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Fresh and Spray Dried Pitanga (*Eugenia uniflora*) and Jambolan (*Syzygium cumini)* Pulps are Natural Sources of Bioactive Compounds with Functional Attributes

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Abstract

This study approaches the physicochemical and bioactive characterization of fresh (FR), spray dried (SD) and freeze dried (FD) jambolan (JA) and pitanga pulps (red: RP and purple: PP) varieties). In general, the concentration of bioactive compounds followed the tendency FR>FD>SD. All samples presented high proanthocyanidin content (18.7 to 121.9 g QTE/Kg^{-1} DW), ellagic acid (0.203 to 0.943 g/Kg^{-1} DW) and anthocyanins (up to 15.8 g/Kg^{-1} DW). In this study, JA, RP and PP powders showed activity against alpha-amylase, alpha-glucosidase and pancreatic lipase. This is the first report of protocatechuic and p-coumaric acids in fresh and spray dried red pitanga pulps. Also, this is the first time that the colorant potential and proanthocyanidin content of dried PP is shown. Overall, this research presents fresh data about these tropical fruits and demonstrates the multifunctional attributes of these understudied Myrtaceae examples of Brazilian biodiversity.

Keywords: Brazilian berries; Flavonoids; Drying

Introduction

Brazil is home to several tropical fruits, including pitanga (*Eugenia uniflora L.*) and jambolan (*Syzygium cumini L.*). The pitanga tree is a Brazilian native plant which produces exotic berry-like fruits also known as Brazilian cherries. These fruits come in three different color varieties (red, orange and purple), each one with very specific attributes, such as different phenolic profiles, anthocyanins, carotenoids and antioxidant activity [1]. The jambolan, also known as black plum, is a tropical fruit with an intense purplish color and sweet/astringent flavor. Besides being bioactive-rich fruits, pitanga and jambolan present outstanding colorant properties, due to their vivid exotic colors [2].

Some scientific studies have shown the health-relevant effects of pitanga [3] and jambolan fruits [4], but very few have focused on the technological exploitation of derived products. The high perishability of both pitanga and jambolan associated with poor post-harvest procedures restricts the popularity and market of these fruits worldwide. Thus, technological processes have to be established for a rational use of these potential food ingredients. We have successfully shown that drying techniques are efficient alternatives to producing high-value tropical dried fruits and residues, besides obtaining final products with preserved bioactive compounds and extended shelf life [5-7].

Therefore, this research investigates the physicochemical and bioactive characterization of jambolan and the two best known pitanga varieties (red and purple) dried by two different techniques: spray drying and freeze drying. The impact of these drying processes is discussed and fresh data concerning the phenolic profile and important *in vitro* functional attributes such as the inhibition of key metabolism enzymes and antimicrobial activity is presented. This biochemical rationale will help to clarify the real value of these understudied Myrtaceae fruits of Brazilian biodiversity.

Materials and Methods

Materials

Red pitanga (RP), purple pitanga (PP) (*Eugenia uniflora L.*) and jambolan (JA, *Syzygium cumini L.*) fruits used in this study were harvested at physiological maturity, which was established according to the color of fruit skins (red for RP, purple for PP and JA), using visual observation. The fruits were sanitized, screened for uniformity and processed into fruit pulp using a depulping machine (model Compacta, Itametal, Brazil). Several batches of pitanga and jambolan pulps were obtained and homogenized in order to constitute a single batch of each fruit that was used for all experiments.

Experimental drying

Two drying processes were applied: spray drying and freeze drying. The spray drying process was performed using a spray dryer model LM MSD 1.0 (Labmaq, Ribeirão Preto, Brazil). For this, 540 g of each fruit pulp (jambolan, red pitanga or purple pitanga) were mixed with 60 g of Arabic gum (AG) and injected into the equipment, which operated at 110°C (inlet air temperature) with a 1.2 mm diameter atomizing nozzle. The air flow (45 L/min) and feed flow (0.53 mL/min) were kept constant. These operational conditions were selected based on previous experiments (data not shown). The obtained fruit powders were immediately weighed and kept refrigerated in amber bottles until analyzed. The freeze drying process was performed using a freeze drier model L101 (Liobras, Campinas, Brazil). The frozen pulps (-18°C)

were freeze-dried for 48 hours at -40°C with a constant speed of 1 mm/h, vacuum of 0.5 mmHg and final pressure of 0.05 mmHg.

