Simulated gastrointestinal digestion of Brazilian açaí seeds affects the content of flavan-3-ol derivatives, and their antioxidant and anti-inflammatory activities

Priscilla Siqueira Melo a, c,*, Adna Prado Massarioli a, Josy Goldoni Lazarini b, Jackeline Cintra Soares a, Marcelo Franchin b, Pedro Luiz Rosalen b, Severino Matias de Alencar a

a Department of Agri-food Industry, Food and Nutrition, ‘Luís de Queiroz’ College of Agriculture, University of São Paulo, Páduas Dias Avenue, P.O. Box. 9, 13418-900, Piracicaba, SP, Brazil
b Piracicaba Dental School, Department of Physiological Sciences, University of Campinas, 901 Limeira Avenue, 13414-903, Piracicaba, SP, Brazil
c Center of Nature Sciences, Lagoa do Sino Campus, Federal University of São Carlos (UFSCar), Lauri Simões de Barros Highway, Km 12, SP-189, 18290-000, Buri, SP, Brazil

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A B S T R A C T
Açaí seeds (Euterpe oleracea Mart.) are the major residue generated during industrial extraction of açaí fruit pulp—a popular and typical Amazon fruit rich in bioactive compounds and nutrients. In this study, we investigated the bioaccessibility of an açaí seed extract using an in vitro simulated gastrointestinal digestion model. Catechin, epicatechin and procyanidins B1 and B2 were identified and quantified in the açaí seed extract and monitored by HPLC-DAD through the digestion phases. Bioaccessibility of these flavan-3-ols and deactivation of reactive oxygen species decreased after the intestinal phase, except for peroxyl radical (ROO•). RAW 264.7 macrophages treated either with the digested or undigested açaí seed extract showed reduced NF-κB activation and TNF-α levels, even following gastrointestinal digestion. Thus, the ROO• scavenging capacity and anti-inflammatory activity of the extract were found to be still remarkable after digestion, suggesting that açaí seeds could be explored as a source of bioactive compounds for functional foods, cosmetic or pharmaceutical purposes.

1. Introduction

Açaí (Euterpe oleracea Mart.) is a palm tree native to the Brazilian Amazon (Kang et al., 2012) whose fruit has been considered a “super fruit” due to its nutritional and functional characteristics (Schauss et al., 2006a; Schauss et al., 2006b; Hogan et al., 2010; Kang et al., 2011; Yamaguchi et al., 2015), which have contributed to its popularity worldwide.

In 2018, Brazil produced 1.5 million tons of açaí fruit (IBGE, 2018) for industrial pulp extraction – its main form of commercialization. While açaí seeds account for 70%–95% of the whole açaí fruit, a high amount of seeds are discarded in the process (Teixeira et al., 2005; Pompeu et al., 2009; Wycoff et al., 2015; EMBRAPA, 2018; Monteiro et al., 2019). A small part of this material has been reused as fertilizer, cultivation of new trees, or handicraft, but most of it is treated as an agro-industrial residue (Rodrigues et al., 2006). Açaí seeds have been reported to have a high content of flavan-3-ols, such as catechin, epicatechin and their polymers – procyanidins (Melo et al., 2016). These compounds are well known for their health benefits, such as antioxidant, antiproliferative and anti-inflammatory properties (Barros et al., 2015; Melo et al., 2016).

Most of the studies on the bioactivity of flavan-3-ols have been performed using in vitro assays and synthetic chemical compounds. Nevertheless, some methods closer to real-life conditions have been recently developed to accurately determine the bioactivity of these compounds. One of these methods was developed to simulate in vitro gastrointestinal digestion in the human body (Minekus et al., 2014; Brodkorb et al., 2019).

This is the first study investigating the bioaccessibility of flavan-3-ols from an açaí seed extract using a simulated in vitro digestion model. Changes in the phytochemical profile, compound concentration and bioactivity (antioxidant and anti-inflammatory properties) of the extract were analyzed throughout the digestion process. Our study hypothesis

* Corresponding author.
E-mail addresses: priscilla.melo@alumni.usp.br, priscilla.esalq@gmail.com (P.S. Melo).

