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FOOD TECHNOLOGY

Energy and lipid contents, and polyphenols composition of pequi pulp according to the fruit native area

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ABSTRACT: Pequi (*Caryocar* spp.) is a fruit native to the Brazilian Savannah (Cerrado), and *Caryocar brasiliense* Camb. is one of the most prevalent species in this biome. The consumption of the pequi pulp has been associated with health benefits, such as antioxidant, anti-inflammatory, hypolipidemic, hepatoprotective, and anticarcinogenic effects. These benefits have been attributed to its high density in nutrients and bioactive compounds. However, there is evidence about considerable variation in the nutritional profile among pequi pulps of different species, and among pulps of the same species, but from different areas of the Cerrado. In addition, there is no information in literature regarding the polyphenols profile of *C. brasiliense* pulp, neither about the influence of the native area of the fruit on the composition of these phytochemicals. So, this study analyzed the nutrient composition and identified the phenolic compounds in the pulp of *C. brasiliense* fruits native to four different areas of the Cerrado. There was a remarkable variation in the contents of energy (176.3-387.2 kcal/100 g) and lipids (13.0-37.5 g/100 g) among samples. In contrast, no difference was observed in the polyphenols composition, since gallic acid, protocatechuic acid, catechin, epicatechin, p-coumaric acid, and ellagic acid were identified in all pequi pulps. *C. brasiliense* pulp shows potential to be used as a functional ingredient rich in bioactive compounds, but with different contents of energy and lipids according to the fruit's native area to attempt distinct health allegations of the product.
Key words: native fruit, functional food, nutrients, bioactive compounds, phenolic compounds.

Teores energético e lipídico, e composição em polifenóis da polpa de pequi de acordo com a área nativa do fruto

RESUMO: O pequi (*Caryocar* spp.) é um fruto nativo do cerrado brasileiro e *Caryocar brasiliense* Camb. é uma das espécies mais prevalentes neste bioma. O consumo da polpa de pequi tem sido associado a benefícios à saúde, como efeitos antioxidantes, anti-inflamatórios, hipolipidêmicos, hepatoprotetores e anticarcinogênicos. Esses benefícios têm sido atribuídos à sua alta densidade em nutrientes e compostos bioativos. No entanto, há evidências de considerável variação no perfil nutricional entre polpas de pequi de diferentes espécies, e entre polpas da mesma espécie, mas de diferentes áreas do cerrado. Além disso, não há informações na literatura sobre o perfil de polifenóis da polpa de *C. brasiliense*, tampouco sobre a influência da área nativa do fruto na composição desses fitoquímicos. Assim, este estudo analisou a composição de nutrientes e identificou os compostos fenólicos na polpa de frutos de *C. brasiliense* nativos de quatro diferentes áreas do Cerrado. Houve notável variação nos teores de energia (176,3-387,2 kcal/100 g) e lipídios (13,0-37,5 g/100 g) entre as amostras. Em contrapartida, não foi encontrada diferença na composição em polifenóis, uma vez que ácido gálico, ácido protocatecuico, catequina, epicatequina, ácido p-cumárico e ácido elágico foram identificados em todas as polpas de pequi analisadas. A polpa de *C. brasiliense* tem potencial para ser utilizada como ingrediente funcional rico em compostos bioativos, porém com diferentes teores de energia e lipídios de acordo com a área nativa do fruto para atender a distintas alegações de saúde do produto.
Palavras-chave: fruto nativo, alimento funcional, nutrientes, compostos bioativos, compostos fenólicos.

INTRODUCTION

Caryocar brasiliense Camb. is the most prevalent species of the genus *Caryocar* spp. in the Brazilian Savannah (Cerrado) (NASCIMENTO-SILVA & NAVES, 2019). The edible pulp (internal mesocarp) of the *C. brasiliense* fruit, known as pequi, is largely consumed by the regional population as the

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primary ingredient in many traditional dishes, and is used by the agroindustry in the production of oil, ice cream, liquor, and cosmetics (ARAÚJO, 1995). Thus, the commercialization and the sale value of the fruit have been increasing over the years (SILVA et al., 2020a), as well as the research and reports on the nutritional value and functional properties of the pequi pulp (NASCIMENTO-SILVA & NAVES, 2019).