Physicochemical characterization

The pitanga (red and purple) and jambolan powders were analyzed for water activity (Aqualab Decagon, Pullman, WA, USA) and pH (Hanna Instruments, Woonsocket, RI, USA). The powder solubility was determined according to Cano-Chauca et al. [8].

Colorimetric analysis

The color measurements (L^*, a^* and b^*) of fruit powders were conducted in a Color Quest XE colorimeter (Hunter Lab, Reston, VA, USA). The parameters C^* (chroma) and H^* (hue angle) were calculated according to the equations $C^*=(a^*2 + b^*2)$ 1/2 and $h^*=\arctan (b^*/a^*)$, respectively. In addition, the total color difference (ΔE) was calculated according to $\Delta E=(\Delta L^*2 + \Delta a^*2 + \Delta b^*2)1/2$ (Pathare et al.) [9].

Preparation of fruit extracts

Initially, samples of dried pulp (1 g) or fresh pulp 5 g were homogenized in 40 mL of extraction solution consisting of methanol/water/acetic acid (70:30:0.5, v/v) using an ultra-turrax® (IKA®Labortechnix, T25 Basic, Wilmington, USA) for 3 min. After this, the samples were centrifuged at 7500 g at 10°C for 10 min. The supernatant was filtered under vacuum using Whatman no. 6 filter paper and further used for the determination of total phenolic content (TPC), total monomeric anthocyanins (TMA), diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity and enzymatic inhibition assays, and for the analysis of flavonoids and free ellagic acid (EA) using high-performance liquid chromatography (HPLC).

Total phenolic content (TPC) and ascorbic acid

The analysis was performed according to Nóbrega et al. [6] and the absorbance was measured at 725 nm using a spectrophotometer (Genesys 10S UV-VIS Thermo Scientific, Waltham, MA, USA). Results were expressed as g gallic acid equivalents (GAE)/Kg^{-1} dry weight (DW). The analysis of ascorbic acid was performed according to AOAC [10].

Total carotenoids (TC)

The analysis of total carotenoids was performed according to Lichtenthaler and Buschmann [11]. The carotenoid extraction was conducted by mixing the samples (0.5 g) with 15 mL of acetone (VETEC, Brazil) for 20 min in the absence of light at room temperature (25°C). The results were expressed as g/Kg^{-1} DW.

Total monomeric anthocyanins (TMA)

This was measured by the pH differential method by Giusti and Wrosltad [12] with samples absorbance readings at 510 nm (A510) and 700 nm (A700). Anthocyanin concentration was calculated as [(A510-A700)] pH 1–[(A510-A700)] pH 4, 5 × (MW × DF × 1000 × ε-1), using the values MW 449.2 $g.mol^{-1}$ and ε 26,900 $L^{-1}mol^{-1}$. The results were expressed as g eq. cyanidin 3-glycoside (CYE)/Kg^{-1} DW.

Total proanthocyanidin content (TP)

The determination was made according to Porter [13]. Aliquots of 250 µL of extracts of dried and fresh pulp were added to 2500 µL of Porter's Reagent (154 mg of $FeSO_4.7H_2O$ per liter of 3:2 n-butanol:chloridric acid), mixed thoroughly, and heated for 30 min in a water bath at 95°C. After cooling, the absorbance was measured at 550 nm using a spectrophotometer (Genesys 10S UV-VIS Thermo Scientific, Waltham, MA, USA). The results were expressed as g of quebracho tannin equivalents (QTE)/Kg^{-1} DW.

2.2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activity

This was performed according to Nóbrega et al. [6]. Methanolic solutions of Trolox at different concentrations (20, 40, 60, 80 and 100 µM) were used to build a calibrating curve and results were expressed as µmol Trolox equivalents (TE)/Kg^{-1} DW.

High-performance liquid chromatography (HPLC) analysis of flavonoids and free ellagic acid (EA)

