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Chemical composition of Brazilian chia seeds grown in different places

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ABSTRACT

This study investigated and compared the occurrence and concentration of macronutrients, moisture, ash, dietary fiber, fatty acids, minerals, carotenoids, vitamins, flavonoids, phenolic compounds, antioxidant activity, phytate and tannin in Brazilian chia seeds grown in the states of Mato Grosso (MT) and Rio Grande do Sul (RS). High concentrations of lipids (31.2 g.100 g^{-1}, on average), proteins (18.9 g.100 g^{-1}, on average), dietary fiber (35.3 g.100 g^{-1}, on average), vitamin E (8,203.6 μg.100 g^{-1}, on average) were observed. Similar values for total phenolic compounds and phytic acid in chia seeds from both regions were observed. Chia grown in RS showed higher antioxidant activity than chia grown in MT, and the tannin concentrations were higher in chia seeds grown in Mato Grosso (19.08 ± 1.08 eq.catequina/g sample). In conclusion, Brazilian chia seeds showed high concentrations of lipids, proteins, total dietary fiber, minerals and vitamin E.

1. Introduction

The genus Salvia, native of southern Mexico and northern Guatemala, includes approximately 900 species of Lamiaceae seeds (Ixtaina, Nolasco, & Tomás, 2008). Of these species, 47 are endemic in Brazil and 61 are grown in the Northeast, Midwest, Southeast and South of the country. The biggest crop seed of this sort occurs in temperate to subtropical mountainous regions (Capitani, Spotorno, Nolasco, & Tomás, 2012; Harley, 2012).

Among the species of the genus Salvia, chia (*Salvia hispanica* L.) is a 2 mm length herbaceous plant which stands out due to its high nutritional and functional values. The chemical composition and nutritional value of the chia seed can vary according to the climatic condition and location of growing. Studies show that the geographical location and climate can influence the concentration of nutrients in chia seeds (Ayerza & Coates, 2009).

In Brazil, chia is consumed mainly in the form of flour and seeds that can be added to preparations such as fruit, yogurt, cakes, among others. Its consumption has increased due to its beneficial effects related to obesity, cardiovascular disease, diabetes and some types of cancer (Ixtaina et al., 2011; Poudyal, Panchal, Waanders, Ward, & Brown, 2012; Vázquez-Ovando, Rosado-Rubio, Chel-Guerrero, & Betancur-Ancona, 2009). Those benefits result primarily of the high concentrations of essential fatty acids, dietary fiber, antioxidants, flavonoids, anthocyanins, vitamins, carotenoids and minerals present in this seed (Ayerza & Coates, 2011; Reyes-Caudillo, Tecante, & Valdivia-López, 2008).

To date, few studies have assessed the chemical characterization and bioactive compounds in Brazilian chia seed. Thus, the purpose of this study is to chemically characterize and compare the

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occurrence and concentration of macronutrients, moisture, ash, dietary fiber, minerals, carotenoids, vitamins, flavonoids, phenolic compounds, antioxidant activity, phytate and tannins of a single Brazilian chia genotype grown in different places.

2. Materials and methods

2.1. Raw material, description of growing regions, storage and preparation of chia flour

Chia seeds (*Salvia hispanica* L.) grown in Brazil were obtained from two distinct regions: Rio Grande do Sul (RS) and Mato Grosso (MT). The seeds used in this study showed brown pericarp and approximate diameter of 2 mm.

The state of RS has temperate climate, with relative humidity ranging from 60 to 80%, while the state of MT presents dry tropical climate, with relative humidity ranging from 15 to 30%. The agronomic variables of each region is shown in Table 1.

The samples were packed and transported to the laboratory in cardboard boxes, by land. Samples were stored in hermetically sealed plastic bags and protected from light, frozen (−18 ± 1 °C) until the time of analysis, which occurred within 30 days. All analyzes were performed in chia flour, obtained by grinding the grain with a multiprocessor (Walita, Brazil), during three minutes. This time was sufficient to prevent formation of paste when ground, since chia has high concentration of lipids and dietary fiber.