The consumption of the *Caryocar* spp. pulp has been associated with health benefits, such as antioxidant, anti-inflammatory, hypolipidemic, hepatoprotective, and anticarcinogenic effects (NASCIMENTO-SILVA & NAVES, 2019). These benefits, in turn, are related to the high nutritional value of the pequi pulp, which has been well documented in literature. In general, the pequi pulp has a high content of energy, lipids, dietary fibers, and minerals, such as zinc, magnesium, and calcium (ALVES et al., 2014; CARDOSO et al., 2013; CORDEIRO et al., 2013), and is a rich source of carotenoids (CHISTÉ & MERCADANTE, 2012; RIBEIRO et al., 2014). Nevertheless, there is evidence in literature about considerable variation in the nutritional profile among pequi pulps of different species (NASCIMENTO-SILVA & NAVES, 2019), and among pulps of the same species (*C. brasiliense*), but native to different areas of the Cerrado (ALVES et al., 2014; RIBEIRO et al., 2014).

In contrast to the nutritional profile, so far, few studies have addressed the profile of phenolic compounds in the pequi pulp, with available data only for the *C. villosum* species (ALMEIDA et al., 2013; CHISTÉ & MERCADANTE, 2012; YAMAGUCHI et al., 2017). These reports showed that *C. villosum* pulp is a source of polyphenols, mostly gallic acid and ellagic acid, with antioxidant properties. However, there is a scarcity of reports on the polyphenols profile of the *C. brasiliense* pulp, and on the influence of the native area of the fruit on the composition of these phytochemicals. Considering these gaps in literature and the functional and nutraceutical properties of the phenolic compounds (ALVES-SANTOS et al., 2020; PANZELLA, 2020), this study analyzed the nutritional composition and the content and profile of polyphenols in the pulp of *C. brasiliense* native to four different areas of the Cerrado.

MATERIALS AND METHODS

Pequi fruit samples

The experimental design was completely randomized. The fruits were collected from six plants in four different areas of the Cerrado biome with representative occurrence of the *Caryocar brasiliense* species, located in the following states of Brazil and respective municipalities: Tocantins (Presidente Kennedy - S 8° 32’ 20” W 48° 30’ 19”) – PEK-TO; Goiás (Silvânia - S 16º 39’ 32” W 48º 36’ 29”) – SIL-GO; Mato Grosso (Santo Antônio do Leverger - S 15º 51’ 17,3” W 56º 02’ 05,2”) – SAN-MT; and Minas Gerais (Campo Azul - S 16º 30’ 13” W 44º 48’ 38”) – CAM-MG. Twenty four (24) kg of sample (approximately 150 fruits) were collected in each area, during the harvest: PEK-TO, in November; and SIL-GO, SAN-MT, and CAM-MG, in January. After harvesting, fruits were selected according to color, maturation, texture, and absence of injuries, cleaned, and shelled. Then, the pulp was manually removed with a stainless-steel knife, vacuum-packed in low-density polyethylene packages covered with aluminum foil, and stored at -20 °C.

Proximate composition

The proximal composition was evaluated according to the methodology described by AOAC (2019) for moisture (method No. 934.06), ashes (method No. 940.26) and proteins (method No. 920.152). The lipids content was quantified according to BLIGH & DYER (1959). The total carbohydrates were estimated by difference, subtracting the moisture, ashes, lipids and proteins values from 100. The total energy value was calculated considering the Atwater conversion factors of 9 kcal per g of lipids, and 4 kcal per g of proteins and carbohydrates. Results were expressed in g per 100 g of fresh weight of sample.