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was that flavan-3-ols present in the açai seed extract can withstand gastrointestinal digestion and preserve their antioxidant and anti-inflammatory properties. Taken altogether, our findings may shed light on the dynamics of phenolic compounds and their bioactivity in biological processes, such as gastrointestinal digestion in the human body. Our results may further contribute to the prospect of açai seeds as a natural source of bioactive compounds for food, pharmaceutical or cosmetic purposes.

2. Material and methods

2.1. Chemicals

The following chemicals were used in this study: Folin-Ciocalteau reagent (Dinâmica Química Contemporânea, Diadema, SP, Brazil); sodium carbonate, potassium chloride, ethanol (Synth, Diadema, SP, Brazil); monobasic and dibasic potassium phosphate; standards of 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), catechin, epicatechin, procyanidins B1 and B2; reagents such as fluorescein sodium salt, 2,2’-azobis(2-methylpropionamide) dihydrochloride (AAPH), β-nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), nitrotetrazolium blue chloride (NBT), sodium hydroxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bile salts; and enzymes such as α-amylase, pepsin, pancreatic. Escherichia coli LPS, DMSO and RPMI were purchased from Sigma-Aldrich (St. Louis, MO, USA); acetonitrile and methanol were purchased from J. T. Baker (Phillipsburg, NJ, USA); formalic acid was purchased from Tedla (Fairfield, OH, USA); fetal bovine serum (FBS), and antibiotics (streptomycin/Tween 20 was obtained from Amresco, Inc. (West Chester, Pennsylvania, USA).

2.2. Sample collection

Açai seeds were obtained from an agro-industrial company located in the city of Valença, Bahia, Brazil, in March 2018. The material was transported in frozen state to the laboratory, lyophilized, and stored in a freezer inside bags protected from light, until extraction.

2.3. Extraction of flavan-3-ols from açai seeds

First, lyophilized açai seeds were frozen with liquid nitrogen and then immediately milled in a stainless-steel mill at 28000 rpm (Basic IKA A11, IKA, Campinas, SP, Brazil) to obtain a powder. Immediately after, aliquots of 100 mL of ethanol:water (57:43, v/v) were added to 10 g of material to extract flavan-3-ols from the açai seeds. The mixtures were shaken and maintained at room temperature for 20 min. After, the mixtures remained 15 min added in an 2.8 L ultrasonic bath (model USC 1400A, 40 kHz of ultrasound frequency, 135 W RMS power, Indaiatuba, SP, Brazil) at room temperature and were centrifuged in an Eppendorf 5810R centrifuge (Eppendorf AG, Hamburg, Germany) at 5000 g for 15 min. The supernatants (solvent ethanol:water containing the compounds extracted from açai seeds) were filtered through a qualitative paper filter to obtain the açai seed extract.

2.4. In vitro simulated digestion

The in vitro gastrointestinal digestion of açai seed extract consisted of three phases (oral, gastric and intestinal) by using enzymes and fluids specific to each phase, according to Minekus et al. (2014) and Rodrigues et al. (2016), with modifications.

Oral phase: an aliquot of 5 mL of synthetic salivary fluid (SSF) containing 0.013 g/L of α-amylase was added to lyophilized açai seed extract (200 mg) in Falcon-type tubes (50 mL). The mixture was incubated at 37 °C in a thermostatic bath under shaking (100 rpm) for 2 min and then placed immediately in an ice bath to stop the enzymatic reaction.

Gastric phase: an aliquot of 10 mL of synthetic gastric fluid (SGF) containing 1.05 g/L of pepsin was added to the tubes from the oral phase. The pH was adjusted to 3.0 using 1 M HCl, and the mixture was incubated at 37 °C in a thermostated bath under shaking (100 rpm) for 120 min. The tubes were placed immediately in an ice bath in order to stop the enzymatic reaction.

