



Nutrition

In vitro gastrointestinal digestion to evaluate the total, bioaccessible and bioavailable concentrations of iron and manganese in açai (*Euterpe oleracea* Mart.) pulps

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ABSTRACT

Açai pulp consumption has increased in Brazil and worldwide. Recently, a high average content of manganese (450 mg/kg) was observed in açai pulp, raising the hypothesis of toxicological effects associated to its ingestion. However, the total concentration of an element does not reflect the real benefits and risks of consuming a food. In this context, the total, bioaccessible and bioavailable concentrations of Fe and Mn were assessed in 9 açai pulps. Fe and Mn contents ranged from 27.6 to 73 and from 145 to 1197 mg kg⁻¹, respectively. Fe and Mn bioaccessibilities represented from 29 to 40 and from 39 to 55% of total amounts. Fe bioavailabilities were lower than LOQ and those of Mn varied from 8 to 17% of total. A daily consumption of 100 g of açai pulp exceeds by at least 1.5-fold the recommended Mn daily intakes for adults whereas poorly contributes to Fe intakes. Since the lowest Mn bioaccessible and bioavailable fraction corresponded to a Mn intake value higher than the tolerable upper intakes for children and that high amounts of Mn intake may impair Fe absorption, higher açai consumption may be worrisome. Future nutritional, toxicological and speciation studies must be undertaken.

1. Introduction

Açai is a native South American tropical palm (*Euterpe oleracea* Mart.), which occurs primarily in the Amazon region [1,2]. This multi-stemmed palm tree produces edible spherical fruits, which are dark purple when fully ripe (about 175 days) and reach 10–12 mm of diameter [1,3–4]. Usually, açai fruits are consumed as pulps, liquids with thick, soft, and smooth texture obtained by macerating the pericarp with different amounts of water [5].

In the last years, açai had a remarkable growth in its popularity being acclaimed as a “superfruit” and its consumption has been increasing all over the world due to its unique flavor and nutritional properties such as high energy, antioxidant, anti-inflammatory and cardioprotective [2,6–8]. Açai is valuable in human nutrition as source of energy, fiber, polyphenols in the form of flavonoids and anthocyanins, minerals and fatty acids. The main constituents found in dry matter are lipids (50%), fibers (25%) and proteins (10%) [7].

Açai is also a good source of macro and micronutrients, such as phosphorus, sodium, zinc, iron, manganese, copper, boron, chromium,

calcium, magnesium, potassium and nickel [1,2,9–13]. An earlier study carried out by our group [13] observed relatively high contents of essential elements, especially manganese, in açai pulps.

Manganese is essential for most life on earth, including human beings. Various enzymes have Mn in their composition, for example superoxide dismutase, pyruvate carboxylase and glutamine synthetase. Additionally, Mn is fundamental for proper brain function and for normal bone structure [14,15]. However, elevated exposure to Mn is associated to neurological impairments which cause several symptoms, and, although the inhalation is the primary route of concern, the main source of exposure is the diet [16]. Therefore, the high amounts of Mn in açai pulps may contribute to a considerably level of exposure. For instance, the northern Brazilian population, which usually consume reasonable amounts of açai every day (may reach 500 mL/person/day or more) may be exposed up to 28 mg per day of Mn [13].

Another crucial point is that Mn may interfere with Fe absorption, due to similar physicochemical properties, and shared absorptive pathways [17]. Manganese may affect on Fe absorption, circulating transporters like transferrin, and regulatory proteins, then influencing

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on Fe homeostasis. Some data show a tight interaction between Mn and Fe, especially in Fe-deficient state [18]. Thus, high levels of Mn ingestion likely perturb the absorption of Fe [19].

As the total content of an essential/toxic element in a food does not represent its beneficial/toxic effects after the food consumption, it is very important to evaluate its bioaccessible and bioavailable fractions. The bioaccessible fraction of an element indicates the soluble fraction from the food matrix in the gastro intestinal (GI) tract and that is available for subsequent processes of absorption through the intestinal mucosa (*i.e.*, enters the blood stream). The bioavailable fraction is the amount of an element which is released from food, absorbed through the intestinal barrier, passing into the bloodstream or an organ, and is available to promote its action in one organism [20]. *In vitro* and *in vivo* approaches trying to mimic the most important processes occurring during human gastrointestinal digestion have been used to assess bioaccessible and bioavailable fractions of the elements [21–24].

In this context, the main goal of the present study is to assess the total, bioaccessible and bioavailable fractions of Fe and Mn in açai pulps using an *in vitro* gastrointestinal digestion. According to our knowledge, no study assessing the bioaccessible and bioavailable fractions of Fe and Mn in this fruit has been exploited so far. With this knowledge, a real contribution of açai pulp for the daily Mn and Fe intakes can be taken and the findings may indicate if a high daily ingestion of this pulp is of toxicological concern.

2. Materials and methods

2.1. Reagents, solutions, samples and materials

For preparing all solutions, it was used deionized water (resistivity 18.2 M Ω cm) from a Milli-Q Gradient system (Merck Millipore). A quartz sub-boiling equipment (Kürner Analysetechnik) was employed to purify the Nitric acid from Synth. Hydrogen peroxide 30% (m/m) (Merck) was used to sample digestion. Hydrochloric acid (Merck) was applied in the *in vitro* digestion. Clean procedures were performed to prepare and analyze the samples. Plastic bottles, conic tubes and glassware were washed by submerging them in 10% (v/v) HNO₃ for 24 h and rinsing carefully with deionized water.

The 1000 mg L⁻¹ stock solutions of Fe and Mn were acquired from PerkinElmer. Aliquots from these stock solutions were diluted with Milli-Q water to daily prepare all working standard solutions. The enzymes α -amylase from *Bacillus subtilis*, porcine pepsin from gastric mucosa P7012, porcine pancreatin from pancreas P1750 and the other reagents, NaHCO₃, NaCl, bile salts and piperazine-*NN*-bis (2-ethanesulfonic acid) di-sodium salt (PIPES), were obtained from Sigma-Aldrich. The dialysis cellulose membranes (14 kDa MWCO) were also from Sigma-Aldrich.