The analysis was performed according to Azevedo et al. [5]. Dried and fresh pulp extracts were concentrated under vacuum at 40°C on a rotary evaporator (Rotavapor RE 120, Buchi, Flawil, Switzerland). The samples were re-suspended in 10 mL of distilled water and passed through polyamide columns (CC 6, Macherey-Nagel, Germany) previously conditioned with 50 mL of methanol and 60 mL of distilled water. The impurities were washed out with 120 mL of distilled water and the retained flavonoids were eluted with 60 mL methanol followed by 60 mL solution of methanol:ammonia (99.5:0.5, v/v). The flow rate through the columns was controlled by a vacuum manifold Visiprep 24 DL (Supelco, Bellefonte, USA). Each eluate was rotary evaporated once again, under reduced pressure at 40°C. After this, the samples were re-suspended in 1 mL of HPLC grade methanol and filtered through a 0.22 mm tetra fluoro ethylene (PTFE) filter (Millipore Ltd., Bedford, USA) prior to the HPLC analysis. The identification and quantification of flavonoids and free EA were done using a Hewlett-Packard 1100 system (automatic sample injector, quaternary pump and diode array detector (DAD) controlled by the Chem Station software (Hewlett-Packard, Palo Alto, CA, USA)). The samples (20 µL) had their phenolic compounds identified by comparing the retention and spectra time with appropriate standards (myricetin, cyanidin, luteolin, apigenin, kaempferol, quercetin, catechin, epicatechin, ellagic acid, protocatechuic, p-coumaric and syringic acid; Sigma-Aldrich, St. Louis, MO, USA and Extra synthèse, Genay, France). The results were expressed as g/Kg^{-1} DW.

Total ellagic acid (EA) content

This was determined after extraction and acid hydrolysis according to Pinto et al. [14]. Aliquots (2 mL) of the crude extracts in 80% acetone were dried under nitrogen. Hydrolysis was initiated by the addition of trifluoroacetic acid (2N) and was carried out at 40°C for 90 min. The hydrolyzed samples were evaporated under nitrogen, re-suspended in methanol and filtered for HPLC analysis.

Enzymatic inhibition assays

Extracts purification for enzyme analysis: Dried and fresh pulp extracts were rotary evaporated under nitrogen and the samples were re-suspended in 5 mL of distilled water. Aliquots of 5 mL were passed through polyamide (PA) (CC 6, Macherey-Nagel, Germany) and C18 (solid-phase extraction (SPE), Supleco, Bellefonte, PA, USA) columns previously conditioned with 50 mL of methanol and 60 mL of distilled water. The impurities were washed out with 80 mL of distilled water.

The extracts after PA columns were eluted with 50 mL methanol followed by 50 mL methanol:ammonia (99.5:0.5, v/v) and the extracts after C18 columns were eluted with 100 mL methanol. Each eluate was rotary evaporated once again under reduced pressure at 40°C and re-suspended in 2 mL of water.

Pancreatic lipase inhibition assay

This was determined by a modified method described by Nakai et al. [15]. Pancreatic lipase (EC 3.1.1.3, type II from porcine pancreas) and 4-methylumbelliferyl oleate (4-MU oleate) served as the reaction enzyme and substrate, respectively (Sigma-Aldrich, St. Louis, MO, USA). The results were calculated as % lipase inhibition=100-[(AS/AC)*100), where AS and AC are the absorbance of sample (S) and control (C), respectively. The IC_{50} values were determined by the linear regression of dose-effect curves and the results expressed both as mg sample/mL reaction. Orlistat was used as the positive control.

Alfa-glucosidase (EC 3.2.1.20) inhibition assay

This was determined using p-nitrophenyl alpha-D-glucopyranoside (pNPG) as the substrate, according to Azevedo et al. [5]. The results were calculated as % alpha glucosidase inhibition=[((Ct5–Ct0)-(St5–St0))/(Ct5–Ct0)] × 100, where St0, Ct0, St5 and Ct5 are the absorbance of sample (S) and control (C) at the beginning (t0) and after 5 min (t5) of reaction, respectively. Acarbose was used as the positive control. The IC50 values were determined by the linear regression of dose-effect curves and the results expressed both as mg sample/mL reaction.

Alpha-amylase (EC 3.2.1.2) inhibition assay

The assay was conducted according to the chromogenic method described by Ali et al. [16]. Results were calculated as % alpha-amylase inhibition=[(AC)-(AS-AB)/(AC)] × 100, where AS, AB and AC are the absorbance of sample (S), blank (B) and control (C), respectively. Acarbose was used as the positive control. The IC_{50} values were determined by linear regression of dose-effect curves and results were expressed as mg sample/mL reaction.

Antimicrobial activity and determination of Minimum Inhibitory Concentration (MIC)

The extracts were tested for activity against *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 8739, *Enterobacter aerogenes* ATCC 13048, *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* ATCC 29213 according to CLSI. Results were evaluated according to Fujita et al. [7]: <9 mm, inactive; 10-12 mm, partially active; 13–18 mm, active; >18 mm, very active. The microdilution method was used to determine the MIC (CLSI, 2010) which corresponds to the lowest concentration that inhibited the visible growth of the microorganism after 24 h. Ampicillin was used as the positive control.

