml)	18 ± 6	26 ± 2	640 ± 20	250 ± 80	3 ± 1
AC 44 (red)	[1]Total phenolic content*	1.93 ± 0.04 c	10 ± 0.3 a	0.16 ± 0.02 d	4.64 ± 0.02 b	0.40 ± 0.10d
	[2]Total antioxidant activity	1.10 ± 0.02a	0.82 ± 0.10b	0.18 ± 0.06cd	0.78 ± 0.04b	0.27 ± 0.06c
	[3] α-Amylase IC_{50} (μg/ml)	2.6 ± 0.5 (x10^3)	870 ± 60	630 ± 80	2.0 ± 1.0 (x10^3)	82 ± 1
	[3] α-Glucosidase IC_{50} (μg/ml)	14 ± 1	32 ± 3	1.19 ± 0.04 (x10^3)	270 ± 30	9 ± 1
AC 87 (red)	[1]Total phenolic content*	2.09 ± 0.02bc	7.1 ± 1.5a	0.08 ± 0.00c	4.5 ± 0.6b	0.57 ± 0.02c
	[2]Total antioxidant activity	1.26 ± 0.04a	0.95 ± 0.12b	0.18 ± 0.03e	0.60 ± 0.03c	0.37 ± 0.05d-e
	[3] α-Amylase IC_{50} (μg/ml)	1.9 ± 0.3 (x10^3)	21 ± 1	770 ± 40	177 ± 4	10 ± 1
	[3] α-Glucosidase IC_{50} (μg/ml)	15 ± 3	6 ± 1	540 ± 40	160 ± 20	3 ± 1

Samples	Part of the fruit, extracts and fractions						
	Pulp-peel	Seed					
	F-IV	Crude	Purified	F–I	F– II	F– III	F– IV
Bicudo (yellow)	0.94 ± 0.04d	1.42 ± 0.04b	2.08 ± 0.27a	1.20(x10^{-3}) ± 0.00 d	0.59 ± 0.03c	1.27 ± 0.17b	0.02 ± 0.00d
	0.38 ± 0.01d	0.33 ± 0.03c	0.19 ± 0.03c	5.8 ± 0.1a	2.9 ± 0.9b	0.41 ± 0.14c	1.03 ± 0.01c
	–	–	336 ± 3	85 ± 1	530 ± 3	141 ± 1	–
	1 ± 1	51 ± 5	32 ± 6	100 ± 20	72 ± 14	3.0 ± 0.4	7 ± 1
AC 44 (red)	0.05 ± 0.01d	1.49 ± 0.02a	1.4 ± 0.1a	1.60(x10^{-3}) ± 0.00c	1.11 ± 0.04b	0.89 ± 0.12b	0.9(x10^{-3}) ± 0.00c
	0.01 ± 0.01d	0.33 ± 0.01c	0.10 ± 0.02d	0.47 ± 0.02b	3.50 ± 1.61a	0.44 ± 0.05b	0.03 ± 0.02d
	41 ± 2	–	170 ± 10	–	150 ± 10	12 ± 1	–
	–	67 ± 11	10 ± 1	22 ± 4	–	–	–
AC 87 (red)	0.13 ± 0.00c	1.53 ± 0.03b	0.62 ± 0.19c	0.90(x10^{-3}) ± 0.00d	0.78 ± 0.10c	2.1 ± 0.2a	0.06 ± 0.01d
	0.50 ± 0.03c-d	0.15 ± 0.01d	7.7 ± 2.2a	0.17 ± 0.00d	0.19 ± 0.01cd	0.60 ± 0.05b	0.46 ± 0.03bc
	17 ± 1	–	36 ± 5	–	56 ± 3	5 ± 2	–
	4 ± 1	73 ± 10	6 ± 1	–	30 ± 6	2 ± 1	5 ± 1

[1] Total phenolic content expressed in g of chlorogenic acid equivalent/100 g dry basis. [2] Antioxidant activity using the DPPH radical expressed in mg of trolox equivalents/g dry basis. Results are expressed as the mean value of three experiments. Means followed by the same lowercase letter did not differ significantly concerning the same accession, fruit parts and assay ($p < 0.05$). [3] IC_{50} values for α-amylase and α-glucosidase inhibition expressed as mean values of three experiments done in triplicate ± standard deviation.