Four commercial medium açai pulps (12.5% of total solids) were bought in the local market of Ribeirão Preto City, Brazil. Other five samples of açai mature berries were harvested at different locations of São Paulo and Pará states, Brazil. The certified reference materials from the National Institute of Standards and Technology (NIST) 8415 *Whole Egg Powder*, 1567a *Wheat Flour*, 1547 *Peach Leaves* and 1568a *Rice Flour* were analyzed to certify the accuracy of analytical procedure.

2.2. Açai berries harvesting and pulp preparation

The açai berries were harvested at different locations of São Paulo and Pará states, Brazil, during the periods representing the middle and the end of the açai harvest season. In total, 5 samples of mature fruits were harvested and approximately 500 g were used in the pulping process after a careful selection. The açai samples were immediately stored in a freezer at -20 °C until the pulping process. The selected fruits were washed in deionized water and the pulping process employed was based on the one used to prepare commercial pulps in Brazil with some modifications [13]. The modifications were carried out in order to

avoid contamination and comprised the use of deionized water, decontaminated plastic materials and an ultra-turrax homogenizer instead of an industrial machine.

The pulping process consisted of soaking the fruits in water at a 3:2 ratio (fruit mass/water volume) at 40 °C for 1 h. Then, the açai berries were pulped by fruit/fruit friction with the aid of mechanical stirring for 5 min, and they were filtered and frozen at -80 °C for subsequent freeze-drying. The freeze-dried açai pulp samples were finally stored in polypropylene conic tubes at room temperature and protected from light prior to sample preparation for analysis.

2.3. Instrumentation

To prepare the açai pulps, an Ultra-Turrax homogenizer (Fisatom) equipped with a metal-free plastic material coated propeller was used. The pulps were frozen at -80 °C and subsequent freeze-dried in a lyophilizer Liotop L101 (Liobras, Brazil). The digestion of samples and certified reference materials were carried out in a microwave oven Start D model (Milestone, Italy). A thermostatic bath (Sppencer, Brazil), an ultracentrifuge (Eppendorf, Brazil), and a pH meter (MS Tecno, Brazil) were applied in the *in vitro* digestion test.

The sample analyses were performed in a quadrupole-based ICP-MS which has a dynamic reaction cell (Elan DRC-e, PerkinElmer, USA). High-purity argon (99.99%; White Martins, Brazil) was used in the analyses. Methane was employed in the dynamic reaction cell to remove the interferences in the Fe and Mn detected isotopes. The sample introduction was carried out by a Meinhard concentric nebulizer directly connected to a cyclonic spray chamber and a peristaltic pump. The determination of Fe and Mn was carried out in an ISO 7 clean room.

2.4. Determination of total Fe and Mn in açai pulps

After freeze-drying all commercial and lab-prepared açai pulps, 200 mg of the samples were added to closed teflon vessels and digested with 6.0 mL of HNO₃ 2.8 mol L⁻¹ and 2.0 mL of H₂O₂ 30% (w/w) using a microwave oven. The microwave oven program was: 5 min at 100 °C, 15 min at 100–200 °C and 15 min at 200 °C. Triplicates of blanks and samples were run. After cooling, the volumes of blanks and decomposed samples were completed to 25 mL with deionized water, diluted 100-fold more and the total contents of Fe and Mn were measured by ICP-MS, employing the instrumental conditions shown in the Table 1. The analytical curves for Fe and Mn were from 0.0 to 100 μ g L⁻¹. Polyatomic interferences for the isotopes ⁵⁴Fe and ⁵⁵Mn were eliminated using Methane to fill the dynamic reaction cell. A dynamic reaction cell conditioning of 45 min was necessary before the measurements.

The certified reference materials 8415 *Whole Egg Powder*, 1567a *Wheat Flour*, 1547 *Peach Leaves* and 1568a *Rice Flour* were analyzed, in triplicate, to verify the accuracy of the proposed method. The decomposed certified reference materials were cooled and diluted to 25 mL with deionized water. Further dilution (4 or 20-fold) was applied to determine Fe and Mn by ICP-MS.

2.5. *In vitro* gastrointestinal digestion of açai pulps

The simulated *in vitro* gastrointestinal digestion employed to assess the bioaccessibility and bioavailability of Fe and Mn in açai pulps was based on a previous publication, with a little modification. Briefly, the amount of pepsin was reduced from 50 to 20 mg [25]. The procedure tried to reproduce the food digestion process in humans encompassing the mouth, stomach and intestine steps. The membrane dialyzable fractions of Fe and Mn in the intestinal phase represented the bioavailabilities of these elements. The method was carried out in triplicate, each one in a different day. A mass of 300 mg of powdered açai pulp sample was added into a 50 mL conic polypropylene tube. To simulate the first stage, the mouth digestion, 5 mL of saliva solution [1% (w/v) α -amylase and 150 mmol L⁻¹ NaCl dissolved in a 100 mmol L⁻¹

Table 1

Operating conditions of the ICP-MS for quantitative determination of Fe and Mn.

