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ARTICLE

Chemical Composition and Antioxidant/Antidiabetic Potential of Brazilian Native Fruits and Commercial Frozen Pulps

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Foods provide essential and bioactive compounds with health-promoting properties such as antioxidant, anti-inflammatory, and hypocholesterolemic activities, which have been related to vitamins A, C, and E and phenolic compounds such as flavonoids. Therefore, the aim of this work was to identify potential sources of bioactive compounds through the determination of flavonoids and ellagic acid contents and the in vitro antioxidant capacity and α-glucosidase and α-amylase inhibitory activities of Brazilian native fruits and commercial frozen pulps. Camu-camu, cambuci, uxi, and tucumã and commercial frozen pulps of cambuci, cagaita, coquinho azedo, and araçá presented the highest antioxidant capacities. Cambuci and cagaita exhibited the highest α-glucosidase and α-amylase inhibitory activities. Quercetin and kaempferol derivatives were the main flavonoids present in most of the samples. Ellagic acid was detected only in umbu, camu-camu, cagaita, araçá, and cambuci. According to the results, native Brazilian fruits can be considered as excellent sources of bioactive compounds.

KEYWORDS: Brazilian native fruits; commercial frozen pulps; flavonoids; diabetes; α-amylase/α-glucosidase inhibitor

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both (*1*). The prevalence of diagnosed diabetes, in particular type 2, and heart disease has increased dramatically over the past 40 years, mainly in developing countries due to factors such as growing population, aging, urbanization, lifestyle changes, and consumption of nutrient-deficient and carbohydrate-rich foods (*2*). In 1998, Brazil had about 4.9 million adults with diabetes. In 2025, that number is expected to reach about 11.6 million (*3*). Southern and southeastern regions have the highest number of deaths caused by diabetes (9%), women and nonwhite individuals being the most afflicted (*4*).

One therapeutic approach for treating diabetes is to decrease the postprandial hyperglycemia by suppressing glucose absorption through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract, and consequently blunting the postprandial plasma glucose rise (*5*, *6*).

Some studies have suggested that fruits and vegetables commonly consumed worldwide represent excellent sources of bioactive compounds, and their consumption has been associated with reduced risk of developing diabetes (*7*). Particularly, native Brazilian fruits were shown to display high in vitro antioxidant capacity and expressive amounts of flavonoids and vitamin C (*8*). One good strategy that has been proposed recently is the use of natural inhibitors of α-amylase and α-glucosidase to control postprandial hyperglycemia, without the side effect caused by available drugs such as acarbose, miglitol, and voglibose (*9*−*11*).

However, no information is available about Brazilian native fruits and frozen pulps related to α-amylase and α-glucosidase inhibitory activity and, therefore, their potential as sources of beneficial compounds in type 2 diabetes management. This information could be useful for promoting agroindustrial utilization of these fruits, nowadays mostly consumed locally.

Therefore, with the purpose of identifying potential health-promoting effects of nonexplored native Brazilian fruits, the objective of this study was to characterize different native fruits and commercial frozen pulps from Brazil in relation to α-amylase and α-glucosidase inhibitory activity and correlate such bioactivity to the content of flavonoids. It is noteworthy that, to the best of the authors' knowledge, this is the first research that reports the inhibitory activity of such fruits and fruit pulps toward α-amylase and α-glucosidase.

MATERIALS AND METHODS

Materials. Two kilograms of each native fresh fruit, at the ripe stage, and of the commercial frozen pulps was obtained from an experimental plantation of a local frozen pulps producer (Sitio do Bello) in Sao Paulo state, Brazil. Some fruits were acquired directly from producers in the Amazon region,

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according to each harvest season. All samples were cut into pieces, lyophilized, and stored at −20 °C until analyses. The moisture was measured by drying at 70 °C under vacuum, according to the AOAC (1995). All chemicals and solvents were of reagent or HPLC grade. Porcine pancreatic α-amylase (EC 3.2.1.1, VI-A type), yeast α-glucosidase (EC 3.2.1.20, I type), and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO).

Methods. *Total Phenolics*. The determination was performed according to the method of Singleton, Orthofer, and Lamuela-Raventos (*12*). The samples were extracted in a solvent mixture comprising methanol/water/acetic acid (70:30:5). The homogenate was filtered under reduced pressure through filter paper (Whatman no. 1). A 0.25 mL aliquot was mixed with 0.25 mL of the Folin−Ciocalteu reagent and 2 mL of distilled water. After 3 min at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture placed at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm using a model Ultrospec 2000 UV−visible spectrophotometer (Amersham Biosciences, Cambridge, U.K.). The results were expressed as milligrams of catechin equivalents (CE) per 100 g of sample dry weight (DW).

DPPH Radical Scavenging Activity. The extracts obtained above were used to assess the antioxidant capacity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to the method of Brand-Williams, Cuvelier, and Berset (*13*), with some modifications (*14*). A 50 μL aliquot of the extract previously diluted and 250 μL of DPPH (0.5 mM) were shaken, and after 25 min, the absorbance was measured at 517 nm using a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA). The control consisted of a methanolic solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at different concentrations. The antioxidant capacity was expressed as micromoles of Trolox equivalents per gram of sample DW.

Oxygen Radical Absorbance Capacity (*ORAC*) *Assay*. The determination was performed according to the method of Dávalos, Gómez-Cordovés, and Bartolomé (*15*). The extracts obtained on "total phenolics analysis" were used to assess the antioxidant capacity by ORAC assay. The antioxidant capacity was expressed as micromoles of Trolox equivalents per gram of sample DW.

Obtaining Crude Methanolic Extracts (*CME*) *and Polyamide-Purified Phenolic Extracts* (*PAE*). CME were obtained by extraction (1:20 w/v) with a solvent mixture comprising methanol/water (70:30 v/v). The homogenate was filtered under reduced pressure through filter paper (Whatman no. 1). PAE were obtained by solid-phase extraction (SPE) of CME on polyamide SC6 column (Macherey-Nagel GmbH and Co., Düren, Germany). The column was washed with water (20 mL) and further eluted with 20 mL of methanol, to elute the neutral flavonols, and with 20 mL of methanol/ammonia (99.5:0.5), to elute the acidic flavonols and ellagic acid.