Table 2
Characterization of phenolic compounds found in araçá fruit extracts and fractions by chemical name, molecular formula, molar mass, retention time, experimental $[M-H]^-$, theoretical $[M-H]^-$, error and mSigma.

Compounds	Molecular formula	Molar Mass	Retention time	$[M-H]^-$ Experimental	$[M-H]^-$ Theoretical	Error (ppm)	mSigma
Catechin	$C_{15}H_{14}O_6$	290.26	13.40	289.07	289.07	−2.2	5.1
Kaempferol	$C_{15}H_{10}O_6$	286.23	17.90	285.04	285.04	4.1	14.9
Myricetin	$C_{15}H_{10}O_8$	318.24	15.60	317.03	317.03	3.3	12.6
Quercetin	$C_{15}H_{10}O_7$	302.24	10.50	301.03	301.03	4.8	20.1
Cyanidin-3-*O*-glucoside	$C_{21}H_{21}O_{11}^+$	449.39	8.57	449.11	449.11	−1.9	6.3
Malvidin-3-*O*-glucoside	$C_{23}H_{25}O_{12}^+$	493.44	8.24	493.13	493.13	1.2	6.9
Vanillic acid	$C_8H_8O_4$	168.14	8.32	167.03	167.03	0.0	22.3
Ellagic acid	$C_{14}H_6O_8$	302.19	10.48	301.00	301.00	2.1	7.0
Syringic acid	$C_9H_{10}O_5$	198.17	8.66	197.04	197.04	−1.3	3.6
p-Coumaric acid	$C_9H_8O_3$	164.05	9.47	163.06	163.06	−0.9	8.2
4-Hydroxybenzoic acid	$C_7H_6O_3$	138.12	8.43	137.02	137.02	−1.3	1.2

measured in the pulp-peel of purified extract of all genotypes. In addition, 4-hydroxybenzoic acid was measured in the Bicudo and AC 87 while quercetin was measured in AC 44. Quercetin, the two anthocyanins and ellagic acid were found in the purified seed extracts of all genotypes. Other acids, such as vanillic, were found only in the seed of Bicudo and AC 87; the syringic acid only in Bicudo; and the *p*-coumaric only in AC 44.

Given the purification methodology used, it was expected that the phenolic acids would be found in F–I; however, none were found. These results were probably because phenolic acids are at low concentrations in the crude extracts (Table 3), and they were retained in the SPE cartridge as some compounds were found in the purified extract. Two anthocyanins, cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside, already reported as araçá constituents, were found in F-II. Cyanidin-3-*O*-glucoside was present in considerable amounts in the pulp-peel of red genotypes (AC 44 and AC 87), while smaller quantities were found in the yellow genotype (Bicudo). Amounts of malvidin-3-*O*-glucoside were lower than cyanidin-3-*O*-glucoside in araçá pulp-peel, while similar values were observed for these compounds in seeds (Table 3).

With F-III of araçá pulp-peel, quercetin was the unique compound found in the pulp-peel of Bicudo, and this compound and ellagic acid were present in AC 87. The same compounds were measured in seeds of all genotypes in addition to *p*-coumaric acid (Bicudo and AC 87). These compounds were observed in more significant amounts in seeds than in pulp-peel.

High amounts of cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside were found in F-IV for both the pulp-peel and seed of all genotypes.