2.2. Macronutrients, moisture, ash, total dietary fiber and minerals analysis

The analysis of content of moisture, ash, proteins, lipids and total dietary fiber in chia were performed in three repetitions. Moisture was determined using an oven (Nova Ética®, model 400/6ND, São Paulo, Brazil) at 105 °C and ash was quantified using a muffle furnace (Quimis, Q320 M model, Brazil) at 550 °C. Protein content was determined though micro-Kjeldhal method, total dietary fiber was determined by the gravimetric non-enzymatic method and lipids were determined by Soxhlet method (AOAC, 2012). Carbohydrates were calculated as the difference, using the following equation: [100 − (% moisture + % lipids + % proteins + % total dietary fiber + % ash)]. The total energy value of chia was estimated considering the conversion factors of 4 kcal·g^{-1} for protein or carbohydrate and 9 kcal·g^{-1} per lipid. Concentrations of Fe, Zn, Ca, Mg, Mn, Cu, B, Pb, Cd, Cr, Na, K, S, Al and N were determined according to the methodology proposed by Gomes (1996).

2.2.1. Extraction and analysis of fatty acid composition

To analyze fatty acids, 150 mg of chia were weighed in a test tube. 1 mL of the internal standard triglyceride tridecanoic acid, 50 mg of pyrogallic acid, 1 mL of 95% ethanol were added and glass beads. The samples were subjected to acid hydrolysis with 5 mL of HCl and stirred in a thermostated bath (75 °C/40 min). After cooling to room temperature, 12 mL of ethyl ether were added and each tube was shaken on a vortex mixer for 1 min. The tubes were centrifuged (2865*g*/10 min) (Hermle®, model Z216MK, Germany), and the supernatant was transferred to another tube. The solvent was slowly evaporated in a thermostat at a temperature below 40 °C using N_2 gas; then 1 mL of 7% boron trifluoride in methanol and 0.5 mL toluene were added. The tubes were covered and placed in boiling bath for 45 min. After cooling to room temperature, 2.5 mL water, 1 mL hexane and approximately 0.5 g of anhydrous Na_2SO_4 were added. After mixing, the tubes were left to stand until phase separation and then the supernatant was transferred to a vial. A 1 μL sample was analyzed by gas chromatography GC 2010 Plus (Shimadzu, Workstation GC solution), equipped with an autosampler AOC-20, flame ionization detector (FID) and capillary column of fused silica from Supelco, SP-2560 (Bis cyanopropyl polisiloxana). The column temperature program was 140 °C for 5 min, heat at 4 °C/min to 240 °C for 20 min; injector and detector temperatures were 250 °C and 260 °C, respectively; helium carrier gas, flow of 1 mL/min and the splitting ratio 1/50. Fatty acid retention times were compared with the standard, and the calculations were based on area and concentration of the internal standard, using theoretical response factors of flame ionization detector (FID).

Table 1
Agronomic variables of the two production zones of chia seeds in Brazil, 2015.

Agronomics variables	RS Chia	MT Chia
Temperature	26 °C/78.8°F	20 °C/68°F
Relative humidity	62%	30%
Soil type	Clay soil	Mixed soil
Rainfall	Regular rainfall	Irregular rainfall
Length growing season	Plantation: January Harvest: June	Plantation: April Harvest: July
Climate	Temperate	Tropical

RS: Rio Grande do Sul, MT: Mato Grosso.

2.3. Extraction and analysis of carotenoids, vitamins and flavonoids

The preparation and analysis of carotenoids, vitamin E, vitamin C and flavonoids content in chia seeds were performed in five repetitions. During analysis, the samples and the extracts were protected from sunlight and artificial light using amber glassware, aluminum foil and blackout curtains, and protected from oxygen by using lids and environments with nitrogen gas in glass bottles.

2.3.1. Carotenoids

The occurrence and the concentration of lutein and zeaxanthin in chia seeds were investigated. Carotenoids were extracted in accordance with Rodriguez-Amaya (1996) with modifications. About 5 g of chia flour were homogenized with 30 mL of acetone for approximately 5 min in a micro grinder. The extract was vacuum filtered on Buchner funnel using filter paper. Then, the filtrate was transferred to a separatory funnel containing 25 mL of petroleum ether to transfer the pigments from acetone to petroleum ether. Each fraction was washed three times with distilled water, to remove all acetone. Anhydrous sodium sulfate was added to the extract to remove any residual water. The pigments were then redissolved in petroleum ether in a 25 mL volumetric flask and stored in amber glass vials at −18 ± 1 °C until the time of, which occurred in a maximum of one hour.