Intestinal phase: an aliquot of 20 mL of the synthetic intestinal fluid (SIF) containing 0.8 g/L of pancreatin and 0.45 g/L of bile salts was added to the tubes from the gastric phase. The pH was adjusted to 7.0 using 1 M NaOH, and the mixture was incubated at 37 °C in a thermostated bath under shaking (100 rpm) for 120 min. The tubes were placed immediately in an ice bath to stop the enzymatic reaction.

Two controls were included under the same conditions, one containing just the synthetic fluids and enzymes with no extract, and another containing only the extract, with no addition of enzymes. In vitro simulated gastrointestinal digestion was carried out in triplicate for each phase (oral, gastric and intestinal), totaling nine tubes, in addition to the controls. At the end of each digestion phase, three tubes were taken and centrifuged in an Eppendorf 5810R centrifuge (Eppendorf AG, Hamburg, Germany) at 10000 g for 5 min at 4 °C. The supernatants were collected and kept at −20 °C until the analysis. At the end of the process, the last three tubes corresponded to the intestinal phase and, therefore, had received all fluids and enzymes throughout the whole digestion process.

2.5. Phenolic composition

2.5.1. Total phenolic content (TPC)

The analysis of TPC was performed following the spectrophotometric method of Folin–Ciocalteau, as previously described by Melo et al. (2015). Aliquots of 20 μL of açai seed extracts (crude or digested) or gallic acid (standard curve ranging from 20 to 120 µg/mL) and 100 μL of the Folin–Ciocalteau aqueous solution (10%) were pipetted into microplate wells. After 5 min, 75-μL aliquots of 7.5% sodium carbonate aqueous solution were added. A control was included in which the extract was replaced by distilled water. After 40 min, the absorbance was measured at 740 nm in a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g of açai seed extract (crude or digested).

2.5.2. Quantification of flavan-3-ols by HPLC-DAD

The quantification of flavan-3-ols in açai seed extracts was carried out using an analytical Shimadzu HPLC unit, equipped with a Shimadzu ODS-A column (4.6 mm × 250 mm, 5 μm) and a photodiode array detector (DAD) (SPD-M10A VP, Shimadzu Co., Kyoto, Japan). Açai seed extracts (crude or digested) were filtered using a 0.22 μm PTFE membrane filter and aliquots of 20 μL were injected into the HPLC system at a flow rate of 1.0 mL/min at a constant temperature of 28 °C. The mobile phase used was composed of water/formic acid (99.9/0.1, v/v) (A) and acetonitrile/formic acid (99.9/0.1, v/v) (B). The elution gradient started with 5% B and increasing to 7% B (7 min), reaching 20% B (50 min), 45% B (70 min), 100% B (85 min), held at 100% until 95 min, and finally decreasing to 5% B (100 min). The authentic standards procyanidin B1, procyanidin B2, catechin, and epicatechin (Sigma-Aldrich, St. Louis, MO, USA) were examined. A standard curve was prepared for each standard to perform the quantification of the compounds in the samples. The Limits of Detection (LOD) and Limits of Quantification (LOQ) of the standards were as follows: Procyanidin B1 (LOD = 4.6 × 10−6; LOQ = 1.4 × 10−5); Procyanidin B2 (LOD = 5.9 × 10−6; LOQ = 1.8 × 10−5); Catechin (LOD = 3.8 × 10−5; LOQ = 1.1 × 10−4); Epicatechin (LOD = 8.7 × 10−6; LOQ = 2.6 × 10−5).
2.6. Biological analysis

2.6.1. Antioxidant activity

The peroxyl (ROO\textsuperscript{•}) scavenging capacity of açai seed extract was determined by ORAC assay according to Melo et al. (2015). Aliquots of the samples (30 μL), fluorescein (60 μL of 508.25 mM solution), and AAPH (110 μL of 76 mM solution) were added to a microplate. Potassium phosphate buffer (75 mM, pH 7.4) was used to dilute the solutions and as a blank. The reaction was performed at 37 °C to promote the generation of peroxyl radical. The fluorescence was measured at 485 nm (excitation) and 528 nm (emission) in a microplate reader ( Molecular Devices, LLC, Sunnyvale, CA, USA) every minute for 2 h. The results were expressed as μmol Trolox equivalents per g of açai seed extract (crude or digested).