Statistical analyses

Three drying batches were performed for each drying process and all analyses were performed at least in triplicate (N=9). The results were expressed as mean ± standard deviation and the differences between means were first analysed by ANOVA test and then the Tukey's test (P<0.05).

Results and Discussion

Physicochemical characterization

As expected, both drying processes led to final products with reduced water activity (P<0.05) when compared to the fresh fruit pulps (Table 1). Both the freeze dried and spray dried fruit powders are considered to be microbiologically stable, since they have aw <0.6 [17].

The pH of dried samples was higher when compared to that of fruit pulps (P<0.05). The SD samples reached the highest pH among samples. The Arabic gum only found in SD samples might have played a role on the increased pH, since it is able to reduce the concentration of organic acids in solution [18,19]. The spray dried powders are highly soluble (82.9 to 91.6%) as a consequence of the high water solubility of Arabic gum [20].

		Red Pitanga	Purple Pitanga	Jambolan
Water activity	FR	0.99 ± 0.1^{aA}	0.98 ± 0.1^{aA}	0.98 ± 0.3^{aA}
	FD	0.43 ± 0.2^{aB}	0.43 ± 0.4^{aB}	0.44 ± 0.3^{bB}
	SD	0.23 ± 0.5^{aC}	0.23 ± 0.3^{aC}	0.29 ± 0.3^{bC}
pH	FR	3.0 ± 0.1^{aA}	3.4 ± 0.1^{bA}	3.4 ± 0.2^{bA}
	FD	3.2 ± 0.5^{aB}	3.6 ± 0.7^{bB}	3.41 ± 0.1^{cB}
	SD	3.9 ± 0.9^{aC}	4.2 ± 0.5^{bC}	3.6 ± 0.1^{cC}
Solubility, %	FR	ND	ND	ND
	FD	70.7 ± 1.1^{aB}	71.1 ± 1.0^{bB}	56.6 ± 1.1^{cB}
	SD	86.7 ± 1.0^{aC}	91.6 ± 1.2^{bC}	82.9 ± 1.2^{cC}
L*	FR	23.4 ± 0.6^{aA}	19.4 ± 0.1^{bA}	11.7 ± 0.3^{cA}
	FD	26.3 ±0.5^{aB}	24.3 ± 0.4^{bB}	36.7 ± 0.6^{cB}
	SD	77.9 ± 1.1^{aC}	53.2 ±0.1^{bC}	47.8 ± 0.1^{cC}
C*	FR	17.6 ± 0.3^{aA}	24.1 ± 0.1^{bA}	22.7 ± 0.1^{cA}
	FD	22.5 ± 0.1^{aB}	23.5 ± 0.0^{bB}	24.6 ± 0.3^{cB}
	SD	18.8 ± 0.5^{aC}	31.3 ± 0.1^{bC}	27.7 ± 0.1^{bC}
h*	FR	1.4 ± 0.0^{aA}	0.3 ± 0.0^{bA}	359.6 ± 0.1^{cA}
	FD	1.3 ± 0.1^{aA}	0.3 ± 0.1^{bA}	359.4 ± 0.1^{cA}
	SD	1.0 ± 0.4^{aA}	0.2 ± 0.0^{bA}	359.5 ± 0.0^{cA}
ΔE	FR	ND	ND	ND
	FD	34.7 ± 0.1^{aA}	33.8 ± 0.7^{bA}	44.8 ± 0.1^{cA}
	SD	80.6 ± 0.1^{aB}	61.7 ± 0.1^{bB}	55.3 ± 0.1^{cB}

Table 1: Physicochemical characterization and color parameters (L*, C*, h* and ΔE) of fresh fruit pulp (FR), freeze dried (FD) and spray dried (SD) red pitanga, purple pitanga and jambolan powders.

Results expressed as mean ± standard deviation (N=9); ND: not determined; L*: lightness (+100=white, −100=black); C*: chroma; h*: hue angle; ΔE: Total color difference. a, b, c: Means in the same line followed by different superscripts are significantly different by Tukey's

test (P<0.05); A, B, C: Means in the same column followed by different superscripts are significantly different by Tukey's test (P<0.05).

Colorimetric analysis

The SD samples showed the highest luminosity (L^*) values (P<0.05), which is an evident influence of white colored Arabic gum used as the drying carrier agent in SD samples only (Table 1). A similar tendency was observed by Souza et al. [21]. No effect of drying was observed on the chroma results. The h^* values of red pitanga, purple pitanga and jambolan are clearly different (P<0.05) and depend on inherent fruit color properties. The hue angles of fresh FD and SD samples within each fruit were similar (P>0.05), which means that the drying process did not significantly affect their color tonality. The ΔE results can be interpreted as very distinct (ΔE>3), distinct (1.5<ΔE<3) and slightly different (ΔE<1.5) [9]. Based on that, all samples reached very distinct colors when compared to the original fresh pulp, similarly to dried camu-camu residue [5].