Total phenolic compounds

The total phenolic compounds were determined as described by GENOVESE et al. (2008). The pulp extract was prepared with a 0.1 g sample homogenized in a vortex with 0.5 mL of ethanol and 0.5 mL of ultrapure water. Then, the solution obtained was defatted using petroleum ether. After that, 1 mL of acetone, acetic acid, and water solution (70:29.5:0.5) was added and the extract was subjected to ultrasound for 5 min at 37 ºC followed by homogenization in a vortex. The extract was obtained after the centrifugation at 9000 rpm and 18 ºC, for 10 min, with disposal of the lipid supernatant. The extracts (0.25 mL) were mixed with 0.25 mL of Folin-Ciocalteu reagent, 2.5 mL of ultrapure water, and 0.25 mL of sodium carbonate (10%). Subsequently, the solution was kept at room temperature for 60 min, in a dim light environment. The total phenolic compounds were quantified using a standard curve for gallic acid (Concentration = [Absorbance + 0.0448]/0.0069; r = 0.9961) at 725 nm by a UV/Visible spectrophotometer (V-630, Jasco, Easton, USA). Results were expressed in mg of gallic acid equivalents (GAE) per 100 g of fresh weight of sample.

Identification of phenolic compounds by HPLC-ESI-HRMS/MS

Samples (0.1 g) were extracted in 3 mL of methanol for 30 min using an ultrasonic bath (AltSonic Clean 3IA). Each sample and a blank (methanol) were filtered through a cellulose acetate filter (0.20 μm) and injected 10 μL into the system. The HPLC-ESI-HRMS/MS system consisted of an HPLC Ultimate 3000 (Thermo Scientific, Waltham, USA) coupled to a high-resolution mass spectrometer (HRMS) Q-Exactive (Thermo Scientific). The H-ESI in negative mode source was used to monitor the compounds by HRMS and in the parallel reaction monitoring (PRM) experiments. The chromatographic separation was carried out using ACE C18 column (4.6 mm × 100 mm, 3.0 μm) at 20 °C column temperature and 0.3 mL/min flow rate, with mobile phase of deionized water (A) and acetonitrile (B), both acidified with 0.1% formic acid. The applied gradients were 93-70 (A%) from 0 to 10 min, 70-50 (A%) from 10 to 15 min, 50-30 (A%) from 15 to 18 min, 30-20 (A%) from 18 to 20 min, 20-0 (A%) from 20 to 23 min, 100% B from 23 to 26 min, 0-93 (A%) from 26 to 28 min, and 93% A from 28 to 33 min. The MS parameters were used in the following conditions: spray voltage 4 kV, sheath gas flow rate 30, auxiliary gas flow rate 10, capillary temperature 350 °C, auxiliary gas heater temperature 300 ºC, S-lens 55 and mass range m/z 150-700.

The phenolic compounds were identified by injecting a stock solution with 14 standards (gallic acid, protocatechuic acid, gentisic acid, caffeic acid, *p*-coumaric acid, vanillic acid, ellagic acid, catechin, epicatechin, rutin, quercetin, naringenin, luteolin, and kaempferol). The data were processed using the software Xcalibur™. The analyses for the identification of phenolic compounds were carried out in the negative ESI ionization mode, and identified by the retention times and fragments generated in the PRM experiments, compared to the commercial standards. The chemicals for the HPLC analyses were purchased from Sigma-Aldrich (St. Louis, USA).

Statistical analysis

Results were expressed as mean ± standard deviation of three replicates (20 fruits per replicate). Data were analyzed using the Analysis of Variance (ANOVA one-way), and the Tukey's test for comparisons of means, and a 5% probability was considered statistically significant. Statistical procedures were performed using the software IBM SPSS Statistics, version 20.