The capacity of açai seed extract (crude or digested) to scavenge the superoxide radical (O\textsubscript{2}\textsuperscript{−}) was determined and expressed as EC\textsubscript{50}, as previously described by Melo et al. (2015). In a microplate, 166 μM NADH, 107.5 μM NBT, açai seed extracts, and 2.7 μM PMS were added to a microplate, totaling a final volume of 300 μL. Potassium phosphate buffer (19 mM, pH 7.4) was used to dilute solutions and samples. After 5 min of reaction the absorbance was measured at 560 nm.

The HOCl-scavenging capacity of açai seed extract (crude or digested) was measured and expressed as EC\textsubscript{50}, according to Melo et al. (2015). The solution of HOCl at pH 6.2, prepared with 1% NaOCl solution and 10% H\textsubscript{2}SO\textsubscript{4} solution for pH adjusting, was diluted in 100 mM phosphate buffer (pH 7.4) and its concentration was measured at 235 nm using the molar absorption coefficient 100 M\textsuperscript{−1} cm\textsuperscript{−1}. The reaction mixture (final volume of 300 μL) was composed of açai seed extract (crude or digested) at different concentrations, phosphate buffer (100 mM, pH 7.4), 1.25 mM DHR, and 5 μM HOCl. The fluorescence was measured immediately at 528 ± 20 nm (emission wavelength) and 485 ± 20 nm (excitation wavelength) in a microplate reader ( Molecular Devices, LLC, Sunnyvale, CA, USA) at 37 °C.

2.6.2. Anti-inflammatory activity

2.6.2.1. Cell culture. Macrophage RAW 264.7 stably bearing the luciferase reporter gene controlled by an NF-κB-sensitive promoter (NF-κB-pLuc) were cultured in RPMI ( Gibco®, United Kingdom) supplemented with 10% FBS, penicillin (100 U/mL), and 2 mM L-glutamine at 37 °C in 5% CO\textsubscript{2}/95% air atmosphere.

2.6.2.2. Cell viability. Briefly, macrophages (2 × 10\textsuperscript{5} cells/well) were seeded onto 96-well plates and incubated overnight (37 °C, 5% CO\textsubscript{2}). After 24 h, aliquots of açai seed extract (crude or digested) at 10, 30, 100, and 300 μg/mL were added. The negative control group was treated with 0.9% saline (vehicle). After 24 h, 100 μL of MTT solution (1 mg/mL in RPMI) was added to each well and the plates were further incubated for 3 h. Subsequently, aliquots of 200 μL of ethanol were added to each well. The optical density (OD) of each well was measured at 540 nm in a microplate reader ( Molecular Devices, LLC, Sunnyvale, CA, USA).

2.6.2.3. NF-κB activation assay. Macrophages were cultured (3 × 10\textsuperscript{5} cell/well) in 24-well plates and incubated overnight (37 °C, 5% CO\textsubscript{2}). The cells were treated with the extracts (crude or digested) at 10, 30, and 100 μg/mL for 30 min before LPS stimulation (100 ng/mL). The negative control group was treated with 0.9% saline (vehicle). After 4 h, cells were lysed with 50 μL of TNT buffer and an aliquot of the suspension was mixed with 25 μL of the Luciferase Assay Reagent containing luciferin (Promega Corporation, Madison, WI, USA). Luminescence was quantitated in a microplate reader (SpectraMax M3, Molecular Devices, LLC, Sunnyvale, CA, USA).

2.6.2.4. Quantification of TNF-α. After 30 min exposure of the macrophage culture to açai seed extract (crude or digested) at 10, 30, and 100 μg/mL, cells were stimulated with LPS (100 ng) and incubated for 4 h (37 °C, 5% CO\textsubscript{2}). TNF-α quantification was performed by ELISA in culture supernatants using protocols provided by the manufacturer (R&D Systems, Inc). The results were expressed as pg/mL.

2.7. Statistical analysis

The assays were carried out in triplicate or quadruplicate, and the results were expressed as mean ± standard deviation (SD). The data were analyzed by one-way ANOVA (p < 0.05), followed by Tukey’s post-hoc test. A 5% significance level was considered in all statistical tests.