Bioactive compounds and antioxidant activity

Table 2 shows the results of bioactive compounds and DPPH radical scavenging activity of fresh (FR), freeze dried (FD) and spray dried (SD) samples expressed in dry weight. The TPC of fruit powders was significantly lower (P>0.05) than fresh pulps. Despite this fact, the dried fruits showed TPC 10 to 25 times higher when compared to fruits such as apple and pear, recognized as relevant sources of bioactive compounds [22]. Our results are also higher than freeze dried pulp, peel and pomace of jaboticaba (*Myrciaria cauliflora*) [23], an anthocyanin-rich tropical fruit. Previously published TPC values of spouted bed dried jambolan and red pitanga pomaces [24], prove that the spray drying and freeze drying techniques are more efficient in preserving the phenolic compounds of these two exotic fruits.

		Red Pitanga	Purple Pitanga	Jambolan
Total phenolic content (g GAE/Kg^{-1} DW)	FR	211.6 ± 1.9^{aA}	310.3 ± 3.1^{bA}	275.4 ± 1.3^{cA}
	FD	168.1 ± 0.4^{aB}	254.3 ±1.3^{bB}	220.0 ± 0.6^{cB}
	SD	139.3 ± 0.9^{aC}	218.3 ± 1.1^{bC}	188.2 ± 1.3^{cC}
Total anthocyanins (g eq. CYE /Kg^{-1} DW)	FR	2.2 ± 0.2^{aA}	15.82 ± 0.5^{bA}	11.23 ± 0.3^{cA}
	FD	0.7 ± 0.4^{aB}	5.5 ± 0.2^{bB}	5.7 ± 0.2^{cB}
	SD	0.3 ± 0.2^{aC}	4.9 ± 0.3^{bC}	3.6 ± 0.4^{cC}
Ascorbic acid (g/Kg^{-1} DW)	FR	7.8 ± 0.7^{aA}	20.9 ± 0.3^{bA}	23.9 ± 0.7^{bA}
	FD	5.4 ± 0.2^{aB}	18.1 ± 0.4^{bB}	19.8 ± 0.6^{aB}
	SD	3.7 ± 0.4^{aC}	11.9 ± 0.3^{bC}	8.9 ± 0.3^{bC}
Total carotenoids (g/Kg^{-1} DW)	FR	32.5 ± 0.2^{aA}	27.3 ± 0.5^{bA}	18.4 ± 0.3^{cA}
	FD	19.8 ± 0.4^{aB}	11.2 ± 0.2^{bB}	1.2±0.2^{cB}
	SD	15.1 ± 0.2^{aC}	8.9 ± 0.4^{bC}	ND
Proanthocyanidins (g QTE/Kg^{-1} DW)	FR	23.9 ± 0.2^{aA}	88.0 ± 1.2^{bA}	121.9 ± 1.3^{cA}
	FD	23.4 ± 0.3^{aA}	86.1 ± 1.1^{bA}	119.8 ± 1.1^{cA}
	SD	18.7 ± 0.2^{aB}	80.9 ± 0.9^{bB}	110.6 ± 0.9^{cB}
DPPH radical scavenging activity (μmol TE/Kg^{-1} DW)	FR	185.9 ± 1.4^{aA}	291.2 ± 1.3^{bA}	164.3 ± 1.1^{cA}
	FD	15.4 ± 0.8^{aB}	26.6 ± 0.7^{bB}	25.4 ± 0.9^{bB}
	SD	13.6 ± 0.1^{aC}	25.1 ± 0.1^{bB}	25.1 ± 0.7^{bB}

Table 2: Bioactive compounds and DPPH radical scavenging activity of fresh (FR), freeze dried (FD) and spray dried (SD) red pitanga, purple pitanga and jambolan pulps expressed in dry weight.

Values are expressed as means ± standard deviation (n=9); ND: not detected. a, b, c: Means in the same line followed by different superscripts are significantly different by Tukey's test (P<0.05); A, B, C: Means in the same column followed by different superscripts are significantly different by Tukey's test (P<0.05).