Instrumental parameters				
Spray chamber				Cyclonic
Nebulizer				Meinhard*
RF power (W)				1200
Nebulizer Ar flow (L min ⁻¹) daily optimized				0.77-0.80
Auxiliary Ar flow Ar (L min ⁻¹) daily optimized				1.00-1.20
Measurements				
Scan mode				Peak hopping
Dwell time (ms)				50
Sweeps/reading				50
Integration time (ms)				2500
Replicates				5
m/z monitored				⁵⁴ Fe ⁺ and ⁵⁵ Mn ⁺
Correction equation				
⁵⁴ Fe ⁺ - 0.028226 * ⁵² Cr				
Dynamic reaction cell operational conditions				
Element	m/z monitored	Reaction Gas	Gas flow (mL min ⁻¹)	RPq (V)
Fe	⁵⁴ Fe ⁺	Methane	1.0	0.75
Mn	⁵⁵ Mn ⁺	Methane	1.0	0.85

NaHCO₃ buffer (pH 6.8)] was added to the tube. Then, the mixture was settled in a thermostatic bath for 5 min at 37 °C and 150 rpm of shaking. After the mouth stage, 5 mL of gastric solution (20 mg of porcine pepsin and 150 mmol L⁻¹ NaCl at pH 2.0) was added to start the gastric digestion part. Concentrated HCl was applied to adjust the pH. During this stage, the mixture remained in the thermostatic bath under the same previously cited conditions for 120 min. The enzymatic gastric digestion was stopped by placing the flask in an ice-water bath for 15 min. To carry out the last step, the intestinal one, the addition of 10 mL of intestinal solution [3% (w/v) porcine pancreatin and 2.5% (w/v) bile salts dissolved in a 100 mmol L⁻¹ NaHCO₃ buffer at pH 7.4] was performed. A dialysis membrane (14 kDa MWCO), filled with a 75 mmol L⁻¹ PIPES solution (pH 7.4) was also inserted in the tube. After 120 min in the thermostatic bath at 37 °C and 150 rpm of shaking, the tube was placed in an ice-water bath for 15 min to stop the enzymatic intestinal digestion. After extracting the dialysis membrane, its outer surface was washed with deionized water and its internal solution (bioavailable fraction) was transferred to a polypropylene conic tube to complete the volume to 25 mL with deionized water. Finally, the remained slurry in the flask was centrifuged for 10 min at 13,800 x g (4 °C). The supernatant collected after centrifugation (bioaccessible fraction) was added to a conic tube and the volume adjusted to 25 mL with deionized water. The açai pulp remaining residue (solid) was digested using the previously described microwave heating program and the decomposed residue was diluted to 25 mL with deionized water. The contents of Fe and Mn in the bioaccessible, bioavailable and residual fractions were determined after further dilution by ICP-MS following the operating parameters described in the Table 1. To prevent enzyme degradation and/or inhibition, each digestion fluid was prepared just before starting the digestion procedure.

Trueness of the current *in vitro* gastrointestinal digestion study was assessed through a mass balance approach. After the *in vitro* procedure, metal concentrations were determined in the bioaccessible fraction and dialyzate, while the residual fraction (non-dialyzable fraction) was acid digested in triplicate and then analyzed 2.

Mass balance was performed by comparison between the metal concentration as the sum of metal concentrations in the bioaccessible, bioavailable and residual fractions, and the total metal concentration obtained, according to the following equation:

Mass Balance (%)

$$= \frac{([Element]_{Bioaccessible} + [Element]_{Bioavailable} + [Element]_{Residue}) * 100}{[Element]_{Total}}$$

3. Results and discussion

3.1. Determination of total contents of Fe and Mn in certified reference materials and in açai pulps by ICP-MS

A suitable nutrition is crucial to ensure a good health. Therefore, it is important to evaluate food composition for a trace essential and toxic elements profile. Recently, it was demonstrated that açai pulp is a good source of Fe (average total concentration of 178 mg kg⁻¹) providing significant portions of the recommended daily intake of this mineral. Moreover, a high average concentration of Mn was also found (450 mg kg⁻¹), which raised the hypothesis of a possible toxicological risk associated with the consumption of this pulp [13]. Additionally, it is known that Fe and Mn can compete each other to be dietary absorbed, and, then, it is possible that high amounts of Mn ingestion influence on Fe absorption [18]. In this context, the total concentrations of Fe and Mn in açai pulp samples were obtained by ICP-MS after microwave assisted acid digestion.

Due to unavailability of certified reference materials of açai pulp, three certified reference materials of food and one of leaves from NIST - 8415 Whole Egg Powder, 1567a Wheat Flour, 1568a Rice Flour and 1547 Peach Leaves were analyzed to check data accuracy. Found values for Fe and Mn were in agreement with the certified values at 95% confidence level, according to the paired *t*-test (Table 2). Furthermore, precision was always < 10% for both Fe and Mn in all certified reference materials evaluated. Linear correlation coefficient (R²) for Fe and Mn were > 0.99 and detection (LOD) and quantification limits (LOQ) were 6.7 and 22.4 µg kg⁻¹ and 3.0 and 9.9 µg kg⁻¹, for Fe and Mn, respectively.

The verified method was applied for the determination of Fe and Mn in nine lyophilized açai pulp samples, five lab-made (3 from São Paulo state and 2 from Pará state) and four commercial (from different locations of Pará state), and the results are in the Table 3. The total concentrations of Fe did not vary considerably (27.6 to 73 mg kg⁻¹) among the açai pulp samples while for Mn a high variation was found (145 to 1197 mg kg⁻¹). Precision was < 9% for both Fe and Mn. Moreover, the levels of Mn in all analyzed samples were always expressively higher than those found for Fe, varying from 4 to 27 times.

In general, the highest contents of Mn were found for the samples from Pará state. Probably, this is due to the genetic variability as well as the soil composition and/or environmental conditions. The samples 5 and 9 presented the highest concentrations of Mn. The fruits that gave

Table 2

Total concentrations (mg kg⁻¹) of Fe and Mn (average value ± standard deviation, n = 3) in the certified reference materials 8415 Whole Egg Powder, 1567a Wheat Flour, 1568a Rice Flour and 1547 Peach Leaves using ICP-MS.