Flavonoids and Hydroxycinnamic Acids Contents. Extraction was performed according to the method of Arabbi et al. (*16*) with some modifications. Samples of homogenized fruits and commercial frozen pulps (1 g) were extracted three times in a solvent mixture (100 mL the first time, 50 mL the next two times) comprising methanol/water (70:30) or methanol/water/acetic acid (70:30:5), for anthocyanin-containing samples, at speed 4 for 1 min (Brinkmann homogenizer, Polytron; Kinematica GmbH), while cooled in ice. The homogenate was filtered under reduced pressure through filter paper (Whatman no. 1), and the combined fractions were evaporated under vacuum at 40 °C to ∼20 mL in a rotatory evaporator and made up to 50 mL with water. An aliquot of 25 mL of the extract was added to a 1 g of polyamide SC6 column preconditioned with methanol (20 mL) and water (60 mL). The column was washed with water (20 mL) and further eluted with methanol (40 mL), to elute the neutral flavonols, and with methanol/ammonia (99.5:0.5), to elute the acidic flavonols and ellagic acid. These fractions were evaporated to dryness under pressure at 40 °C, redissolved in HPLC grade methanol (1 mL), filtered through 0.22 μm polytetrafluoroethylene (PTFE) filters (Millipore Ltd., Bedford, MA), and analyzed by HPLC.

Total Ellagic Acid Content. Total ellagic content was determined in 80% (v/v) aqueous acetone extracts according to the method of Pinto et al. (*17*).

HPLC Quantitation of Flavonoids and Hydroxycinnamic and Ellagic Acids. Identification and quantification of flavonoids were achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector according to the method of Arabbi et al. (*16*). The column used was 250 × 4.6 mm, i.d., 5 μm, Prodigy ODS3 reversed phase silica (Phenomenex Ltd., Torrance, CA), and elution solvents were (A) water/tetrahydrofuran/trifluoroacetic acid (98:2:0.1) and (B) acetonitrile. The solvent gradient was the same as in ref *16* except for the separation of acidic flavonols, where the initial % B was 25% to allow separation of ellagic acid from quercetin derivatives, as described in Pinto et al. (*18*). Samples were injected in duplicate. Calibration was performed by injecting the standards three times at five different concentrations. Results were expressed as milligrams of aglycon per 100 g of sample DW. Hydroxycinnamic acids were expressed as milligrams of chlorogenic acid per 100 g of sample DW.

α-*Amylase Inhibition Assay*. Two extracts were evaluated for α-amylase inhibition: CME, obtained through extraction (1:20 w/v) in a solvent mixture comprising methanol/water (70:30 v/v) or methanol/water/acetic acid (70:30:5), for anthocyanin-containing samples, and PAE, obtained after polyamide SC6 cleaning extraction, described previously. The α-amylase inhibitory activity was determined by the chromogenic method described by Ali et al. (*19*). Porcine pancreatic α-amylase (EC 3.2.1.1, type VI) was dissolved in ice-cold distilled water to give a concentration of 4 units/mL of solution. Potato starch (0.5% w/v) in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride was used as the substrate. In this experiment, 40 μL of sample extract, 160 μL of distilled water, and 400 μL of starch were mixed in a screw-top plastic tube. The reaction was started by the addition of 200 μL of the enzyme solution. The tubes were incubated at 25 °C for a total of 3 min. Final concentrations in the incubation mixture were plant extract, 1 mg/mL, 0.25% (w/v) starch, and 1 unit/mL enzyme. At intervals from addition of the enzyme (1, 2, and 3 min), 200 μL of mixture was removed and added into a separate tube containing 100 μL of DNS color reagent solution (96 mM 3,5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 M NaOH) and placed into a 85 °C water bath. After 15 min, this mixture was diluted with 900 μL of distilled water and removed from the water bath. α-Amylase activity was determined by measuring the absorbance of the mixture at 540 nm. The absorbance (*A*) due to maltose generated was calculated as

$$A_{540\text{nm}}(\text{control or plant extract}) = A_{540\text{nm}}(\text{test}) - A_{540\text{nm}}(\text{blank})$$

From the net absorbance obtained, the percent (w/v) of maltose generated was calculated using a maltose standard calibration curve (0−0.1%, w/v). Percent of inhibition was calculated as 100 − % reaction at t = 3 min, whereby the % reaction = (mean maltose in sample/mean maltose in control) × 100. Results were expressed as micrograms of sample (DW) per milliliter of reaction and as micrograms of catechin equivalent per milliliter of reaction necessary to inhibit 50% of maltose production.

α-*Glucosidase Inhibition Assay*. Both CME and PAE were evaluated. The α-glucosidase inhibitory assay was based on the chromogenic method described by Watanabe et al. (*20*), with some modifications (*21*). Briefly, yeast α-glucosidase (0.7 U) was dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/L bovine serum albumin and 0.2 g/L sodium azide. The substrate solution, 5 mM *p*-nitrophenyl-α-D-glucopyranoside, was prepared in the same buffer (pH 7.0). Enzyme solution (100 μL) and sample (20 μL) were mixed, and the absorbance was read at 405 nm (time zero) (Benchmark Plus, Bio-Rad Laboratories, Hercules, CA). After 5 min of incubation at 37 °C, the substrate solution (100 μL) was added and incubated for another 5 min at 37 °C, and the absorbance at 405 nm was determined (10 min). The extracts were diluted in several rates (1/2, 1/3, 1/5, 1/10, 1/20 and 1/40) and compared to a control (100 μL of methanol instead of fruit extract). The α-glucosidase inhibitory activity was expressed as percentage of inhibition and was calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{Abs}_{405\text{nm}}\ (\text{control}) - \text{Abs}_{405\text{nm}}\ (\text{extract})}{\text{Abs}_{405\text{nm}}\ (\text{control})} \times 100$$

Results were expressed as micrograms of sample (DW) per milliliter of reaction and as micrograms of catechin equivalent per milliliter of reaction necessary to inhibit 50% of glucose production.