Some compounds such as catechin, kaempferol and myricetin were measured in the crude extracts, but after fractionation, they were no longer observed. A possible explanation can be due to the instability of phenolic compounds depending on pH, temperature, presence of oxygen, light and metallic ions (Albuquerque et al., 2017; Canada et al., 1990; Dall'Acqua et al., 2012). The presence of quercetin in the whole araçá fruit was also found by other authors (Biegelmeyer et al., 2011), who reported high amounts in red (0.20 g of quercetin/100 g) and lower amounts in yellow genotypes (0.04 g of quercetin/100 g). Quercetin was reported to inhibit α-glucosidase and α-amylase (Tadera et al., 2006).

Ellagic acid was measured in crude and purified extracts in all genotypes. This compound has already been reported in native fruits from the Myrtaceae family (Abe et al., 2012). In araçá, the reported concentration of ellagic acid was 2.21–3.82 μg/100 g of fresh fruit (Ribeiro et al., 2014). Ellagic acid is described as having chelating properties, inhibiting lipid peroxidation, maintaining endogenous antioxidant defense systems, anti-inflammatory, antiproliferative and antimicrobial activities among others (Badhani et al., 2015; Chanwitheesuk et al., 2007; García-Niño & Zazueta, 2015; Jagan et al., 2008; Kaur et al., 2009; Long et al., 2016; Shay et al., 2015; Tadera et al., 2006; Wang et al., 2016). Other compounds such as the ellagic deoxyhexyl derivative (1.48–2.07 μg/100 g of extract), an ellagitannin compound (0.08–1.02 μg/100 g of extract), vanillic acid hexoside (8.1 mg/100 g of pulp) and quercetin-hexoside (6.4 mg/100 g of pulp) have also been reported for araçá (Ribeiro et al., 2014; Silva et al., 2014). Concentrations of cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside found in the present study were higher than those reported in the literature (Dalla Nora et al., 2014).

3.3. Antioxidant activity and inhibition of α-amylase and α-glucosidase

The antioxidant activity was measured using the capacity of the extracts to neutralize free radicals. All extracts and fractions showed antioxidant activity. The crude extracts for pulp-peel were those with higher antioxidant activity. This activity was correlated with the TPC of crude extracts and can also be associated with the large variety of phenolic compounds quantified in these samples (Table 3), which are known to have antioxidant properties. Pulp-peel purified extracts of all genotypes were also effective antioxidants, probably because of their highest TPC contents (Table 2) and even the presence of individual phenolic compounds (Table 3) as described for the crude extract. Pulp-peel fractions showed lower antioxidant values.

The antioxidant capacity of araçá seed crude extract was lower compared with pulp-peel results; however, the purified AC 87/seed extract showed the highest antioxidant activity among all samples studied. The presence of high concentrations of quercetin, anthocyanins, vanillic and ellagic acids in this extract (Table 3) may be responsible for this result. Although F–I of the Bicudo/seed also showed high antioxidant capacity, but no correlation was observed with TPC (Table 2), or with individual phenolic compounds (Table 3). Therefore, other compounds not measured or below the limit of detection of the method used might be involved in the observed activity. In addition, F-II of AC44 showed half of the antioxidant capacity of AC87/seed extract, but higher than other samples, which might be associated with the considerable level of TPC (Table 1) and the presence of anthocyanins (Table 3).

The inhibition of α-amylase activity by araçá pulp-peel showed that values of IC_{50} for crude extracts were in the same range (Table 1), however for purified extracts and fractions it varied considerably. Fractions III and IV were the most active and the crude extract the least active. F-III of all genotypes, F-IV of red genotypes and purified extract of AC 87 (Table 1), showed lower IC_{50} values than the positive control acarbose (IC_{50} 140 ± 20 μg/ml). The considerable TPC value for these samples (Table 1) and the presence of compounds such as quercetin (Bicudo and AC 87) and ellagic acid (AC 87) (Table 3) might be responsible for the inhibitory properties observed for these extracts. F-III of AC 87 was the most active one, followed by F-III of AC44, purified extract of AC 87 and F-II of AC 87 all with IC_{50} values lower than acarbose.