Certified Reference Material	Concentration (mg kg ⁻¹)			
	Fe		Mn	
	Certified	Found	Certified	Found
Whole Egg Powder	112 ± 16	101 ± 8	1.78 ± 0.38	2.12 ± 0.22
Wheat Flour	14.1 ± 0.5	13.0 ± 0.9	9.4 ± 0.9	8.8 ± 0.5
Rice Flour	7.4 ± 0.9	7.4 ± 0.6	20.0 ± 1.6	18.6 ± 1.1
Peach Leaves	218 ± 14	180 ± 19	98 ± 3	89 ± 7

Paired *t*-test: Whole Egg Powder - Fe - *t*_{calculated} = 2.38; Mn - *t*_{calculated} = 2.68; *t*_{critical} = 4.30; Wheat Flour - Fe - *t*_{calculated} = 2.12; Mn - *t*_{calculated} = 2.08; *t*_{critical} = 4.30; Rice Flour - Fe - *t*_{calculated} = 0; Mn - *t*_{calculated} = 2.20; *t*_{critical} = 4.30; Peach Leaves - Fe - *t*_{calculated} = 3.46; Mn - *t*_{calculated} = 2.23; *t*_{critical} = 4.30

Table 3

Total concentrations (mg kg⁻¹) of Fe and Mn (average value ± standard deviation, n = 3) in açai pulps.

Sample	[Fe], mg kg ⁻¹	[Mn], mg kg ⁻¹
Lab-made		
1*	35.0 ± 2.0	145 ± 3
2*	41.4 ± 1.5	262 ± 7
3*	49.8 ± 2.9	286 ± 10
4**	27.6 ± 0.4	354 ± 12
5**	44.4 ± 3.2	1197 ± 114
Commercial***		
6	49.6 ± 1.4	329 ± 4
7	49.3 ± 1.0	573 ± 26
8	46.8 ± 1.5	637 ± 23
9	73 ± 1	940 ± 43

Samples from: *São Paulo state and **Pará state; ***All commercial pulps are from Pará state.

origin to these samples came from the state of Pará, region where the fruit is native. The soil of these locations may have a high Mn concentration. This agrees with the fact that some Mn ores have been still exploited in the soil of this state [26,27]. On the other hand, the pulping processes of the fruits did not contribute to sample contamination since high concentrations of Mn were found for both the lab-made and commercial samples.

The iron concentrations found in the pulp samples are in good agreement with some previous studies with açai pulps [1,2,10,11]. On the other hand, it was slightly lower than those found by Santos et al. [13] and higher than those found by Rogez [12]. Possibly, these differences resulted from the variability of samples analyzed by the different studies. For Mn, the found values are in agreement with the concentrations previously reported [1,10,12,13], but higher than those found by Menezes [11].

It is also important to mention that the observed levels of Fe in the present study for the açai pulps were higher than the concentrations reported to other berry fruits in the literature, such as blackberry - 10.9 and 12.8 mg kg⁻¹, strawberry - 7.3 and 10.6 mg kg⁻¹, blueberry - 8.2 and 10.4 mg kg⁻¹ and raspberry - 13.3 and 15.9 mg kg⁻¹ [28,29]. Nevertheless, the Fe concentration range agreed with the concentration found to goji berry - 39.41 mg kg⁻¹ [30].

Despite the levels of Mn in some berry fruits be considerably high, ex. blackberry - 28.7 and 169 mg kg⁻¹, strawberry - 4.1 and 35.1 mg

Table 4

Total concentrations (mg kg⁻¹) of Fe and Mn (average values ± standard deviations, n = 3) determined in bioaccessible, bioavailable and residual fractions of açai pulp samples. Values in parenthesis represent the % of the total concentration.

Sample	Element	Total, mg kg ⁻¹	Bioaccessible, mg kg ⁻¹ (%)	Bioavailable, mg kg ⁻¹ (%)	Residue, mg kg ⁻¹ (%)	Mass Balance, mg kg ⁻¹ (%)
Lab-made						
1	Fe	35.0 ± 2.0	12.1 ± 0.7 (34)	< LOQ	22.7 ± 3.5 (65)	34.8 ± 3.1 (99)
	Mn	145 ± 3	58 ± 3 (40)	15.7 ± 1.1 (11)	42.2 ± 0.8 (29)	116 ± 1 (80)
2	Fe	41.4 ± 1.5	13.8 ± 1.1 (33)	< LOQ	22.5 ± 4.2 (54)	36.3 ± 3.1 (87)
	Mn	262 ± 7	112 ± 10 (43)	40.7 ± 0.8 (15)	60 ± 1 (23)	213 ± 11 (81)
3	Fe	49.8 ± 2.9	17.1 ± 2.1 (34)	< LOQ	32.1 ± 0.3 (64)	49.2 ± 1.8 (99)
	Mn	286 ± 10	157 ± 6 (55)	45.3 ± 0.9 (16)	78 ± 5 (27)	280 ± 22 (98)
4	Fe	27.6 ± 0.4	8.0 ± 0.8 (29)	< LOQ	19.5 ± 1.3 (71)	27.6 ± 2.2 (100)
	Mn	354 ± 12	184 ± 1 (52)	54 ± 7 (15)	138 ± 11 (39)	376 ± 19 (106)
5	Fe	44.4 ± 3.2	8.9 ± 0.5 (20)	< LOQ	31.9 ± 1.6 (72)	40.8 ± 1.1 (92)
	Mn	1197 ± 114	134 ± 6 (11)	98 ± 3 (8)	997 ± 89 (83)	1229 ± 62 (102)
Commercial						
6	Fe	49.6 ± 1.4	17.4 ± 1.4 (35)	< LOQ	28.9 ± 1.9 (58)	46.3 ± 3.3 (93)
	Mn	329 ± 4	139 ± 8 (39)	42.8 ± 0.6 (13)	102 ± 8 (31)	283 ± 15 (86)
7	Fe	49.3 ± 1.0	19.1 ± 1.2 (39)	< LOQ	30.8 ± 0.3 (62)	49.9 ± 1.5 (101)
	Mn	573 ± 26	230 ± 24 (40)	70 ± 9 (12)	216 ± 1 (38)	516 ± 32 (90)
8	Fe	46.8 ± 1.5	16.3 ± 1.6 (35)	< LOQ	31.7 ± 0.3 (68)	48.0 ± 5.9 (103)
	Mn	637 ± 23	345 ± 12 (54)	94 ± 6 (15)	160 ± 12 (25)	599 ± 19 (94)
9	Fe	73 ± 1	29.2 ± 2.7 (40)	0.58 ± 0.01 (0.8)	38.6 ± 1.9 (53)	68 ± 5 (94)
	Mn	940 ± 43	133 ± 6 (14)	160 ± 8 (17)	581 ± 52 (62)	874 ± 38 (93)

kg⁻¹, blueberry - 2.2 and 95 mg kg⁻¹, raspberry - 4.3 and 73 mg kg⁻¹ and goji berries - 9.99 mg kg⁻¹ [28–30] the concentration range found for the açai pulps was substantially higher. Unfortunately, the comparison between the found contents of Fe and Mn in our study with those reported for other kinds of fruits or for manufactured açai juices [31,32] and even for açai pulps [33] is impossible, since most of the previous studies did not report the data based on dry matter and the amount of total solids varies significantly between samples.