For analysis, an aliquot of 1 ml of extract was evaporated under nitrogen gas flow, and then recovered in 1 mL of HPLC grade acetone. The extract was filtered through a filter unit with porosity of 0.45 μm (Millipore, Brazil). Carotenoids were analyzed using a high performance liquid chromatography system (HPLC) (Shimadzu, SCL 10AT VP model, Japan) comprised of a high-pressure pump (Shimadzu, LC-10AT VP model, Japan), an autosampler with a loop of 500 μL (Shimadzu, SIL-10AF model, Japan) and a diode array detector (DAD) (Shimadzu, SPD-M10A model, Japan). The following chromatographic conditions were used: chromatographic column RP-18 (Phenomenex Gemini, 250 mm × 4.6 mm, 5 μm), fitted with a guard column (C18), (Phenomenex ODS 4 mm × 3 mm); mobile phase composed of hexane: isopropanol (HPLC grade, Tedia, Brazil), in proportions of 95:5, with flow rate of 2.0 mL·min^{-1} and injection volume of 50 μL (Pinheiro-Sant'Ana, Stringheta, Brandão, & Azeredo, 1998). The chromatograms were obtained at 450 nm.

2.3.2. Vitamin E

We investigated the occurrence and the concentration of the eight components of vitamin E (α-, β-, γ- and δ-tocopherols and tocotrienols) in chia seeds. The extraction of the components was carried out according to Pinheiro-Sant'ana et al. (2011) with modifications. Approximately 4.0 g of chia flour were weighed and added to 4.0 mL of heated ultrapure water (80 ± 1 °C). Then 10.0 mL of isopropyl alcohol, 1.0 mL of hexane containing 0.05% BHT, 5 g of anhydrous sodium sulfate and 25 mL of extraction solvent mixture (hexane: ethyl acetate, 85:15 v/v) were added. The sample was homogenized using a micro grinder for 1 min. The extract was vacuum filtered on Buchner funnel using filter paper. Then, the extract was transported to a rotating evaporator (70 ± 1 °C/2 min) until it was concentrated. After this procedure, it was transferred to a volumetric flask and the volume was completed to 25.0 ml using the solvent mixture.

For the analysis, an aliquot of 5 mL of extract was evaporated under nitrogen gas flow, and then recovered in 2.0 mL of HPLC grade hexane (Tedia, Brazil) and filtered through a filter with porosity of 0.45 μm (Millipore, Brazil) and 5 μL injected onto the chromatographic column for analysis. The total vitamin E content was calculated by adding the vitamin E identified components.

The chromatographic conditions used included: HPLC system (Shimadzu, SCL 10AT VP model, Japan); fluorescence detector (Shimadzu, RF10AXL); (290 nm excitation and 330 nm emission); chromatographic column Phenomenex Luna Si100 (250 × 4 mm, 5 μm) coupled with a Si100 Phenomenex guard column (4 × 3 mm); mobile phase – hexane: isopropanol: glacial acetic acid (HPLC grade, Tedia, Brazil) (98.9: 0.6: 0.5, v/v/v); flow rate of 1.0 mL/min and 22 min run time (Guinazi, Miranda Milagres, Pinheiro-Sant'Ana, & Chaves, 2009). The identification of the components of vitamin E was performed by comparing the retention time of commercial standards with those obtained for the samples analyzed under the same conditions.

2.3.3. Vitamin C

The vitamin C extraction and analysis (in AA form) were carried out according to the conditions proposed by Campos, Ribeiro, Della Lucia, Pinheiro-Sant'Ana, and Stringheta (2009), with modifications. About 5 g of chia were homogenized for 5 min with 15 ml of extraction solution (3% metaphosphoric acid, 8% acetic acid, H_2SO_4 0.3 N and 1 mM EDTA) using a microgrinder. The extract was centrifuged (Hermle®, modelo Z216MK, Alemanha) at 2865g for 15 min, and filtered through Buchner funnel using filter paper. The filtrate was transferred to a 25.0 mL volumetric flask and completed to volume with ultrapure water.