FD samples reached higher TPC when compared to SD samples (P<0.05), tendency previously observed by Sogi et al. [25] in dried mango. Better phenolic extraction caused by enhanced porosity in freeze dried samples may also play a role in the observed TPC content [26].

The anthocyanin contents of purple pitanga and jambolan pulps and powders are truly high and reveal their potential as food colorants. Our results are higher than jambolan fruits [27] and comparable to dried jambolan pomace [24]. To the best of our knowledge, this is the first

report about the colorant potential of dried purple pitanga pulp in the literature. Although SD samples reached lower anthocyanin concentration when compared to FD samples ($P<0.05$), both processes produced intense purplish powders and therefore, choosing the best technique should take into account additional economic and performance aspects.

The ascorbic acid values of fruit powders (Table 2) are higher than pineapple (*Ananas comosus* (L.) Merr.) and other exotic fruits [28]. Yet, ascorbic acid losses were detected in both FD and SD samples, which is in agreement with results elsewhere [29].

The carotenoid contents of RP and PP powders are higher than dried camu-camu and acerola residues [5,6]. Freeze drying technique produced samples with superior carotenoid content ($P<0.05$) when compared to SD samples. The carotenoid SD losses are explained by the thermal degradation and oxidation of lipid-like carotenoid pigments induced by drying conditions [30].

Proanthocyanidins are flavonoid-derived polymeric structures with health properties [31]. Jambolan, red and purple pitanga pulps and powders presented remarkably high proanthocyanidin content (Table 2). In fact, similarly to what Fujita et al. [7] and Azevêdo et al. [5] observed for dried camu-camu, freeze drying did not affect the proanthocyanidins of fruit powders ($P>0.05$). PP and JA presented outstanding proanthocyanidin levels. This is the first report of the proanthocyanidin content of dried purple pitanga and unveils the bioactive potential of this underexploited tropical fruit.

The DPPH radical scavenging activity was high for all fruits, mainly for purple pitanga and jambolan fruits. Interestingly, FD and SD samples reached similar results (Table 2). Antioxidant activity is a complex attribute affected by several food compounds and factors, but the high bioactive content previously discussed might play an important role in explaining the experimental results [28].

Flavonoids and total EA content

Overall, several phenolic compounds were detected in all fruits (Figure 1), following the tendency FR>FD>SD (Table 3). The red pitanga samples presented a diversified range of compounds, including a significant concentration of protocatechuic and p-coumaric acids. In fact, this is the first report of these phenolic acids in fresh and spray dried red pitanga. In spite of the losses caused by drying, both SD and FD products presented high concentration of both phenolic acids, recognized by several biological activities [32,33].

Fresh and dried PP and JA proved to be outstanding sources of cyanidin and ellagic acid (Table 3) with higher content than several other exotic fruits [34]. Purple pitanga and jambolan powders presented higher total EA content when compared to red pitanga ($P<0.05$). The expressive ellagic acid content observed for all samples agrees with Abe et al. [34] who found relevant ellagic acid concentration in other Myrtaceae fruits.

Enzymatic inhibition

The metabolic syndrome is characterized by several pathological disorders such as visceral obesity, hyperglycaemia, and hypertension and has been considered as a worldwide health disorder [35]. Natural products with multiple actions would be ideal to manage this chronic condition. In this study, JA, RP and PP showed effective activities against alpha-amylase and alpha-glucosidase, key enzymes of the carbohydrate metabolism, and lipase, responsible for the triglyceride hydrolyses that induces the uptake of fat and consequent obesity (Table 4).

		Red Pitanga	Purple Pitanga	Jambolan
Phenolic acid				
Protocatechuic acid	FR	0.016 ± 0.2^{A}	ND	ND
	FD	0.014 ± 0.1^{B}	ND	ND
	SD	0.009 ± 0.1^{C}	ND	ND
p-coumaric Acid	FR	0.051 ± 0.2^{A}	ND	ND
	FD	0.043 ± 0.5^{B}	ND	ND
	SD	0.025 ± 0.3^{C}	ND	ND
Flavonoids				
Quercetin	FR	0.097 ± 0.2^{aA}	0.157 ± 0.5^{bA}	ND
	FD	0.083 ± 0.2^{aB}	0.137 ± 0.7^{bB}	ND
	SD	0.046 ± 0.3^{aC}	0.98 ± 0.3^{cC}	ND
Myricetin	FR	0.069 ± 0.7^{aA}	ND	0.61 ± 0.2^{bA}
	FD	0.047 ± 0.4^{aB}	ND	0.41 ± 0.2^{bB}
	SD	0.029 ± 0.2^{aC}	ND	0.25 ± 0.1^{bC}
Anthocyanins				
Cyanidin	FR	ND	1.935 ± 1.1^{aA}	1.593 ± 0.9^{bA}
	FD	ND	1.693 ± 1.2^{aB}	1.352 ± 1.1^{bB}
	SD	ND	1.548 ± 0.6^{aC}	1.148 ± 0.7^{cC}
Ellagic acid				
Total Ellagic acid	FR	0.592 ± 0.6^{aA}	0.678 ± 0.3^{bA}	0.943 ± 0.8^{cA}
	FD	0.349 ± 0.5^{aB}	0.511 ± 0.5^{bB}	0.789 ± 0.7^{cB}
	SD	0.253 ± 0.3^{aC}	0.203 ± 0.2^{bC}	0.299 ± 0.4^{cC}