According to this study, açai berries can be considered an important natural food source of these two elements, mainly Mn. The concentration of Mn in foodstuffs varies widely and the highest concentrations can be found in foods of plant origin, such as wheat, rice, tea leaves - between 10 and 100 mg kg⁻¹ [34] and in different types of nuts, such as almond (4780 mg kg⁻¹), peanut (2110 mg kg⁻¹), pine nuts (*Pinus pinea*) (26–559 mg kg⁻¹), black walnut (576.2 mg kg⁻¹), pecan nut (143 mg kg⁻¹) and white walnut (68 mg kg⁻¹) [35]. Moreover, in general, dairy products, fruit and meat contain less than 1 mg kg⁻¹ [34].

3.2. Bioaccessibility and bioavailability of Fe and Mn in açai pulps

Fruits and vegetables are important to the diet because they are rich sources of vitamins, minerals, phytochemicals (especially antioxidants) and dietary fiber [36]. At the same time, fruits and vegetables may contain toxic elements or essential elements at high concentrations. Taking into account the total concentrations of Fe and Mn found in açai pulps, it is reasonable to state that açai pulps are good sources of Fe and that they accumulate significant amounts of Mn. However, although the total amount of a mineral is important, it does not reflect the real benefits and risks of consuming a food. Therefore, an *in vitro* gastrointestinal digestion procedure was carried out to evaluate the bioaccessibility and bioavailability of Fe and Mn in the 9 açai pulp samples. It is important to comment that the bioavailabilities of Fe and Mn in açai pulps were estimated using the dialyzable fractions.

Mass balance recoveries varied from 87 to 103% and 80 to 106% for Fe and Mn, respectively. On the other hand, the mass balance precision was acceptable with the relative standard deviations (RSD) lower than 12 and 8% for Fe and Mn, respectively. According to these results, the developed method was suitable to measure the bioaccessible, bioavailable and residual fractions of Fe and Mn in açai pulps.

The concentrations of Fe and Mn in the bioaccessible, bioavailable and residual fractions of the açai pulp samples are in the Table 4. The

Table 5

Dietary reference intakes (DRI) of Fe and Mn (mg per day) and contribution of açai pulp consumption – 100 g (%) to supply human essential Fe and Mn requirements according to gender, age, and pregnancy-lactation.

Life Stage	Age (years)	Fe ^a		Mn ^b	
		DRI (mg/day)	Contribution of Açai Pulp – 100 g (%)	DRI (mg/day)	Contribution of Açai Pulp – 100 g (%)
Children	1-3	7	2.4 – 3.3	1.2	279 – 388
Children	4-8	10	1.7 – 2.3	1.5	223 – 311
Adult (Male)	19-70	8	2.1 – 2.9	2.3	146 – 203
Adult (Female)	19-50	18	0.9 – 1.3	1.8	186 – 259
Pregnancy	All ages	27	0.6 – 0.8	2.0	167 – 233
Lactation	All ages	10	1.7 – 2.3	2.6	129 – 179

^a RDA: Recommended Dietary Allowance.

^b AI: Adequate Intake.

highest concentrations of Fe were obtained in the residual fractions for all açai pulp samples analyzed. The concentration range represented approximately 53–72 % of the total Fe. The bioaccessible fractions of Fe did not vary significantly for all samples (29–40 % of the total Fe), except for the sample 5 (20% of the total Fe), which contained the highest total Mn concentration (1197 mg kg⁻¹). These results indicate that the absorption of Fe after gastrointestinal digestion is reasonable (up to 40% is available for absorption) and that high Mn contents may influence on the bioaccessibility of Fe in açai pulps. In fact, Fe was not bioavailable in the açai pulp samples evaluated since all found concentrations were lower than the LOQ or very low as that one found to sample 9, which represented approximately 0.8% of the total Fe.

The bioaccessible fractions presented the highest concentrations of Mn for all samples, representing from 39 to 55% of the total Mn. However, two samples, 5 and 9 (which are the two samples with the highest total concentrations of Mn), showed lower bioaccessible fractions of this element (11 and 14%, respectively). The hypothesis is that these two samples have accumulated the excess of Mn in the form of non bioaccessible compounds. The residual fractions of Mn did not vary significantly for all samples (23–39 % of the total Mn), except for the samples 5 and 9 (83 and 62% of the total Mn). The bioavailable concentrations of Mn represented from 8 to 17% of the total Mn concentration. The sample 5 showed the lowest bioavailable percentage of Mn and coincidentally this sample showed the highest total concentration of Mn. Although the Mn total concentration is very high, the bioavailable fraction is low. Adding the bioaccessible and the bioavailable fractions, the concentrations of Mn represented from 51 to 71% of the total concentration, except for the samples 5 and 9 (19 and 31%, respectively). Thus, the highest total concentrations of Mn presented by the samples 5 and 9 presented the lowest sums of bioaccessible and bioavailable fractions (in %) of this element.