The extracts were filtered through filter units with porosity of 0.45 μm (Millipore, Brazil). For AA analysis chromatographic the following conditions were used: chromatographic column Lichrospher RP 18 (100, 250 x 4 mm, 5 μm), HPLC system (Shimadzu, SCL 10AT VP model, Japan), DAD, ultrapure water mobile phase containing 1 mM NaH_2PO_4, 1mMde EDTA and adjusted to pH 3.0 with H_3PO_4; flow rate of 1.0 mL/min and injection volume of 50 μL. Chromatograms were obtained at 245 nm.

2.4. Determination of flavonoids

The main 3-DXAs (luteolinidin, apigeninidin, 7-methoxy-apigeninidin and 5-methoxy-luteliolinidin), flavones (luteolin and apigenin) and flavanones (naringenin and eriodictyol) were investigated in chia. The compounds were extracted from 2 g of sample in 20 mL of methanol: HCl (99:1, v:v). Analyzes were performed in a HPLC system (Shimadzu, SCL 10AT VP, Japan) equipped with DAD (Shimadzu, SPD-M10A, Japan), high pressure pump (Shimadzu, LC-10AT VP, Japan), autosampler with a 500 μL loop (Shimadzu, SIL-10AF, Japan), and helium degassing system using the chromatographic conditions described by Yang, Allred, Geera, Allred, and Awika (2012).

Flavonoids were determined by HPLC using the following chromatographic conditions: HPLC system (Shimadzu, SCL 10AT VP model, Japan), Kinetix C-18 column (150 × 4.6 mm id, 5 μm) equipped with C-18 guard column (4 mm × 3 mm. Phenomenex, Torrance, CA), column temperature of 35 °C, injection volume of 100 μl, with detection at 480 nm, 360 nm, 280 nm for 3-deoxyanthocianidins, flavones and flavanones, respectively. The mobile phase consisted of 2% formic acid in ultrapure water (line A) and 2% formic acid in acetonitrile (line B). The elution gradient of B was 0–3 min isocratic 10%; 3–4 min, 10–12%; 4–5 min isocratic 12%; 5–8 min, 12–18%; 8–10 min, isocratic 18%; 10–12 min, 18–19%; 12–14 min, isocratic 19%; 14–18 min, 19–21%; 18–22 min, 21–26%; 22–28 min, 26–28%; 28–32 min, 28–40%; 32–34 min, 40–60%; 34–36 min, isocratic 60%; 36–38 min, 60–10%; 38–45 min, isocratic 10%. The mobile phase was degassed with helium gas at 50 kPa before and during runs, which were performed using the following flow gradient: 0–36 min, 1.0 mL/min; 36–38 min, 1.0–2 mL/min, 38–44 min, 2.0 mL/min; 44–45 min, 2.0–1.0 mL/min. Chromatograms were obtained at 480 nm, 360 nm, 280 nm for 3-deoxiantocianidinas, flavones and flavanones, respectively.

2.5. Preparation and evaluation of the purity of the standards carotenoids, vitamins and flavonoids

Solutions were prepared for each of the investigated components in chia (lutein, zeaxanthin, AA, α-tocopherol, α-tocotrienol, β-tocopherol, β-tocotrienol, γ-tocopherol, γ-tocotrienol, δ-tocopherol, δ-tocotrienol, luteolinidina, apigeninidina, apigeninidina 7-methoxy-5-methoxy-luteliolinidina, luteolin, apigenin, naringenin and eriodictyol).

The purity of the standards was evaluated by HPLC and quantified by performing spectrophotometry, based on the maximum absorbance according to the Lambert-Beer law. To perform the actual quantification of the standards we used the following equation: C (g/ml) × 104 = ABS/E1% 1 cm, where C = concentration; ABS = maximum absorbance; E1% 1 cm = molar absorptivity coefficient.