For the inhibition of the digestive enzyme α-glucosidase, most

Table 3
Phenolic compounds identified in the crude and purified extracts and fractions of red and yellow araçá fruit accessions.

Samples	Compounds	Part of the fruit, extracts and fractions											
		Pulp-peel						Seed					
		Crude	Purified	F–I	F-II	F-III	F-IV	Crude	Purified	F–I	F-II	F-III	F-IV
Bicudo (yellow)	Catechin	2.07 ± 0.001 (x 10^3)	–	–	–	–	–	189 ± 0.1	–	–	–	–	–
	Kaempferol	4.69 ± 0.01	–	–	–	–	–	11.2 ± 0.04	–	–	–	–	–
	Myricetin	0.93 ± 0.01	–	–	–	–	–	1.32 ± 0.03	–	–	–	–	–
	Quercetin	19.4 ± 0.01	–	–	–	310 ± 0.4	–	47.6 ± 0.1	95 ± 1	–	–	730 ± 10	–
	Cyanidin-3-*O*-glucoside	–	720 ± 10	–	14.7 ± 0.03	–	744 ± 1	–	71.3 ± 0.1	–	89.8 ± 0.1	–	4.72 ± 0.004 (x10^3)
	Malvidin-3-*O*-glucuside	38.4 ± 0.2	36 ± 1	–	20 ± 1	–	1.5 ± 0.04 (x10^3)	13.8 ± 0.1	165 ± 4	–	80 ± 5	–	10.5 ± 0.02 (x10^3)
	Vanillic acid	10.3 ± 0.01	430 ± 10	–	–	–	–	6.9 ± 0.1	735 ± 0.3	–	–	–	–
	Ellagic acid	19.1 ± 0.01	57.2 ± 0.04	–	–	–	–	47.7 ± 0.03	201 ± 0.1	–	–	1.4 ± 0.02 (x10^3)	–
	Syringic acid	3.99 ± 0.02	168 ± 0.5	–	–	–	–	2.97 ± 0.02	157 ± 1	–	–	–	–
	p-Coumaric acid	–	14.5 ± 0.04	–	–	–	–	–	–	–	–	11 ± 0.4	–
	4-Hydroxybenzoic acid	5.08 ± 0.03	22.4 ± 0.3	–	–	–	–	7.16 ± 0.01	–	–	–	–	–
AC 44 (red)	Catechin	1.25 ± 0.01 (x10^3)	–	–	–	–	–	40 ± 2	–	–	–	–	–
	Kaempferol	13.7 ± 0.01	–	–	–	–	–	7.2 ± 0.1	–	–	–	–	–
	Myricetin	1.19 ± 0.02	–	–	–	–	–	1.07 ± 0.01	–	–	–	–	–
	Quercetin	29.1 ± 0.1	44 ± 1	–	–	–	–	158 ± 0.3	446 ± 2	–	–	1.46 ± 0.01 (x10^3)	–
	Cyanidin-3-*O*-glucoside	1.45 ± 0.01 (x10^3)	12.9 ± 0.001 (x10^3)	–	3.4 ± 0.002 (x10^3)	–	24.4 ± 0.02 (x10^3)	7 ± 0.5	234 ± 2	–	230 ± 50	–	12.4 ± 0.2 (x10^3)
	Malvidin-3-*O*-glucoside	16.5 ± 0.3	46 ± 3	–	30.4 ± 0.1	–	1.75 ± 0.02 (x10^3)	4.99 ± 0.01	320 ± 30	–	270 ± 190	–	10.6 ± 0.2 (x10^3)
	Vanillic acid	8.05 ± 0.01	182 ± 1	–	–	–	–	2.93 ± 0.05	–	–	–	–	–
	Ellagic acid	29.1 ± 0.02	96 ± 2	–	–	–	–	159 ± 1	854 ± 2	–	–	2.81 ± 0.01 (x10^3)	–
	Syringic acid	2.29 ± 0.01	77.7 ± 0.3	–	–	–	–	0.73 ± 0.02	–	–	–	–	–
	p-Coumaric acid	–	44.9 ± 0.1	–	–	–	–	–	10.0 ± 0.3	–	–	–	–
	4-Hydroxybenzoic acid	4.07 ± 0.02	–	–	–	–	–	3.09 ± 0.04	–	–	–	–	–
AC 87 (red)	Catechin	1.29 ± 0.01 (x10^3)	–	–	–	–	–	29.4 ± 0.1	–	–	–	–	–
	Kaempferol	4.86 ± 0.02	–	–	–	–	–	4.43 ± 0.04	–	–	–	–	–
	Myricetin	1.14 ± 0.03	–	–	–	–	–	2.06 ± 0.02	–	–	–	–	–
	Quercetin	44 ± 1	–	–	–	506 ± 3	–	110 ± 2	201 ± 0.04	–	–	1.99 ± 0.003 (x10^3)	–
	Cyanidin-3-*O*-glucoside	1.2 ± 0.1 (x10^3)	11.6 ± 0.04 (x10^3)	–	2.26 ± 0.01 (x10^3)	–	70 ± 3 (x10^3)	2.46 ± 0.01	310 ± 120	–	240 ± 10	–	2.89 ± 0.01(x10^3)
	Malvidin-3-*O*-glucoside	19.9 ± 0.1	56.8 ± 0.02	–	21.0 ± 0.05	–	2.96 ± 0.001 (x10^3)	4.77 ± 0.02	340 ± 10	–	158 ± 4	–	2.5 ± 0.1(x10^3)
	Vanillic acid	8.8 ± 0.1	192 ± 1	–	–	–	–	1.85 ± 0.01	390 ± 10	–	–	–	–