According to these findings, Mn was more bioaccessible than Fe for the most açai pulps. Moreover, the bioavailable concentrations of Fe were lower than LOQ whereas those of Mn represented from 8 to 17% of the total concentration. These results agree with other works, which shows higher bioaccessible fractions for Mn than for Fe in fruits and vegetables [28,37]. The possible reason is the presence of fibers, phytate, oxalic acid, carbonate and polyphenols which forms insoluble compounds and impairs Fe absorption [37]. In fact, açai contain a high amount of fibers (insoluble fiber with a smaller fraction of soluble fiber) and polyphenols (in the form of flavonoids and anthocyanins) [7]. For Mn, it seems that the formed compounds are more soluble at the pH of the gastrointestinal tract [28]. Other possibility is that Mn is binding to proteins since açai contains a reasonable portion of them. Then, this binding is broken in the gastrointestinal tract due to the action of pepsin and pancreatin, which causes the hydrolysis of proteins via the cleavage of peptide bonds [38]. Additionally, the high concentrations of Mn in the açai samples may have influenced on the bioaccessible and

bioavailable fractions of Fe due to similar physicochemical properties and shared absorptive pathways, as previously commented [17,18]. Also, it is important to mention that there are data demonstrating multiple forms of interactions between Fe and the other essential elements Cu and Zn, besides Mn, which may have some influence on the observed results [18].

3.3. Estimation of Mn and Fe daily intake based on açai consumption

The contribution of açai for Fe and Mn dietary reference intakes (DRI) was calculated assuming a daily consumption of 100 g of açai pulp. The amount as well as the frequency of açai consumption varies significantly according to the region of Brazil and worldwide. In the Pará state, açai is currently consumed daily at main meals, or as a beverage, either simple or mixed with cassava/manioc flour and fish, with or without sugar. However, in other Brazilian regions, the frequency of consumption is lower, once a week, in general and the açai is typically ingested mixed with guarana syrup and with other fruits, such as, banana, strawberry and kiwi as a frozen dessert [7].

Additionally, there was an assumption of an average content of total solids of 12.5%, which corresponds to regular or medium açai (Type B - from 11% to 14% total solids) according to the regulation of the Brazilian Ministry of Agriculture, Livestock and Supply. The bioaccessible and bioavailable fractions (sum range, taking into account all samples, except 5 and 9, which presented lower values) of Fe and Mn were used to calculate the contribution of açai to DRI.

The micronutrients Fe and Mn are essential for the human organism and therefore there are recommendations in terms of nutritional intake for them according to gender and age. Table 5 shows the contribution of consuming 100 g of açai pulp (%) to Fe and Mn dietary reference intakes (DRI), that is recommended dietary allowance (RDA) for Fe and adequate intake (AI) for Mn according to the Food and Nutrition Board [39]. A portion of approximately 100 g of açai pulp is capable of supplying from 2.4 to 3.3%, 1.7 to 2.3%, 2.1 to 2.9%, 0.9 to 1.3%, 0.6 to 0.8% and 1.7 to 2.3% of Fe daily needs for children 1–3 years, children 4–8 years, male 19–70 years, female 19–50 years, pregnant and lactants, respectively. Therefore, considering the bioaccessible fractions of Fe, the ingestion of 100 g of açai pulp represents only a minor contribution to Fe DRI. If the same amount of beef meat (100 g) is consumed, total Fe amount of 49 mg kg⁻¹ and 19% of this bioaccessible [40], the contributions to Fe daily needs correspond to 13.3, 9.3, 11.6, 5.2, 3.4 and 9.3% for children 1–3 years, children 4–8 years, male 19–70 years, female 19–50 years, pregnant and lactants, respectively, which are around 4-fold higher than the contributions from açai pulps. However, the contributions from açai pulps for Fe daily needs are higher than those find for other kinds of berries, blackberries, raspberries, blueberries and strawberries [28].

The same portion of açai pulp (100 g) is able to provide from 279 to 388%, 223 to 311%, 146 to 203%, 186 to 259%, 167 to 233% and 129 to 179% of Mn daily needs for children 1–3 years, children 4–8 years, male 19–70 years, female 19–50 years, pregnant and lactants. These results confirm that açai pulp is a very significant source of Mn. Considering the sum of bioaccessible and bioavailable fractions of Mn, the ingestion of 100 g of açai pulp exceeds at least in 1.5 and 1.8-fold the daily needs for a man and a woman (adults), respectively. Taking into account the highest fraction of bioaccessible and bioavailable Mn (which corresponded to 71% of the total Mn) this element intake (4.66 mg) exceeds the lowest-observed-adverse-effect level (LOAEL) for Mn established at 4.2 mg/day for a 70 kg individual [41] although it is lower than the tolerable upper daily intake for an adult of 11 mg/day, which is the maximum level of daily nutrient intake (by food, water and supplements) that is likely to pose no risk of adverse effects [39].

Extremely high Mn exposure from açai ingestion might be considered of concern, especially for children, since even the lowest fraction of bioaccessible and bioavailable Mn (51% of the total Mn) corresponded to an element intake (3.35 mg) higher than the tolerable

upper daily intakes for children of 1–3 years (2 mg/day) and of 4–8 years (3 mg/day). As previously mentioned, the amount of açai daily consumption may reach values higher than 500 mL in some Brazilian regions, which means even higher levels of Mn exposure. However, future studies are necessary to evaluate possible toxic effects of such high levels of Mn exposure based on ingestion route. Moreover, due to the possible impairment of Fe absorption associated to the high levels of Mn intake, a high consumption of açai may be taken of concern for populations with Fe deficiency.

4. Conclusion

According to the results obtained, açai pulp is a natural good source of Fe and accumulates higher contents of Mn, up to approximately 1 g kg⁻¹. The bioaccessibility and bioavailability findings showed that Mn might be more absorbed than Fe from the açai pulps by the gastrointestinal tract. In fact, the high contents of Mn may influence on Fe absorption. Additionally, Mn exposure by açai pulp ingestion might be carefully considered since the ingestion of 100 g of açai pulp exceeds at least in 1.5-fold the dietary reference intake of this element for adults and also is higher than the tolerable upper daily intakes for children.