3. Results and discussion

3.1. Changes in the TPC and bioaccessibility of flavan-3-ols

In our study, an extract from açai seeds was subjected to simulated in vitro digestion and further tested for its bioaccessibility and bioactivity throughout the process. The changes in the TPC of the extract throughout the digestion can be seen in Figure 1. There was no significant change in the TPC during the oral phase (35.78 mg/g) when compared to the content originally found in the seed extract (35.67 mg/g). Nevertheless, the TPC content (21.93 mg/g) decreased significantly in the gastric phase and increased in the intestinal phase (24.87 mg/g) in relation to the previous phase. At the end of the in vitro digestion, there was a 34% loss in the TPC, which can be explained by structural changes in the compounds that might have occurred during digestion, particularly because phenolic compounds are pH sensitive (Jara-Palacios et al., 2018; Moyo et al., 2020). Previous studies showed a reduced TPC following in vitro digestion of guarana extract (57% less) when compared to its original content (Silva et al., 2018) and after gastrointestinal digestion of fruit juices (24% less) (Rodríguez-Roque et al., 2013a). However, despite the variation following digestion, the remaining TPC of the açai seed extract in the intestinal phase was higher than that found in freeze-dried açai samples (Schauss et al., 2006a).

Flavan-3-ols (catechin and epicatechin) and their derivatives (procyanidin B1 and B2) in the crude and digested extracts were monitored and quantified by HPLC-DAD, as these compounds have important biological properties and are abundant in açai seeds (Melo et al., 2016).

Catechin and epicatechin are bioflavonoids with proven antioxidant and anti-inflammatory properties (Grzesik et al., 2018; Pan et al., 2018; Tremoccoli et al., 2018), while procyanidins are polymeric compounds formed by catechin molecules. There is a wide range of procyanidins in nature, varying in type and number of oxidized catechin monomers, which determines their degree of polymerization and physiological functions in the human body (Matsui, 2015). Procyanidins have high antioxidant and anti-inflammatory activity and are associated with vascular health (Corder et al., 2006; Melo et al., 2015; Bashir et al., 2016; Tremoccoli et al., 2018)
Oldoni et al., 2016). As shown in Figure 2, there was nearly no change in the content of these phenolic compounds throughout the digestion process. Nevertheless, the quantitative analysis of these compounds revealed levels of procyanidins B1 and B2 in the crude extract (16.08 and 1.49 mg/g, respectively) higher than those reported for açaí pulp (Pacheco-Palencia et al., 2009). In addition, the concentrations of catechin (15.67 mg/g) and epicatechin (5.32 mg/g) in the extract were higher than those found in green tea (Naldi et al., 2014). Therefore, açaí seeds proved to be an important source of catechin and epicatechin (Figure 3A).

During gastrointestinal digestion, the amount of catechin, epicatechin and procyanidins B1 and B2 in the extract changed considerably (Figure 3B). Catechin was the flavonoid mostly reduced after digestion.
with a bioaccessibility rate of 47% in relation to its initial concentration. Epicatechin, procyanidin B1 and procyanidin B2 showed bioaccessibility of 55%, 72% and 87%, respectively (Figure 3B). Except for catechin, all other compounds were found in greater amounts in the gastric phase. Catechin showed 79% bioaccessibility through the oral phase, which remained through the gastric phase. The amounts of epicatechin, procyanidin B1 and B2 increased from 82% to 94%, 89%–102% and 92%–128%, respectively (Figure 3B). Consistent with this, the study by Rodríguez-Roque et al. (2013a) showed that phenolic compounds have different stability in the presence of enzymes and depolymerization reactions during simulated conditions of gastric digestion, mainly due to low pH, which might have increased the concentration of most compounds. Moreover, studies have also shown that under these conditions, phenolic compounds linked to matrix proteins or carbohydrates can undergo hydrolysis, which could result in increased compound concentrations (Rodríguez-Roque et al., 2013a; Rodríguez-Roque et al., 2013b).