Statistical Analysis. All analyses were run in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using STATISTICA 5.0 software (StatSoft, Inc., Tulsa, OK). Differences between means were first analyzed by ANOVA test and then

Table 1. Botanical Identification and Moisture of Brazilian Native Fruits

	origin	family	scientific name	moisture (%)
fruits				
cambuci	Atlantic forest	Myrtaceae	*Campomanesia phaea* Berg.	87.1 ± 0.4
araçá-boi	Amazonia	Myrtaceae	*Eugenia stipitata* Mc. Vaugh	87.9 ± 0.1
camu-camu	Amazonia	Myrtaceae	*Myrciaria dubia* Mc. Vaugh	90.2 ± 0.3
araçá	Atlantic forest and Amazonia	Myrtaceae	*Psidium guineensis* Sw.	75.8 ± 0.4
bacuri	Amazonia	Arecaceae	*Scheelea phalerata*	83.3 ± 0.3
buriti	Cerrado	Arecaceae	*Mauritia flexuosa*	64.2 ± 0.6
star fruit	Ásia	Oxalidaceae	*Averrhoa carambola*	91.4 ± 0.8
cupuaçu	Amazonia	Sterculiaceae	*Theobroma grandiflorum* (Willd. ex Spreng) Schum	54.9 ± 0.2
graviola	Central America and Amazonia	Annonaceae	*Annona muricata* L.	80.5 ± 0.4
maná-cubiu	Amazonia	Solanaceae	*Solanum sessiliflorum*	72.1 ± 1.5
tucumã	Amazonia	Arecaceae	*Astrocaryum aculeatum*	37.7 ± 0.4
uxi	Amazonia	Humiriaceae	*Endopleura uchi*	49.8 ± 0.7
abiu	Amazonia	Sapotaceae	*Pouteria caimito* (Ruiz et Pavon) Radlk.	66 ± 2
granadilla	Central America	Passifloraceae	*Passiflora ligularis* Juss	42.9 ± 1.5
tamarindo	Africa	Leguminosae	*Tamarindus indica* L.	38 ± 1
sweet passion fruit	Central America and Atlantic forest	Passifloraceae	*Passiflora alata* Curtis	71.4 ± 1.0
commercial pulps				
araçá	Atlantic forest and Amazonia	Myrtaceae	*Psidium guineensis* Sw.	89 ± 2
cambuci	Atlantic forest	Myrtaceae	*Campomanesia phaea* Berg.	89.6 ± 0.5
umbu	Cerrado	Anacardiaceae	*Spondias tuberosa* Arruda	89.8 ± 0.4
coquinho azedo	Cerrado	Palmaceae	*Butia capitada* Becc.	87.4 ± 0.3
panã	Cerrado	Annonaceae	*Annona crassifolia* Mart.	83.9 ± 0.4
cagaita	Cerrado	Myrtaceae	*Eugenia dysenterica* DC.	91.1 ± 0.1

followed by post hoc Newman−Keuls test. A probability level below 0.05 was considered to be significant. Pearson's correlation coefficients were used to investigate pairwise association between continuous variables.

RESULTS AND DISCUSSION

Botanical Characterization of Native Fruits. The fruits analyzed in this work mainly originate in Amazonia and Cerrado regions. Botanical identification following the classification proposed by the APG II system (Angiosperm Philogeny Group) is presented in **Table 1**. The most representative botanical families of the fruits were Myrtaceae, Arecaceae, and Passifloraceae. Moisture contents of the native fruits and commercial frozen pulps ranged from 38 to 91%, for fruits, and from 84 to 91%, for commercial frozen pulps (**Table 1**). Camu-camu and star fruits presented the highest moisture contents, whereas tucumã presented the lowest value. Among commercial frozen pulps, cagaita (90%) and umbu (91%) presented the highest contents of moisture and panã the lowest (ca. 84%).

Total Phenolics Content and in Vitro Antioxidant Capacity. The results of total phenolics content (Folin−Ciocalteu reducing capacity) and in vitro antioxidant capacity (DPPH radical scavenging capacity and ORAC) of fruits and commercial pulps are presented in **Figures 1** and **2**. Generally, camu-camu presented the highest antioxidant capacity followed by tucumã and uxi. In relation to Folin−Ciocalteu reducing capacity, camu-camu presented the highest value, which was around 10 times higher than those of tucumã and uxi. In the case of fruits rich in ascorbic acid, total phenolics content determined by means of the Folin−Ciocalteu reagent is most correctly referred to as Folin−Ciocalteu reducing capacity, due to the interference of this compound above certain levels. As previously shown (*8*) this would be the case of camu-camu. Ascorbic acid present in crude extracts is also able to scavenge DPPH radicals and presents ORAC.

Camu-camu and maná-cubiu fruits presented the highest values of antioxidant capacity by the ORAC method. Indeed, there was a positive correlation (**Figure 3**) between the antioxidant capacity obtained through the ORAC method and the total phenolic content determined through the Folin−Ciocalteu reducing capacity ($r = 0.795$; $p < 0.001$).

Also, there was a significant correlation between Folin−Ciocalteu reducing capacity and DPPH radical scavenging capacity ($r = 0.989$; $p < 0.001$) (**Figure 3**). The high correlation between Folin−Ciocalteu and DPPH is due to the fact that both are based on similar action mechanisms, that is, in electron transference, whereas the ORAC method is based on the transference of hydrogen atoms from the antioxidant to the radical AAPH (*22*).

Wang et al. (*23*) determined the antioxidant capacity of 12 different fruits through the ORAC method and found high correlation between the antioxidant capacity value and phenolic content. They reported that the contribution of ascorbic acid for the antioxidant activity (ORAC) was <15%. The authors also found a positive correlation between the antioxidant capacity determined by the ORAC method and the total phenolic content present in some other fruits such as blackberry, raspberry, and strawberry. Folin−Ciocalteu reducing capacity and DPPH of uxi and tucumã are similar to those of different cultivars of strawberry reported by Pinto et al. (*17*).