Table 3: Phenolic acids, flavonoids and total ellagic acid content (g/Kg^{-1} DW) of fresh (FR), freeze dried (FD) and spray dried (SD) red pitanga, purple pitanga and jambolan pulps.

Quercetin was detected in both Eugenia varieties. Our results for these fruits are substantially higher than several fresh Brazilian fruits [34]. Similarly to Celli et al. [1], higher quercetin concentration was found in purple pitanga pulps. Myricetin was not detected in PP, but values ranging from 2.5 to 6.9 mg/100 g DW were found for fresh and dried RP and JA pulps. This is substantially higher than the myricetin concentration of camu-camu residue [5].

The RP extracts were especially efficient in inhibiting the lipase activity ($P<0.05$), which might be explained by the phenolic acids detected in RP, acting individually or synergistically, as demonstrated elsewhere [35,36].

In particular, the jambolan extracts presented an impressive alpha-amylase inhibitory activity. This might be related with increased levels of proanthocyanidins found in JA samples (Table 2), since it has been demonstrated a link between proanthocyanidins content and alpha-amylase inhibition [37]. On the other hand, alpha-glucosidase was

better inhibited by PP extracts (P<0.05). The significant concentration of cyanidin, quercetin, proanthocyanidins and EA might be implicated in the observed functional activity [38].

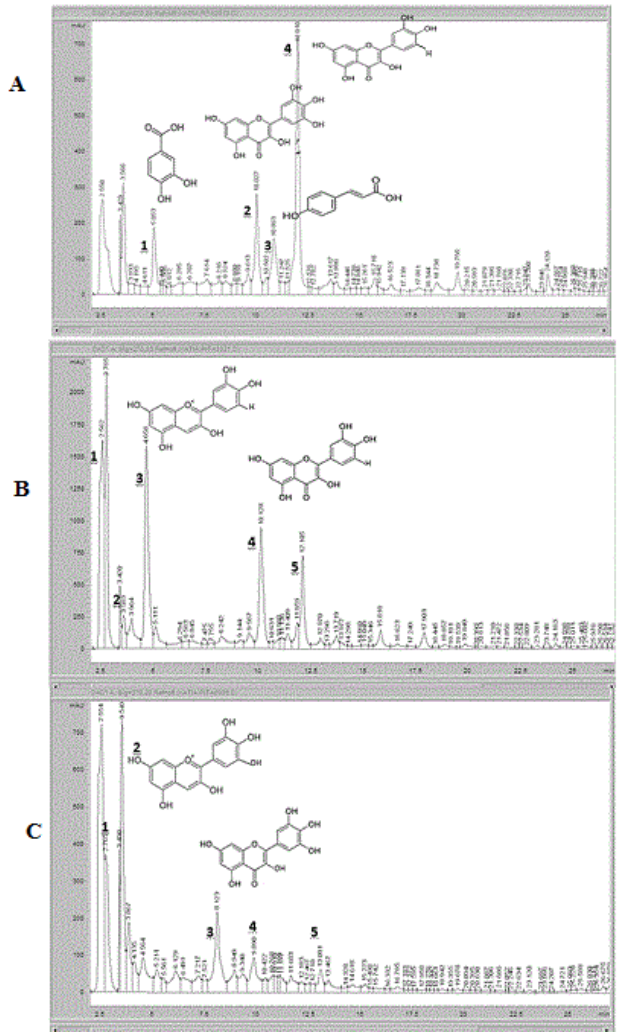

Figure 1: Representative chromatograms showing relevant flavonoids in spray dried A: red pitanga (1- protocathecuic acid; 2- myricitin; 3-p-coumaric acid; 4-quercetin); B: purple pitanga (1,2,3- anthocyanins; 4,5-quercetin) and C: jambolan (1, 2- anthocyanins; 3, 4, 5- myricitin).