RESULTS AND DISCUSSION

The contents of energy and lipids varied significantly among all the samples, and the energy values varied according to the lipid contents of the pulps (Table 1). The pulp of pequi native to the State of Tocantins (PEK-TO) had the lowest energy and lipids contents. These values are compatible with those reported for the *C. brasiliense* pulp from another area of the same State (energy: 149.9 kcal/100g; lipids: 8.39 g/100 g) (ALVES et al., 2014). The CAM-MG and SIL-GO samples showed intermediate concentrations of energy and lipids. The highest energy and lipids contents were observed in the pequi pulp from the State of Mato Grosso (SAN-MT), which were superior to the energy and lipids values usually reported in the pulps of *C. brasiliense* from different Brazilian States, respectively: 294.0 kcal/100 g and 24.27 g/100 g, from Goiás (ALVES et al., 2014); 293.7-325.4 kcal/100 g and 27.1-32.4 g/100 g, from Mato Grosso (CORDEIRO et al., 2013); and 317.2 kcal/100 g and 33.1 g/100 g, from Minas Gerais (CARDOSO et al., 2013). The great lipid concentration in the SAN-MT sample adds economic and nutraceutical value to the fruit, since the pequi pulp oil is traditionally used in the typical cuisine of the Cerrado region, and in folk medicine to treat several diseases (ARAÚJO, 1995). The pequi pulp oil has been associated with several health benefits, such as antioxidant, anti-inflammatory, hypolipidemic, hepatoprotective, and anticarcinogenic effects (NASCIMENTO-SILVA & NAVES, 2019). In addition, the oil extracted from the pequi pulp has been used in the food, cosmetic and pharmaceutical products because of its technological and functional proprieties, as a source of unsaturated fatty acids, vitamin E, carotenoids, and phenolic compounds (ARAÚJO, 1995; BORGES et al., 2021; CARDOSO et al., 2013; PEGORIN et al., 2020).

The contents of the total phenolic compounds of the pequi pulps native to the States of Tocantins, Goiás and Mato Grosso were similar, and higher than those of the fruits harvested in the state of Minas Gerais (Table 1). The phenolic contents of PEK-TO, SIL-GO, and SAN-MT samples were close to those reported in *C. brasiliense* pulp from the state of Minas Gerais (108.56 mg GAE/100 g) (MAGALHÃES et al., 2018), and in the pulp of the giant pequi (101 mg GAE/100 g) (SILVA et al., 2020b), but lower than those of pequi pulp from different regions of Brazil (178 to 334 mg GAE/100 g) (RIBEIRO et al., 2014). However, the total phenolic contents observed in all samples were higher than that reported in *C. villosum* species (58.94 mg/100 pulp)

Table 1 - Proximate composition, energy value and total phenolic compounds in pulps of *C. brasiliense* native to different areas of the Cerrado.

Samples	----------------------Proximate composition (g/100 g)------------------------					Energy value (kcal/100 g)	Total phenolic (mg GAE/100 g)
	Moisture	Proteins	Lipids	Ashes	Total carbohydrates		
PEK-TO	$71.2^{a} \pm 0.1$	$4.1^{a} \pm 0.2$	$13.0^{d} \pm 0.3$	$1.0^{a} \pm 0.0$	$10.7^{c} \pm 0.4$	$176.3^{d} \pm 1.4$	$101.85^{a} \pm 2.12$
SIL-GO	$50.4^{c} \pm 0.4$	$3.7^{b} \pm 0.1$	$28.6^{b} \pm 1.0$	$0.7^{b} \pm 0.0$	$16.6^{a} \pm 1.3$	$338.6^{b} \pm 4.5$	$100.03^{a} \pm 2.64$
SAN-MT	$49.5^{c} \pm 0.6$	$3.3^{b} \pm 0.2$	$37.5^{a} \pm 1.0$	$0.5^{c} \pm 0.0$	$9.2^{c} \pm 0.4$	$387.2^{a} \pm 7.3$	$99.22^{a} \pm 4.53$
CAM-MG	$58.8^{b} \pm 0.2$	$4.3^{a} \pm 0.2$	$22.9^{c} \pm 0.3$	$0.9^{a} \pm 0.0$	$13.1^{b} \pm 0.1$	$276.6^{c} \pm 2.3$	$74.77^{b} \pm 0.77$