Among the ORAC values of fruits, nuts, and vegetables reported by the U.S. Department of Agriculture (*24*), those for apples, pomegranate juice, peach, fig, guava, and tangerine were very close to those obtained for the fruits analyzed here. Camu-camu fruit presented the highest ORAC value, similar to the fruits lemon, plum, cranberry (*Vaccinium* subgenus *cyanococcus*) and blueberry (*Vaccinium* subgenus *oxycoccus*).

Flavonoids and Ellagic and Hydroxycinnamic Acids Contents. Fruits and vegetables are the main dietary sources of flavonoids, and their potential health benefits are associated with the powerful hydrogen-donating and also their reducing properties, which contribute to redox regulation in cells (*25*). Among native Brazilian fruits, the flavonoid contents varied significantly (**Table 2**). Catechin and epicatechin were detected only in tucumã, graviola, and star fruit, ranging from 15 to 79 mg/100 g of sample (DW). Quercetin and its derivatives were detected in all frozen fruit pulps and in some fruits. The highest contents were found in araçá and camu-camu, 40 and 42 mg/100 g of sample (DW), respectively.

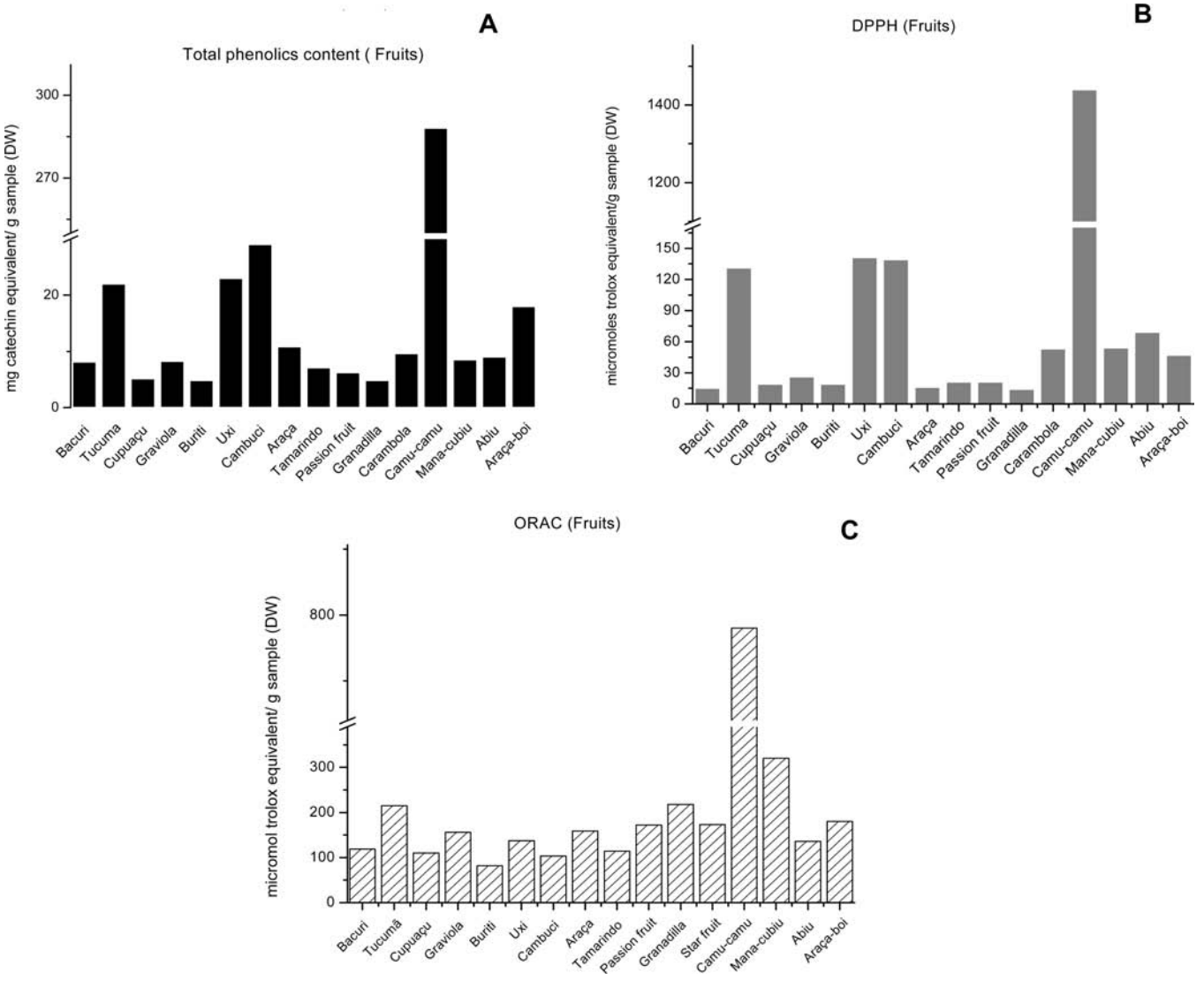

Figure 1. Total phenolics content (**A**) and antioxidant capacity determined by DPPH scavenging activity (**B**) and ORAC method (**C**) of native fruits from Brazil.

In contrast to the fruits, cambuci and araça frozen pulps presented low contents of these compounds. According to Arabbi et al. (*16*), besides the content variation of the fruits, losses can also occur during the fruit pulp manufacturing process, which may also explain the differences found in our study. Kaempferol was detected in low concentrations (0.4−2.5 mg/100 g of DW) in cambuci, araçá-boi, camu-camu, and araça (all fruits from the Myrtaceae family). In relation to the fruit frozen pulps, kaempferol was found only in panã and cagaita, panã presenting the highest concentration of 4.4 mg/100 g of sample (DW). Only camu-camu presented significant amounts of anthocyanins (306 mg/100 g of DW) and mainly as cyanidin derivatives. In its ripe stage, this fruit presents a color that varies from red to purple, as the result of the presence of anthocyanins in its peel.