(*continued on next page*)

Table 3 (*continued*)

Samples	Compounds	Part of the fruit, extracts and fractions													
		Pulp-peel					Seed								
		Crude	Purified	F–I	F-II	F-III	F-IV	Crude	Purified	F–I	F-II	F-III	F-IV		
	Ellagic acid	43 ± 2	158 ± 1	–	–	1.18 ± 0.001 (x10^3)	–	98 ± 1	1.99 ± 0.004 (x10^3)	–	–	4.06 ± 0.003 (x10^3)	–		
	Syringic acid	1.96 ± 0.04	117 ± 0.5	–	–	–	–	0.68 ± 0.06	–	–	–	–	–		
	p-Coumaric acid	–	40.8 ± 0.04	–	–	–	–	–	–	–	–	119 ± 1	–		
	4-Hydroxybenzoic acid	3.64 ± 0.06	35.0 ± 0.1	–	–	–	–	2.07 ± 0.02	–	–	–		–		

*Standard deviation (n = 2). Crude extracts expressed in μg/g and purified extract and phenolic fractions expressed in μg/ml.

extracts were shown to be efficient (Table 1). Values of IC_{50} for pulp-peel extracts and fractions varied considerably. F-IV of Bicudo was the most active one, and fractions I and IV of AC 44 the least active one. All extracts and fractions were more active than acarbose (IC_{50} = 840 ± 100 μg/ml), except F–I of AC 44. F-III of all genotypes, F-IV of Bicudo and AC 87 and the purified extract of AC 87 showed remarkable results with IC_{50} values lower than 10 μg/ml (Table 1). Nevertheless, the purified and crude extracts were also efficient at inhibiting enzyme activity (Table 1). Fractions III and IV of Bicudo and AC 87 and purified extract of seeds of the red genotypes showed IC_{50} values in the same range observed for the most active extracts and fractions of araçá pulp-peel. Native fruits, such as pitanga (*Eugenia uniflora* L), have already been described with properties of α-glucosidase inhibition (Correia et al., 2012), but araçá was more effective when compared to purple, red or orange pitanga (Vinholes et al., 2017). Results for α-glucosidase inhibition were similar to the IC_{50} values reported for red and yellow araçá, (22 ± 1 and 25 ± 1 μg/ml, respectively) but in fresh fruit (Vinholes et al., 2017).