Values (expressed in fresh weight of the sample) are mean ± standard deviation (n= 3). [(a-c)] In the same column, means with different letters differ significantly by Tukey test ($P < 0.05$). PEK-TO: sample from Presidente Kenedy, State of Tocantins; SIL-GO: sample from Silvânia, State of Goiás; SAN-MT: sample from Santo Antônio do Leveger, State of Mato Grosso; CAM-MG: sample from Campo Azul, State of Minas Gerais; GAE: gallic acid equivalents.

(CHISTÉ & MERCADANTE, 2012). According to a comprehensive review on the pequi fruit (NASCIMENTO-SILVA & NAVES, 2019), the total phenolic content of the pequi pulp is variable, even in the same pequi species, and these differences may be explained, at least in part, by different procedures used in the polyphenol extraction.

The following phenolic compounds were identified in the pequi pulp, with no difference among the pulps from different areas of the Cerrado: gallic acid, protocatechuic acid, catechin, epicatechin, *p*-coumaric acid, and ellagic acid (Table 2, Figure 1). So far, the phenolic compounds have been identified only in the pulp of *C. villosum*, and gallic acid, ellagic

Table 2 - Phenolic compounds identified in *C. brasiliense* pulps by HPLC-HRMS through the parallel reaction monitoring (PRM) experiments.

Identified compound	Molecular formula	RT (min)	Standard RT	Detected mass	Calculated mass	Error (ppm)	Fragments (m/z)
				---------------[M – H]--------------			
Gallic acid	$C_7H_6O_5$	14.29	14.27	169.01318	169.01370	0.001	169.01318 125.02316
Protocatechuic acid	$C_7H_6O_4$	19.26	19.22	153.01814	153.01879	0.622	153.01814 109.02817
Catechin	$C_{15}H_{14}O_6$	20.07	20.08	289.07159	289.07122	3.201	289.07159 245.08145 205.04997 179.03389 125.02304
Epicatechin	$C_{15}H_{14}O_6$	22.24	22.23	289.07159	289.07122	3.201	289.07159 245.08145 205.04997 179.03389 125.02304
p-Coumaric acid	$C_9H_8O_3$	25.05	25.08	163.03891	163.03952	0.065	163.03891 119.04893
Ellagic acid	$C_{14}H_6O_8$	26.39	26.38	300.99881	300.99845	2.546	300.99881 283.99646 229.01391 185.02354

RT: retention time.