A variation in the content of anthocyanins between 337 and 606 mg/100 g of DW was found in camu-camu cultivated in two different regions in Brazil (*26*). These authors also reported that cyanidin derivatives were the main types of anthocyanins found in this fruit.

It is known that free ellagic acid contents in fruits are significantly low. However, the highest amounts of this compound are detected after acid hydrolysis of extracts, as a result of ellagitannin breakdown (*27*). Among the fruits analyzed here, ellagic acid was detected in umbu, camu-camu, cagaita, araçá, and cambuci (**Table 2**). Free ellagic acid content ranged from 0.9 to 16 mg/100 g of sample (DW), representing 0.3−3% of total, respectively. In Brazilian diet, strawberry and its derivatives such as jam and juice represent the major sources of ellagic acid. Pinto et al. (*17*) analyzed the content of free ellagic acid in the seven most consumed strawberry cultivars in Brazil and reported values ranging from 150 to 430 mg/100 g of sample (DW).

The highest content of hydroxycinnamic acids, among fruits, was detected in maná-cubiu (239 mg/100 g of DW), the unique representative of the Solanaceae family. Some studies have already reported that the Solanaceae family presents high quantities of phenolic acids, chiefly chlorogenic acids (*28*). Hydroxycinnamic acids were also found in buriti and abiu as well, but in lower contents. Among the frozen fruit pulps, only in umbu and coquinho azedo were these compounds detected, in very low levels.

α-Amylase Inhibitory Activity. The α-amylase inhibition activity of crude methanolic extracts could not be evaluated due to the presence of reducing sugars, glucose and fructose, compounds that react with the dinitrosalicylic acid (DNS), interfering in the determination. In this regard, sugars, vitamins, and minerals possibly present in crude extracts were eliminated and a methanolic fraction rich in flavonoids was obtained through SPE in a polyamide SC6 column. The α-amylase inhibition activity of the

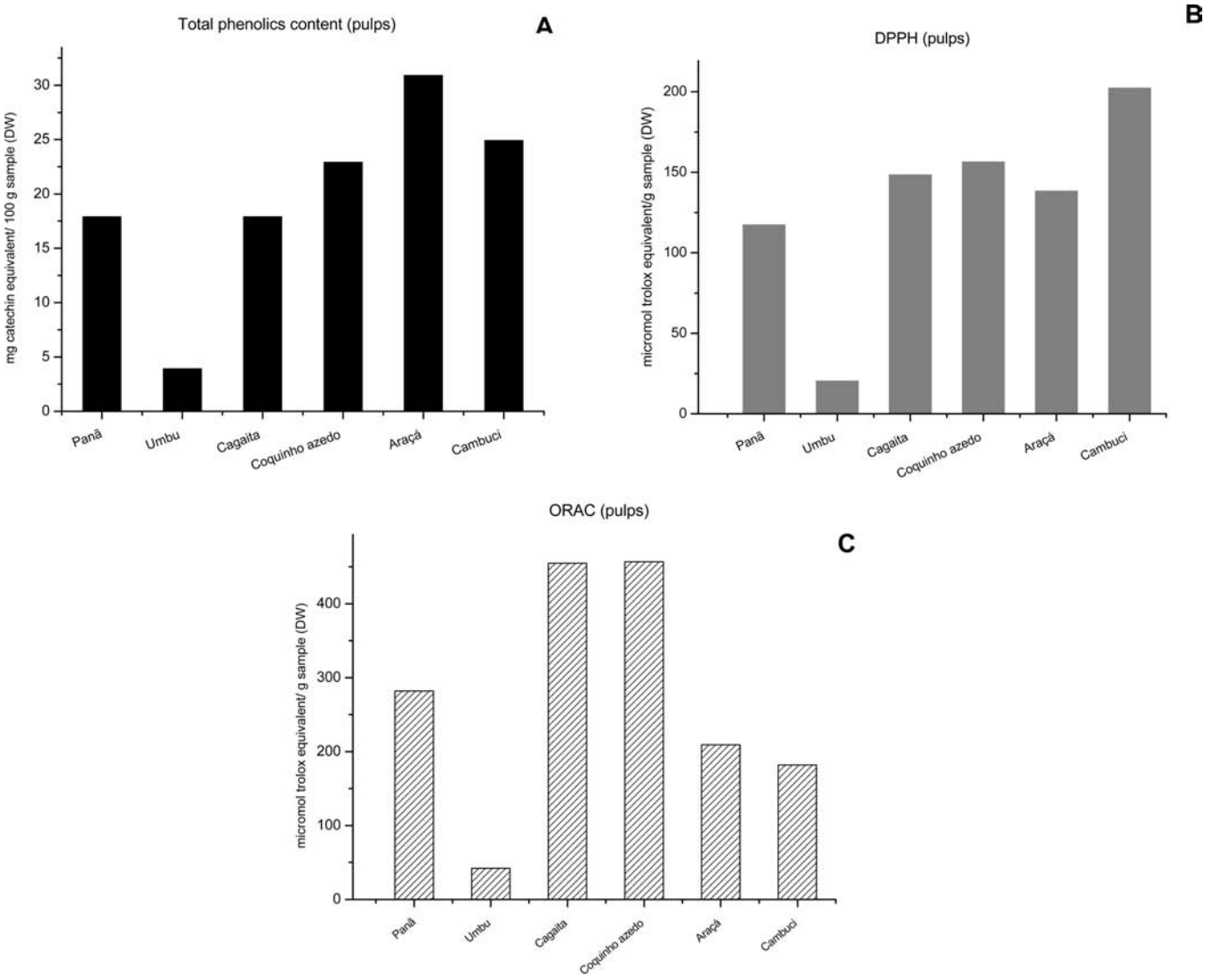

Figure 2. Total phenolics content (**A**) and antioxidant capacity determined by DPPH scavenging activity (**B**) and ORAC method (**C**) of native commercial pulps from Brazil.

resulting PAE is shown in **Table 3**. The results were expressed both on a sample weight basis and as equivalents of catechin required to inhibit 50% of α-amylase activity. All fruits presented inhibitory activity toward α-amylase except for bacuri, uxi, maná-cubiu, and abiu and, among pulps, umbu. The most potent inhibition was observed for cambuci and cupuaçu fruits (2.5−9 times higher than other fruits) and panã frozen pulp (**Table 3**).