In the intestinal phase, there was a decrease in the bioaccessibility of the compounds, which can be explained by the fact that polymerization and oxidation reactions can also occur under alkaline conditions (Rodríguez-Roque et al., 2013a). The increased concentration of procyanidin B1 and B2 in relation to catechin and epicatechin (Figure 3B) suggests that polymerization and oxidation reactions may be occurring during the intestinal digestion phase. The increased amount of B1 and B2 dimers at the end of digestion may also be due to degradation of higher molecular weight procyanidins, such as trimers and tetramers, during the gastric phase (Jara-Palacios et al., 2018).

3.2. Changes in antioxidant activity

The crude and digested acai extracts were tested for their antioxidant capacity against three reactive oxygen species (ROS): peroxyl radical, superoxide anion and hypochlorous radical (Figure 4). ROS are reactive metabolites generated during the aerobic metabolism. In excess, ROS can cause oxidative stress, a condition that can potentially damage DNA, lipids, and proteins, and consequently, lead to cell alterations and diseases (Prior, 2015).

In vitro digestion of acai seed extract increased its peroxyl radical scavenging capacity (142%) (Figure 4A). Conversely, the digestion process decreased the antioxidant activity of the extract against superoxide anion and hypochlorous radicals, with bioaccessibility of 22% and 27%, respectively (Figure 4D, F). In all cases, the antioxidant activity of the extract in the gastric phase was greater than that in the oral phase.
Based on the findings, we reasoned that during the gastric phase, where pH reaches 3, procyanidin may hydrolyze into catechin monomers, whereas the intestinal phase may favor polymerization and oxidation reactions due to alkaline conditions, resulting in the rearrangement of catechin and epicatechin monomers to form procyanidins. Catechin and epicatechin have been reported in the literature to have strong antioxidant activity (Oldoni et al., 2016), which can explain, at least in part, the stronger antioxidant activity of the gastric fraction as compared to the intestinal fraction. Evidence shows that pH has a strong influence on the activity of phenolic compounds as it can induce structural changes (Jara-Palacios et al., 2018). In contrast, a previous study reported increased antioxidant activity of *Ligusticum chuanxiong* Hort. following simulated *in vitro* digestion on DPPH, superoxide anion and hydroxyl radical (Ge et al., 2018). According to the authors, although the TPC content decreased, there was an increase in the content of free phenolics as compared to bound ones, especially of phenolics such as ferulic acid, which may have contributed to the increased antioxidant activity observed after digestion. In our study, the lower concentration of flavonoids in the sample composition after digestion may explain the reduced antioxidant activity observed in most assays. In addition, under alkaline conditions, flavonoids can undergo structural changes such as transformation into chalcones and racemization reactions and, consequently, they may have their antioxidant activity substantially affected (Sun et al., 2019).

As previously mentioned, the digestion of açaí seed extract significantly increased its peroxyl radical scavenging capacity. The ORAC value found for the digested extract (4771 ± 141 μmol/g (Figure 4A) was higher than that reported for wine by-products (Jara-Palacios et al., 2018) and freeze-dried açaí samples (Schaft et al., 2006a). In contrast, an opposite trend was observed for the other reactive oxygen species under analysis. The EC50 of the extract for quenching superoxide anions increased from 32 ± 1.5 to 57 ± 1.6 ppm (Figure 4C) after digestion, which corresponds to a bioaccessibility of only 22% (Figure 4D).

In our study, the açaí seed extract showed decreased superoxide anion scavenging capacity after digestion. Superoxide anion is one of the first ROS produced during aerobic metabolism and is related to early stages of oxidative stress in the human body. It is considered a precursor of other ROS, such as hydrogen peroxide, peroxyxynitrite, hydroxyl radicals, and peroxyl radical (Schaft et al., 2006a; Prior, 2015). In fact, most of the superoxide anion toxicity is related to its derivative ROS, such as peroxyxynitrite, which is a strong oxidant generated by reaction of superoxide anion with nitric oxide, reportedly related to inflammatory stress and carcinogenesis. We reason that the diminished superoxide anion scavenging capacity of the digested açaí seed extract can be counterbalanced by its high capacity to scavenge peroxyl radical, which is the most predominant radical produced in human beings. On the other hand, superoxide anion can also have health benefits by acting against invading microorganisms. Therefore, a weaker potential to scavenge this ROS can render the açaí seed extract more biologically beneficial (Prior, 2015).