The knowledge of the bioaccessible and bioavailable contents of Fe and Mn represented a more reliable method to establish the real nutritional value of the açai pulps compared to total concentrations. A high consumption of açai pulp may be taken of concern mainly by the Brazilian northern population and also by those individuals with Fe deficiency. However, further research is necessary to evaluate the possible toxic effects of ingesting foods containing high levels of Mn. Moreover, more studies to separate and identify the Mn species in açai pulp and in its bioaccessible and bioavailable fractions are required since the chemical speciation of Mn may explain how the chemical forms of this element change and/or modulate its bioaccessibility and bioavailability as well as its toxicity or beneficial health effects.

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Conflict of Interest

All authors declare that they have no conflict of interest.

References

- [1] A. Gordon, A.P.G. Cruz, L.M.C. Cabral, S.C. de Freitas, C.M.A.D. Taxi, C.M. Donangelo, R.A. Mattioto, M. Friedrich, V.M. da Matta, M. Friedhelm, Chemical characterization and evaluation of antioxidant properties of Açai fruits (*Euterpe oleracea* Mart.) during ripening, *Food Chem.* 133 (2012) 256–263.
- [2] A.G. Schauss, X. Wu, R.L. Prior, B. Ou, P. Patel, D. Huang, J.P. Kaback, Phytochemical and Nutrient Composition of the Freeze-Dried Amazonian Palm Berry, *Euterpe oleracea* Mart. (Acai), *J. Agric. Food Chem.* 54 (2006) 8598–8603.
- [3] A.G. Costa, D.F. Garcia-Diaz, P. Jimenez, P.I. Silva, Bioactive compounds and health benefits of exotic tropical red–blackberries, *J. Funct. Foods* 5 (2013) 539–549.
- [4] J. Strudwick, G.L. Sobel, Uses of *Euterpe oleracea* Mart. in the Amazon estuary, Brazil, *Adv. Econ. Bot.* 6 (1988) 225–253.
- [5] R. Lichtenthaler, R.B. Rodrigues, J.G.S. Maia, M. Papagiannopoulos, H. Fabricius, F. Marx, Total oxidant scavenging capacities of *Euterpe oleracea* Mart. (açai) fruits, *Int. J. Food Sci. Nutr.* 56 (2005) 53–64.
- [6] A.G. Schauss, X. Wu, R.L. Prior, B. Ou, D. Huang, J. Owens, A. Agarwal, G.S. Jensen, A.N. Hart, E. Shanbrom, Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, *Euterpe oleracea* Mart. (Acai), *J. Agric. Food Chem.* 54 (2006) 8604–8610.
- [7] K.K.L. Yamaguchi, L.F.R. Pereira, C.V. Lamarão, E.S. Lima, V.F. Veiga-Junior, Amazon acai: chemistry and biological activities: a review, *Food Chem.* 179 (2015) 137–151.
- [8] V.S. Santos, E. Bisen-Hersh, Y. Yu, I.S.R. Cabral, V. Nardini, M. Culbreth, J.B.T. Rocha, F. Barbosa Jr., M. Aschner, Anthocyanin-rich açai (*Euterpe oleracea* Mart.) extract attenuates manganese-induced oxidative stress in rat primary astrocyte cultures, *J. Toxicol. Environ. Health A* 77 (2014) 390–404.
- [9] T. Córdova-Fraga, D.B. de Araujo, T.A. Sanchez, E.J. Júnior, A.A. Carneiro, R. Brandt-Oliveira, M. Sosa, M. Baffa, *Euterpe oleracea* (Açai) as an alternative oral contrast agent in MRI of the gastrointestinal system: preliminary results, *Magn. Reson. Imaging* 22 (2004) 389–393.
- [10] C.V. Lamarão, Estudo da variabilidade do teor em compostos fenólicos e minerais do açai em função de variáveis geográficas e edáficas, Universidade Federal do Pará, Belém, 2009.
- [11] E.M.S. Menezes, A.T. Torres, A.U.S. Srur, Valor nutricional da polpa de açai (*Euterpe oleracea* Mart.) liofilizada, *Acta Amaz.* 38 (2008) 311–316.
- [12] H. Rogez, Açai: preparo, composição e melhoramento da conservação, first ed., UFPA, Belém, 2000.
- [13] V.S. Santos, G.H.A. Teixeira, F. Barbosa Júnior, Açai (*Euterpe oleracea* Mart.): a tropical fruit with high levels of essential minerals—especially manganese—and its contribution as a source of natural mineral supplementation, *J. Toxicol. Environ. Health A* 77 (2014) 80–89.
- [14] B. Michalke, S. Halbach, V. Nischwitz, Speciation and toxicological relevance of manganese in humans, *J. Environ. Monit.* 9 (2007) 650–656.
- [15] B. Michalke, K. Fernsebner, New insights into manganese toxicity and speciation, *J. Trace Elem. Med. Biol.* 28 (2014) 106–116.
- [16] Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Manganese, (2012) (Accessed 14 November 2018), <https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=102&tid=23>.
- [17] L. Rossander-Hultén, M. Brune, B. Sandström, B. Lönnnerdal, L. Hallberg, Competitive inhibition of iron absorption by manganese and zinc in humans, *Am. J. Clin. Nutr.* 54 (1991) 152–156.
- [18] G. Björklund, J. Aaseth, A.V. Skalny, J. Suliburska, M.G. Skalnaya, A.A. Nikonorov, A.A. Tinkov, Interactions of iron with manganese, zinc, chromium, and selenium as related to prophylaxis and treatment of iron deficiency, *J. Trace Elem. Med. Biol.* 41 (2017) 41–53.
- [19] C.J. Mercadante, C. Herrera, M.A. Pettiglio, M.L. Foster, L.C. Johnson, D.C. Dorman, T.B. Bartnikas, The effect of high dose oral manganese exposure on copper, iron and zinc levels in rats, *Biometals* 29 (2016) 417–422.
- [20] J. Moreda-Piñeiro, A. Moreda-Piñeiro, P. Bermejo-Barrera, In vivo and in vitro testing for selenium and selenium compounds bioavailability assessment in food-stuff, *Crit. Rev. Food Sci. Nutr.* 57 (2017) 805–833.
- [21] G. Cano-Sancho, G. Perelló, A.L. Maulvault, A. Marques, M. Nadal, J.L. Domingo, Oral bioaccessibility of arsenic, mercury and methylmercury in marine species commercialized in Catalonia (Spain) and health risks for the consumers, *Food Chem. Toxicol.* 86 (2015) 34–40.
- [22] S.G. Lee, J. Kim, H. Park, W. Holzappel, K. Lee, Assessment of the effect of cooking on speciation and bioaccessibility/cellular uptake of arsenic in rice, using in vitro digestion and Caco-2 and PSI cells as model, *Food Chem. Toxicol.* 111 (2018) 597–604.
- [23] M.A. Herrera-Agudelo, M. Miró, M.A.Z. Arruda, In vitro oral bioaccessibility and total content of Cu, Fe, Mn and Zn from transgenic (through cp4 EPSPS gene) and nontransgenic precursor/successor soybean seeds, *Food Chem.* 225 (2017) 125–131.
- [24] J. Moreda-Piñeiro, A. Moreda-Piñeiro, V. Romarís-Hortas, C. Moscoso-Pérez, P. López-Mahía, S. Muniategui-Lorenzo, P. Bermejo-Barrera, D. Prada-Rodríguez, In-vivo and in-vitro testing to assess the bioaccessibility and the bioavailability of arsenic, selenium and mercury species in food samples, *Trends Analyt. Chem.* 30 (2011) 324–345.
- [25] C.A. Lopes Júnior, S.R. Oliveira, P. Mazzafera, M.A.Z. Arruda, Expanding the information about the influence of cadmium on the metabolism of sunflowers: Evaluation of total, bioavailable, and bioaccessible content and metallobiomolecules in sunflower seeds, *Environ. Exp. Bot.* 125 (2016) 87–97.
- [26] J.M.M. Paixão, J.C. Amaral, L.E. Memória, L.R. Freitas, Sulphation of Carajás manganese ore, *Hydrometallurgy* 39 (1995) 215–222.
- [27] Agência Nacional de Mineração (ANM), Departamento Nacional de Produção Mineral (DNPM), Anuário Mineral Brasileiro 2016 – Metálicos, (2016) (Accessed 14 November 2018), <http://www.anm.gov.br/dnpm/publicacoes/serie-estatisticas-economia-mineral/anuario-mineral/anuario-mineral-brasileiro>.
- [28] C.C. Pereira, E.N. da Silva, A.O. de Souza, M.A. Vieira, A.S. Ribeiro, S. Cadore, Evaluation of the bioaccessibility of minerals from blackberries, raspberries, blueberries and strawberries, *J. Food Anal.* 68 (2018) 73–78.
- [29] A. Nikolic, A. Pavlovic, S. Mitic, S. Tosic, E.P. Marinkovic, M. Đorđević, R. Micic, Optimization and validation of an inductively coupled atomic emission spectrometry method for macro- and trace element determination in berry fruit samples, *Anal. Methods* 8 (2016) 4844–4852.
- [30] J. Wojcieszek, P. Kwiatkowski, L. Ruzik, Speciation analysis and bioaccessibility evaluation of trace elements in goji berries (*Lycium Barbarum*, L.), *J. Chromatogr. A* 1492 (2017) 70–78.
- [31] United States Department of Agriculture (USDA), Agricultural Research Service. USDA Food Composition Databases, (2018) (Accessed 14 November 2018), <https://ndb.nal.usda.gov/ndb/nutrients/index>.
- [32] E.J. Llorent-Martínez, M.L. Fernández-de-Córdova, P. Ortega-Barrales, A. Ruiz-Medina, Characterization and comparison of the chemical composition of exotic superfoods, *Microchem. J.* 110 (2013) 444–451.
- [33] Núcleo de Estudos e Pesquisas em Alimentação (NEPA), Tabela Brasileira de Composição de Alimentos (TACO), (2011) (Accessed 14 November 2018), <http://www.nepa.unicamp.br/taco/tabela.php?ativo=tabela>.
- [34] R. Cornelis, Element by element review, in: R. Cornelis, J. Caruso, H. Crews, K. Heumann (Eds.), Handbook of Elemental Speciation II – Species in the Environment, Food, Medicine and Occupational Health, Wiley, Chichester, 2005,