The efficiency of phenolic compounds for α-amylase inhibition (IC_{50} expressed in mg of catechin equivalent) was cupuaçu = cagaita frozen pulp > cambuci = star fruit > araça > tucumã > sweet passion fruit = granadilla = panã frozen pulp = cambuci frozen pulp. Comparatively, phenolic compounds of coquinho azedo, araçá-boi, camu-camu, and buriti were much less potent. These results show that the α-amylase inhibitory activity does not depend only on the presence of flavonoids, because other phenolic compounds present in cupuaçu, sweet passion fruit, and granadilla also showed significant inhibition. On the other hand, the highest content of hydroxycinnamic acids from maná-cubiu (239 mg/100 g of DW) did not result in α-amylase inhibition. The high flavanol contents observed in tucumã, star fruit, and graviola seem to be directly connected to the inhibitory activity of α-amylase, whereas the same does not occur with quercetin.

Camu-camu, although having a high content of anthocyanins and quercetin, did not inhibit α-amylase as efficiently as star fruit, reinforcing the hypothesis about the influence of the type of flavonoids in this assay. Araça presented a similar content of quercetin but a 3 times higher α-amylase inhibition, indicating that differences in glycosylation may also be involved.

Among the vegetables already studied, green pepper, broccoli, ginger, and carrot presented high in vitro inhibition of α-amylase. Among fruits, red grapes, strawberry, and raspberry presented good inhibition toward α-amylase (*7*, *9*).

Few studies refer to the inhibition activity of α-amylase from fruits or their bioactive compounds. Tadera et al. (*29*) reported that the flavonoids quercetin, myricetin, epigallocatechin gallate, and cyanidin were efficient inhibitors of α-amylase. Tannins, present in green and black teas, grapes, wine, raspberry, and strawberry, also seem to be good α-amylase inhibitors. The removal of tannins from fruit extracts by Sephadex LH-20 suggests that the responsible compounds of α-amylase inhibition are hydrolyzable tannins (*30*). In the present study, the efficiency of tannins was not assessed as they were eliminated through irreversible binding to polyamide.

α-Glucosidase Inhibition Assay. Table 3 shows the α-glucosidase inhibitory activity of crude methanolic extracts and polyamide extracts of fruits and frozen pulps analyzed in this work. In this assay there was no interference from sugars present in fruits and frozen fruit pulps; however, with the goal