Similarly to what was observed for superoxide anion, only 27% of the initial hypochlorous acid scavenging capacity of the extract was conserved, since its EC50 increased from 2 ± 0.0 to 3.4 ± 0.2 ppm after *in vitro* digestion (Figure 4F). Hypochlorous acid (HOCl) is a strong oxidant produced enzymatically by myeloperoxidase which can interact with proteins, DNA, and fatty acids. Recent evidence has shown this ROS may be implicated in the onset of acute coronary diseases (Ma et al., 2018).

Despite the reduced scavenging capacity of açaí seed extract against superoxide anions and hypochlorous acids after *in vitro* digestion, the antioxidant activity of the intestinal fraction was still higher than that reported for avocado peels (52 and 5.2 ppm, respectively) and seeds (70 and 6.7 ppm, respectively) (Tremocolidi et al., 2018). The superoxide anion scavenging capacity of the digested extract was stronger than that of *Ligusticum chuanxiong* extract, a Chinese medicinal herb (821 ppm) (Ge et al., 2018), and it was also stronger than that of catechin (90 ppm) and epicatechin (227 ppm) standards (Tremocolidi et al., 2018), suggesting that procyanidins have a great influence on the sequestration of

![Figure 5](image-url) **Figure 5.** Anti-inflammatory activity of açaí seed extract (S. açaí) before (upper figures) and after (lower figures) simulated *in vitro* gastrointestinal digestion. (A) Cellular viability (MTT); (B) NF-κB activation; and (C) TNF-α release. To evaluate NF-κB activation, macrophages were treated with açaí seed extract before and after *in vitro* digestion at 10, 30, and 100 μg/mL for 30 min before LPS stimulation (100 ng/mL). The negative control group was treated with 0.9% saline (vehicle - V). The results were expressed as mean (n = 4), and bars indicate the standard deviation. Different letters indicate statistical difference (P < 0.05) by one-way ANOVA followed by Tukey’s post-hoc test.
Açaí seeds are an agro-industrial residue rich in bioactive compounds such as proanthocyanidins B1 and B2, catechin and epicatechin. Tracking the presence and bioactivity of these compounds throughout gastrointestinal digestion allowed us to prove our initial hypothesis that the antioxidant and anti-inflammatory properties of açaí seed extract are preserved to some extent throughout gastrointestinal digestion. The compounds present in the extract showed reduced bioaccessibility after digestion as well as a reduced reactive oxygen species scavenging capacity, except for peroxyl radicals, against which the digested extract showed stronger antioxidant activity. While gastrointestinal digestion affected compound bioaccessibility, the antioxidant activity observed at the end of the process was still significant when compared to that of other natural extracts. Moreover, even though a decrease in the anti-inflammatory activity of the açaí seed extract was observed after simulated digestion, the digested extract decreased NF-κB activation and TNF-α levels, suggesting that the compounds present therein may still play a role in immune modulation of inflammatory processes when they reach the intestine. Thus, the biological activities of the crude extract and its bioaccessible digested fraction make açaí seeds an important source of bioactive compounds for the development of new functional foods and for the cosmetic and pharmaceutical industries. In future studies, the bioavailability of the intestinal fraction should be analyzed through absorption assays using Caco-2 cells in order to better estimate the benefits of the açaí seed extract for human health.

Declarations

Author contribution statement

Priscilla Siqueira Melo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Adna Prado Massarioli: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Josy Goldoni Lazarini: Performed the experiments; Wrote the paper.

Jackeline Cintra Soares: Analyzed and interpreted the data.

Marcelo Franchin: Performed the experiments.

Pedro Luiz Rosalen: Contributed reagents, materials, analysis tools or data.

Severino Matias de Alencar: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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