In general, the IC_{50} values for α-amylase were higher than for α-glucosidase. The ideal is an enzyme inhibitor having mild inhibitory activity for α-amylase, and a more effective inhibition for α-glucosidase. Full inhibition of α-amylase is not desired since the undigested starch will be used by the intestinal microflora, causing disorders such as diarrhea, abdominal pain and flatulence (Cho et al., 2011). Therefore, araçá can be considered as a suitable inhibitor of digestive enzymes that might control postprandial hyperglycaemia.

F-III was effective in inhibiting both enzymes. For α-amylase inhibition, more efficient results were obtained for AC 87 seed and pulp-peel, followed by AC44 seed and the Bicudo pulp-peel. Greater inhibition of α-glucosidase was observed for F-III seed and pulp-peel of AC87, Bicudo seed and pulp-peel of AC44. The presence of considerable amounts of quercetin in all genotypes and its fruit parts, except pulp-peel of AC 44, ellagic acid in pulp-peel of AC 87, and seeds of the three genotypes, together with *p*-coumaric acid and other compounds not found using the present methodology, seems to be responsible for the observed activity. Different studies described the inhibition of α-amylase and α-glucosidase by quercetin (Tadera et al., 2006; You et al., 2012) and ellagic acid (You et al., 2012). Moreover, Jadhav and Puchchakayala (2012) report that these compounds have *in vivo* anti-hyperglycemic activity, but their mechanism of action remains unknown. In addition, the strong inhibition observed for F-IV for some genotypes might be related to the high concentration of anthocyanins in this fraction (Table 3).

Thus, due to the presence of such compounds in araçá, this fruit has good potential for glycemic control. The evaluation of the influence of the fruit portion on the inhibition of α-glucosidases activity showed promising results for both pulp-peel and seed. The genotype had no impact on the results. The presence of unidentified compounds or compounds in amounts below the quantification limit may have influenced the results for the measured activities. Overall, the results showed evidence for the potential antihyperglycemic and antioxidant activity of different araçá genotypes and the possible bioactive compounds involved.

4. Conclusion

Fruit extracts and phenolic fractions of three genotypes of araçá (Bicudo, AC44 and AC87) showed high antioxidant and α-glucosidases inhibitory properties. The total antioxidant activity observed for araçá extracts and fractions is linked to their high TPC. Fractions III and IV of araçá pulp-peel for all genotypes were the most active against α-glucosidase and α-amylase, while F-III of araçá seed showed similar behavior. The considerable amounts of TPC and the presence of quercetin, ellagic acid and *p*-coumaric acid in F-III seems to be responsible for its activity whereas the anthocyanins present in F-IV and all compounds present in the purified extract, inhibited the α-glucosidase, with lower

inhibition of α-amylase. In summary, this study indicated that araçá extracts and fractions have bioactive compounds with antioxidant activity and α-glucosidase inhibitory properties that can be added into food formulations or plant-based pharmaceutical products to control blood glucose levels.

CRediT authorship contribution statement

Elisa dos Santos Pereira: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Juliana Rocha Vinholes:** Data curation, Formal analysis, Methodology, Validation, Visualization, Writing - review & editing. **Taiane Mota Camargo:** Formal analysis, Investigation, Methodology, Writing - review & editing. **Fabiana Roos Nora:** Data curation, Validation, Writing - review & editing. **Rosane Lopes Crizel:** Data curation, Formal analysis, Investigation, Writing - review & editing. **Fábio Chaves:** Conceptualization, Supervision, Writing - review & editing. **Leonardo Nora:** Conceptualization, Supervision, Writing - review & editing. **Márcia Vizzotto:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

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