- pp. 277–280.
- [35] A.B. Borrego, T.G. Barrera, J.L. Gómez-Ariza, Speciation of manganese binding to biomolecules in pine nuts (*Pinus pinea*) by two-dimensional liquid chromatography coupled to ultraviolet and inductively coupled plasma mass spectrometry detectors followed by identification by electrospray ionization mass spectrometry, *Rapid Commun. Mass Spectrom.* 22 (2008) 3053–3060.
- [36] J.L. Slavin, B. Lloyd, Health benefits of fruits and vegetables, *Adv. Nutr.* 3 (2012) 506–516.
- [37] R.B. Khouzam, P. Pohl, R. Lobinski, Bioaccessibility of essential elements from white cheese, bread, fruit and vegetables, *Talanta* 86 (2011) 425–428.
- [38] S.J. Hur, B.O. Lim, E.A. Decker, D.J. McClements, *In vitro* human digestion models for food applications, *Food Chem.* 125 (2011) 1–12.
- [39] Institute of Medicine (IOM), Food and Nutrition Board, Dietary Reference Intakes for Vitamin a, Vitamin K, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc, National Academy Press, Washington, DC, 2001, pp. 394–419.
- [40] E.A. Menezes, A.F. Oliveira, C.J. França, G.B. Souza, A.R.A. Nogueira, Bioaccessibility of Ca, Cu, Fe, Mg, Zn, and crude protein in beef, pork and chicken after thermal processing, *Food Chem.* 240 (2018) 75–83.
- [41] J.L. Greger, Dietary standards for manganese: overlap between nutritional and toxicological studies, *J. Nutr.* 128 (1998) 368S–371S.