Values are expressed as means ± standard deviation (n=9); ND: not detected. a, b, c: Means in the same line followed by different superscripts are significantly different by Tukey's test (P<0.05); A, B, C: Means in the same column within the same component followed by different superscripts are significantly different by Tukey's test (P<0.05).

Antimicrobial activity

Only the Gram-positive *S. aureus*, one of the most common bacteria involved in food outbreaks, was inhibited by the fruit extracts. According to the standard references used in this study, the RP extracts were classified as partially active to active, while the PP and JA extracts were considered active (Table 5).

	Pancreatic Lipase		Alpha-glucosidase	Alpha-amylase
	IC_{50} (mg sample/ mL reaction)		IC_{50} (mg sample / mL reaction)	IC_{50} (mg sample/ mL reaction)
	FR	2.0^{a}	12.4^{a}	11.9^{a}
	FD	4.1^{b}	13.3^{b}	12.5^{b}
	SD	4.7^{c}	14.1^{c}	13.9^{c}
	FR	2.6^{d}	8.6^{d}	10.1^{d}
	FD	4.9^{c}	10.7^{e}	11.8^{a}
	SD	6.0^{f}	11.8^{f}	12.3^{b}
	FR	4.4^{b}	10.3^{g}	8.9^{f}
	FD	5.0^{c}	12.9^{h}	10.8^{e}
	SD	5.8^{f}	13.8^{i}	11.2^{e}
Positive control IC_{50} (mg/ml reaction)				
		Orlistat 5.8	Acarbose 12.8	Acarbose 8.8

Table 4: Inhibitory effects on pancreatic lipase, alpha-glucosidase and alpha-amylase of fresh (FR), freeze dried (FD) and spray dried (SD) red pitanga (RP), purple pitanga (PP) and jambolan (JA) pulps.

Values are expressed as means ± standard deviation (N=9). a, b, c: Means in the same column followed by different superscripts are significantly different by Tukey's test (P<0.05).

		Inhibition Zone	MIC
	FR	13 ± 0.9^{a}	0.31^{a}
RP	FD	12 ± 0.9^{b}	0.62^{b}
	SD	10 ± 0.9^{c}	1.25^{c}
	FR	16 ± 0.9^{d}	0.08^{d}
PP	FD	14 ± 0.9^{e}	0.15^{e}
	SD	13 ± 0.9^{a,f}	0.31a,f
	FR	16 ± 0.9d^{,g}	0.11^{g}
JA	FD	15 ± 0.9^{h}	0.22^{h}
	SD	14 ± 0.9^{e,1}	0.45^{i}
Ampicillin	ND		0.25

Table 5: Inhibition zones (mm) and minimum inhibitory concentration (MIC) (mg/mL) of fresh (FR), freeze dried (FD) and spray dried (SD) red pitanga (RP), purple pitanga (PP) and jambolan (JA) pulps against *Staphyloccocus aureus* ATCC 29213.

Values are expressed as means ± standard deviation (n=9); ND: not determined. a, b, c: Means in the same column followed by different superscripts are significantly different by Tukey's test (P<0.05).

The PP and JA extracts showed the highest inhibitory activities ($P<0.05$), and the FR and FD samples of both fruits had lower MIC when compared to ampicillin, used as the control sample. This might be related to the observed concentration of cyanidin aglycones, EA and proanthocyanidins (Table 2 and Table 3), acting synergistically with other compounds in the fruit extracts. It is known that phenolics can cause cell death by affecting the membrane permeability and/or by coagulating internal cell content [39]. Similarly, Fujita et al. [7] and Azevêdo et al. [5] also observed antimicrobial activity of camu-camu extracts against *S. aureus*.

Conclusion

We have shown that spray dried and freeze dried red pitanga, purple pitanga and jambolan are convenient natural sources of bioactive compounds with functional attributes. The fruit powders exhibited high concentrations of proanthocyanidin, anthocyanins, phenolic acids, flavonoids and ellagic acid. To the best of our knowledge, this is the first report of protocatechuic and p-coumaric acids in fresh and spray dried red pitanga pulps and also the first time that the colorant potential and proanthocyanidin content of dried purple pitanga is demonstrated. Overall, we have presented fresh data concerning these underexploited and bioactive-rich tropical powders, which present antimicrobial activity against *S. aureus* and multiple *in vitro* effects against key metabolism enzymes.

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