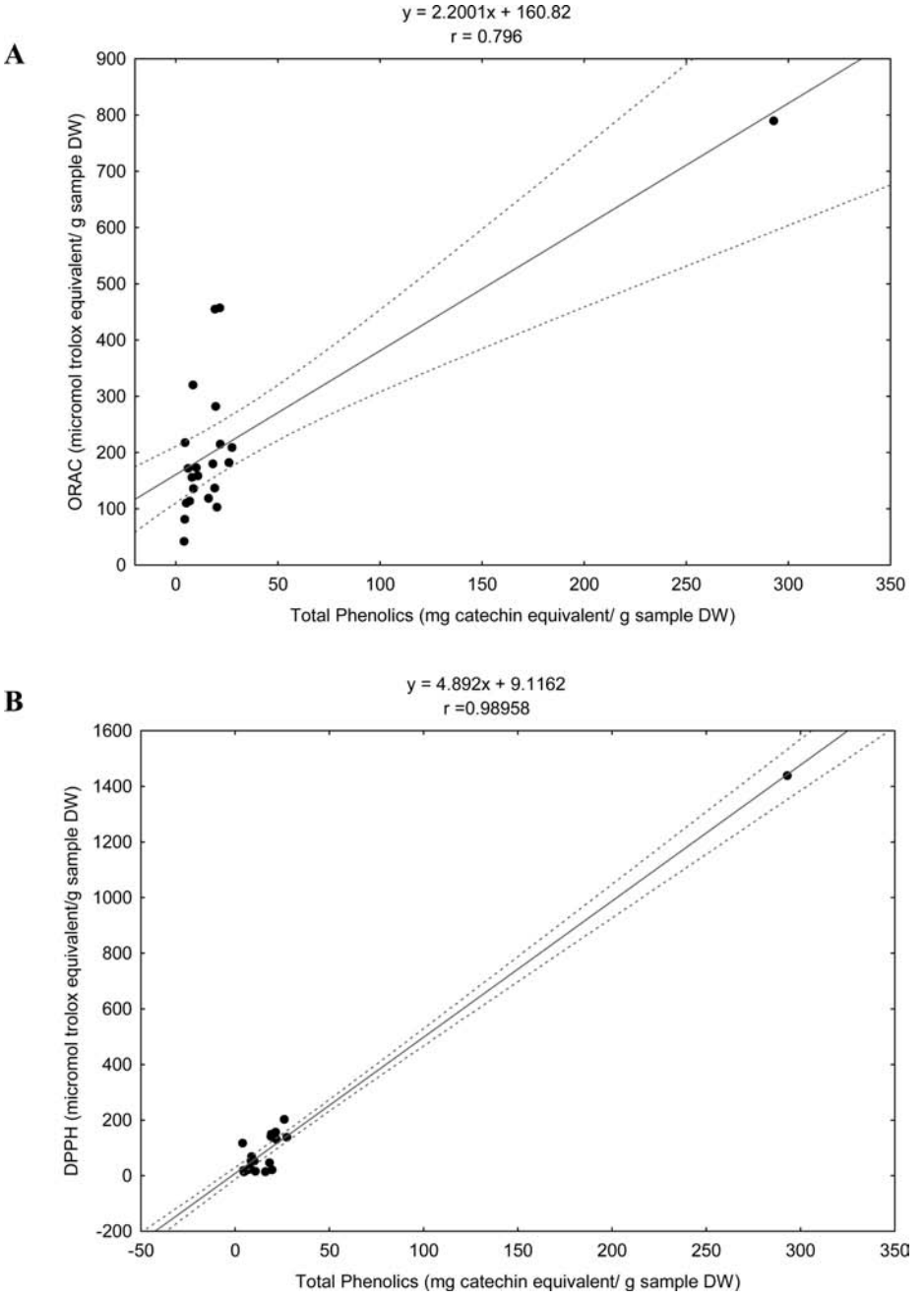

Figure 3. Pearson correlation between total phenolics and ORAC (**A**) and between total phenolics and DPPH scavenging capacity (**B**) of Brazilian native fruits and frozen pulps.

of assessing the efficiency of flavonoids present in these samples, the same extract analyzed in the α-amylase inhibitory activity assay was used.

In contrast to what was observed with α-amylase, the PAE (and also the CME) obtained from bacuri and uxi showed significant inhibition of α-glucosidase activity. As these fruits did not present flavonoids or hydroxycinnamic acids in their composition, it could be possible that other hydroxybenzoic acids besides ellagic acid would be implicated.

The PAE obtained from cambuci and araça fruits were the most effective in inhibiting α-glucosidase, and among the frozen fruit pulps extracts obtained from both cagaita and cambuci were the most efficient. Phenolic compounds from these fruits were also more efficient in the α-glucosidase inhibition, and this fact could be observed on IC_{50} values expressed in catechin equivalent per milliliter of reaction.

Cambuci and araça frozen fruit pulp presented high contents of quercetin derivatives, which were also detected in high amounts in araçá-boi and camu-camu. However, these compounds did not display efficacy as α-glucosidase inhibitors, again indicating that this would be the result of a different pattern of glycosylation.

Cupuaçu, which presented excellent α-amylase inhibition activity, did not demonstrate the same efficacy in the α-glucosidase inhibition.

Sweet passion fruit, granadilla, and maná-cubiu fruits did not present any α-glucosidase inhibition activity after polyamide extract purification, indicating that the inhibition observed for crude extracts was due to nonflavonoid compounds. Hydroxycinnamic acids present in maná-cubiu did not inhibit α-glucosidase, similar to what was observed in the α-amylase assay.

In addition to the fruits and frozen pulp fruits, the α-glucosidase and α-amylase inhibition activities of some pure compounds

Table 2. Flavonoids and Ellagic and Hydroxycinnamic Acids Contents (Milligrams per 100 g of Sample Dry Weight) of Brazilian Native Fruits and Commercial Frozen Pulps[a]

	flavanols		flavonols		anthocyanins			
	catechin	epicatechin	quercetin	kaempferol	cyanidin	hydroxycinnamic acids	free ellagic acid	total ellagic acid
fuits								
cambuci	nd	nd	21.6 ± 0.3a	0.4 ± 0.1a	nd	nd	2.5 ± 0.1a	240 ± 15a
araça-boi	nd	nd	14.4 ± 0.2b	2.5 ± 0.1b	nd	nd	nd	nd
camu-camu	nd	nd	42 ± 4c	2.1 ± 0.1b	306 ± 10	nd	16 ± 1c	490 ± 20b
araça	nd	nd	40 ± 2c	0.7 ± 0.1a	nd	nd	7.4 ± 0.4b	262 ± 12a
maná-cubiu	nd	nd	nd	nd	nd	239 ± 11a	nd	nd
tucumã	79 ± 5a	nd	2.96 ± 0.05d	nd	nd	nd	nd	nd
buriti	nd	nd	0.6 ± 0.1e	nd	nd	9.0 ± 0.4b	nd	nd
abiu	nd	nd	nd	nd	nd	19 ± 1c	nd	nd
star fruit	22 ± 2b	36 ± 2a	nd	nd	nd	nd	nd	nd
graviola	15.9 ± 0.3b	15 ± 1b	nd	nd	nd	nd	nd	nd
bacuri	nd	nd	nd	nd	nd	nd	nd	nd
cupuaçu	nd	nd	nd	nd	nd	nd	nd	nd
uxi	nd	nd	nd	nd	nd	nd	nd	nd
tamarindo	nd	nd	nd	nd	nd	nd	nd	nd
granadilla	nd	nd	nd	nd	nd	nd	nd	nd
sweet passion fruit	nd	nd	nd	nd	nd	nd	nd	nd
commercial pulps								
araçá	nd	nd	4.3 ± 0.2d	nd	nd	nd	7.1 ± 0.4b	218 ± 18b
cambuci	nd	nd	4.0 ± 0.1d	nd	nd	nd	12.8 ± 0.5c	512 ± 35c
umbu	nd	nd	9.4 ± 0.5e	nd	nd	0.2 ± 0.1d	0.9 ± 0.1a	314 ± 20a
coquinho	nd	nd	26 ± 2a	nd	nd	1.9 ± 0.1e	nd	nd
panã	nd	nd	4.1 ± 0.2d	4.4 ± 0.2b	nd	nd	nd	nd
cagaita	nd	nd	27 ± 1f	0.9 ± 0.1a	nd	nd	5 ± 1b	289 ± 24ab

[a] Values are expressed as means ± SD for triplicates. Means in the same column with common letters are not significantly different ($p < 0.05$). nd, not detected.