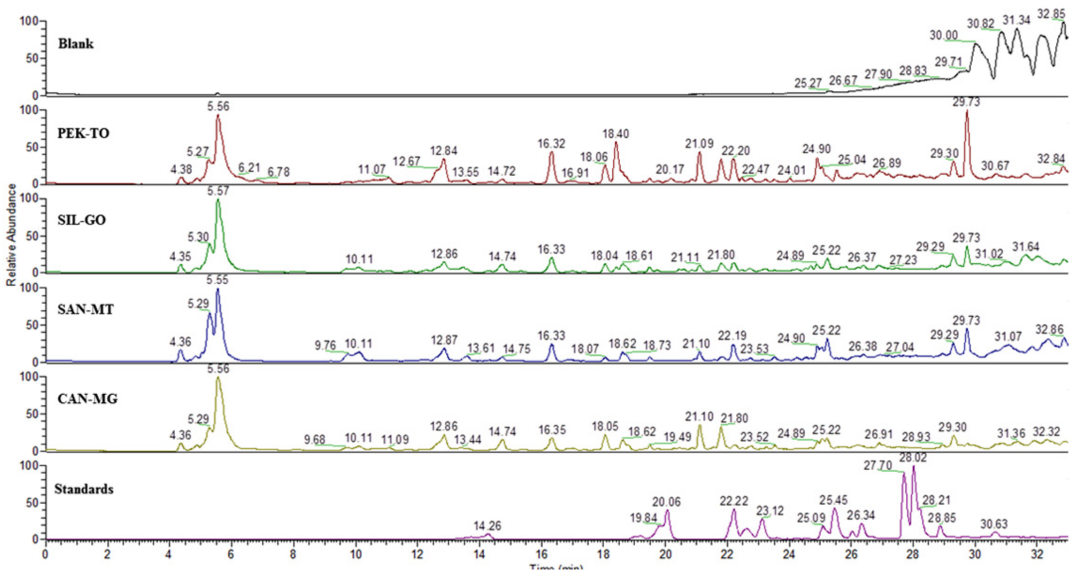

Figure 1 - Total ion chromatograms of pulps of *C. brasiliense* native to different areas of the Cerrado, and phenolic compounds standards, in negative ESI ionization mode.

acid, and ellagic acid rhamnoside were reported as major compounds (CHISTÉ & MERCADANTE, 2012). The presence of gallic and ellagic acids was confirmed in two other studies with the *C. villosum* species (ALMEIDA et al., 2013; YAMAGUCHI et al., 2017). Therefore, in our study, the phenolic compounds protocatechuic acid, catechin, epicatechin, and *p*-coumaric acid were identified for the first time in the pulp of *C. brasiliense*. The phenolic compounds of *Caryocar* spp. pulp have been associated with health benefits, such as antioxidant, anti-inflammatory, and cardioprotective effects (NASCIMENTO-SILVA & NAVES, 2019; YAMAGUCHI et al., 2017). Indeed, antioxidant, anti-inflammatory, and anti-atherosclerotic activity have been attributed to the consumption of ellagic acid and protocatechuic acid (RASHMI & NEGI, 2020), and anti-inflammatory and antiviral activity have been reported for catechin, epicatechin, and gallic acid (MATSUI, 2015; YOU et al., 2018).

Phenolic compounds from edible or non-edible natural sources have gained a lot of attention from the scientific community due to their broad spectrum of effects on health and use in the food, cosmetic and pharmaceutical industries (PANZELLA, 2020). These compounds can be used as functional ingredients in plant-based foods, bioactive supplements, and nutraceuticals, with innovative applications, such as antiviral and prebiotic compounds (ALVES-SANTOS et al., 2020; SILVA, 2021).

It is worth highlighting that the pequi pulp from the state of Tocantins is a source of polyphenols with relatively low contents of calories and lipids. In addition, the pulp of pequi from Mato Grosso, with a remarkable concentration in lipids, is indicated for oil exploitation by the food, cosmetic and pharmaceutical industries. Thus, the pequi pulps have potential to be used as functional ingredients in plant-based foods rich in bioactive compounds, and for different nutraceutical applications.

CONCLUSION

There is a range in energy and lipids contents of the pequi pulps according to the native area of the fruit. However, the native area of pequi seems to have no influence in the profile of phenolic compounds. Gallic and ellagic acids were identified in *C. brasiliense* pulp, as already observed in *C. villosum* pulp, but protocatechuic acid, catechin, epicatechin, and *p*-coumaric acid were identified for the first time in the pequi pulp. The pulp of the pequi native to the State of Tocantins has relatively low contents of lipids and energy, and a considerable content of polyphenols, indicating its use in healthy diets and lower-calorie food products.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this article. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

NRR Nascimento-Silva carried out the data collection and wrote down the original version of the manuscript. AM Alves-Santos helped to design the study, guided the data collection, revised and formatted the manuscript. C Oliveira and AP Terezan performed the HPLC analysis, analyzed the results and reviewed the manuscript. APG Silva analyzed and discussed the results and reviewed the manuscript. MMV Naves conceived and designed the study, guided the research project and carefully ad critically reviewed the manuscript. All authors approved the final version of the manuscript.

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