Table 3. α-Amylase and α-Glucosidase Inhibitory Activity of Methanolic (CME) and Polyamide-Purified Extracts (PAE) Obtained by Solid-Phase Extraction of Native Fruits and Commercial Frozen Pulps[a]

	α-amylase		α-glucosidase			
	PAE		CME		PAE	
sample	IC_{50} (mg of sample dw/mL of reaction)	IC_{50} (mg of CE[b]/mL of reaction)	IC_{50} (mg of sample dw/mL of reaction)	IC_{50} (mg of CE/mL of reaction)	IC_{50} (mg of sample dw/mL of reaction)	IC_{50} (mg of CE/mL of reaction)
fruits						
bacuri	nd	nd	0.5	1.6	6.2	2.6
tucumã	2.9	0.6	1.2	5.9	1.7	1.3
cupuaçu	1.1	0.3	nd	nd	nd	nd
graviola	9.1	0.9	nd	nd	2.7	1.8
buriti	4.6	1.8	1.9	1.8	2.7	1.1
uxi	nd	nd	0.5	2.3	3.2	1.9
cambuci	1.0	0.4	0.3	1.3	0.6	0.4
araçá	6.8	0.5	1.0	2.4	0.6	0.2
tamarindo	6.3	1.0	nd	nd	nd	nd
sweet passion fruit	4.4	0.7	3.5	5.1	nd	nd
granadilla	4.6	0.7	2.1	2.2	nd	nd
star fruit	2.4	0.4	2.0	4.5	3.1	2.7
camu-camu	4.1	1.8	1.3	73.1	3.3	2.9
maná-cubiu	nd	nd	3.1	6.0	nd	nd
abiu	nd	nd	nd	nd	2.5	0.9
araçá-boi	3.3	2.9	0.6	2.4	2.4	1.4
commercial pulps						
panã	1.3	0.7	1.3	5.8	1.8	1.6
umbu	nd	nd	nd	nd	nd	nd
cagaita	3.8	0.3	0.5	1.0	1.0	0.9
coquinho azedo	7.2	3.8	1.2	5.9	3.4	5.0
araçá	5.9	1.2	nd	nd	nd	nd
cambuci	0.8	0.7	0.2	0.9	0.7	0.8

[a] nd, not detected. [b] Milligrams of catechin equivalent.

such as rutin, quercetin, chlorogenic acid, and catechin, which are present in some of the samples, were also tested (**Table 4**). Among them, quercetin was the compound that presented the highest inhibitory activity, for both α-glucosidase and α-amylase enzymes. Glycosylation of quercetin such as in rutin (Q-3-rut) significantly decreases the inhibition potential for both enzymes, mainly α-glucosidase. This could be related with the much lower α-glucosidase inhibition activity of coquinho azedo despite the

Table 4. α-Glucosidase and α-Amylase Inhibitory Activity of Pure Compounds[a]

	IC_{50} (μM)	
pure compound	α-glucosidase inhibitory activity	α-amylase inhibitory activity
acarbose	5.9	nd
rutin	1.3	1.2
quercetin	0.1	0.9
chlorogenic acid	7.6	3.9
catechin	3.1	2.1
ellagic acid	3.3	2.0
rosmarinic acid	nd	1.4

[a] nd, not detected.

6 times higher quercetin content compared to cagaita and cambuci frozen pulps. Most probably, the kind and position of sugars attached to quercetin are responsible for the differences observed. Acarbose, traditionally used in diabetes treatment, when compared to phenolic compounds, was more efficient than only chlorogenic acid in the inhibition of α-glucosidase and did not inhibit α-amylase. Chlorogenic acid, among all tested compounds, was the least efficient in α-glucosidase and α-amylase inhibitory assays.

Tadera et al. (*29*) verified that the most effective compounds in α-glucosidase inhibition were myricetin, epigallocatechin galate (EGCG), and cyanidin and that there was a considerable increase in the inhibitory activity of flavonoids with the increase of the number of hydroxyls linked to the B ring.

Apostolidis et al. (*31*) evaluated the α-glucosidase inhibitory activity of yogurt based on milk and soybeans enriched with fruits and found high correlation with the total phenolic contents. They observed that the yogurts that were enriched with blueberries (*Vaccinium* subgenus *cyanococcus*) were those that presented a higher content of total phenolics and were the most effective in inhibiting α-glucosidase.

In another study, red grapes and cinnamon also presented high antioxidant and α-glucosidase inhibitory activities, due to the high content of total phenolics. However, the same study showed that the extracts with higher antioxidant activity were more efficient in inhibiting α-amylase than in inhibiting α-glucosidase (*9*).

From our results, there seems to be no correlation among antioxidant and α-amylase and α-glucosidase inhibitory activities. Fruits such as uxi with high antioxidant capacity and phenolic content failed to inhibit amylase. Camu-camu, with the highest antioxidant activity and phenolic content, presented medium and low inhibitory activities against α-amylase and α-glucosidase, respectively.

Kim et al. (*21*) assessed the efficacy of 21 phenolic compounds toward α-glucosidase and α-amylase inhibition, among them quercetin, hesperidin, luteolin, rutin, and kaempferol. Luteolin aglycon was the most efficient compound in the α-glucosidase inhibition. The study reported that some glycosylated compounds, such as rutin, can bind to α-glucosidase and increase its activity and are, therefore, considered to be inefficient in inhibiting this enzyme. However, the flavones luteolin and luteolin-7-*O*-glucoside and the glycosylate derived from kaempferol were the most efficient in α-amylase inhibition.

From our results, the best α-glucosidase inhibitory activity was presented by phenolics from fruits with also a high inhibition toward α-amylase, such as cambuci and araça. High amylase inhibition is not a good quality as it can result in undigested starch. In this sense, the best antidiabetic potential would be of those fruit phenolics causing high α-glucosidase inhibition and medium to low α-amylase inhibition, such as buriti and araça-boi.

In conclusion, excellent sources of bioactive compounds can be found among Brazilian fruits, such as uxi, cambuci, and camu-camu. Cambuci, camu-camu, araçá, umbu, and cagaita were, for the first time, shown to be rich sources of ellagic acid, whereas maná-cubiu can be considered a suitable source of hydroxycinnamic acids. Araçá, camu-camu, cambuci, and araçá-boi presented high contents of glycosylated quercetin derivatives, and their potency as inhibitors of enzymes of carbohydrate metabolism seems to be related to the pattern of glycosylation.

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