2.6. Identification and quantification of carotenoids, vitamins and flavonoids

The qualitative identification of the investigated compounds was performed by HPLC, by comparing the retention times obtained for the standards with the interest peaks obtained for the samples, analyzed under the same conditions. Furthermore, the carotenoids, flavonoids and AA were identified by comparing the absorption spectra of the standard and the peaks of interest in the samples, using the DAD (de Morais Cardoso, Oliveria, Bedetti, Martino, & Pinheiro-Sant'Ana, 2013).

For the quantification of the compounds found in chia, standard curves were used. The construction of calibration curves was performed by injection in duplicate in five increasing concentrations of the standard solutions in the range from 0.177 to 2.840 μg to lutein; 0.40 to 3.20 μg to zeaxanthin; 0.06 to 5.88 μg AA; 1.02–104.21 ng to α-tocopherol, 2.01–204.12 ng to α-tocotrienol; 0.44–167.89 to β-tocopherol; 0.32–154.32 to β-tocotrienol; 2.22–107.6 ng for γ-tocopherol; 3.21–157.6 ng for γ-tocotrienol; 0.78–80.62 ng to δ-tocopherol and 0.79–104.56 ng to δ-tocotrienol, 0.70–105.65 ng to luteolinidin; 0.19–114.08 ng to apigeninidin; 0.19–114.08 ng to 7-methoxy-apigeninidin; 0.70–105.65 ng to 5-methoxy-luteliolinidin; 0.8–40.0 ng for luteolin; 0.8–40.0 ng for apigenin; 46.4 to 0.928 ng to naringenin and 0.80–80.00 ng to

eriodictyol. Thus, a linear correlation between the peak areas and the injected concentration of each compound was done.

The quantification of the compounds in chia was performed from standard curves constructed and from the regression equation obtained for lutein ($y = 31{,}547.09x + 4662.43$; $R^2 = 0.99$); zeaxanthin ($y = 23{,}326.28x + 5644.83$; $R^2 = 1.00$); AA ($y = 2{,}967{,}843.75x - 263{,}100.04$; $R^2 = 1.00$), α-tocopherol ($y = 7{,}284{,}877.24x + 58673.13$; $R^2 = 0.98$), α-tocotrienol ($y = 67{,}799{,}527.73x - 2449.21$; $R^2 = 1.0$), β-tocopherol ($y = 109{,}337{,}012.29x - 96.92$; $R^2 = 1.00$), β-tocotrienol ($y = x\ 1{,}052{,}509.7795 + 1838.1232$; $R^2 = 0.99$), γ-tocopherol ($y = 52{,}176{,}064.10x + 5{,}556{,}148.10$; $R^2 = 0.98$), γ-tocotrienol ($y = 10{,}541{,}950.6116 + 15{,}790.3141$; $R^2 = 0.98$), δ-tocopherol ($y = 49{,}747{,}176.34x + 6091.48$; $R^2 = 1.00$), δ-tocotrienol ($y = 10{,}541{,}950.6116 - 15{,}790.3141$; $R^2 = 1.00$) luteolinidin ($y = 8141{,}5x - 2823.4$; $R^2 = 0.99$), apigeninidin ($y = 6345{,}7x + 8276.4$; $R^2 = 0.99$); 7-methoxy-apigeninidin ($y = 6345{,}7x + 8276.4$; $R^2 = 0.99$) and 5-methoxy-luteliolinidin ($y = 7570.4x + 8276.4$; $R^2 = 0.99$), luteolin ($y = 4.293{,}2779x + 7729.2457$; $R^2 = 0.99$); apigenin ($y = 6772{,}2x - 9650.2$; $R^2 = 0.99$), naringenin ($y = 2891{,}4 + 3067.6$; $R^2 = 0.99$) and eriodictyol ($1754{,}7936 + y = 3.9746$; $R^2 = 1.00$). The real concentration was obtained by calculations from the dilutions or concentrations carried out.

2.7. Determination of antioxidant activity

2.7.1. Preparation of extracts

Two grams of chia were added to a 20 mL of acetone 70% solution. Then, the suspension was shaken automatically (10 g, 2 h, 25 °C) and centrifuged (2865 g, 15 min) (Hermle®, model Z216MK, Germany). The supernatant was transferred to a beaker and volume was completed to 20 mL with acetone 70%. The extract was placed in amber bottle and stored in a freezer (−18 ± 1 °C) until the time of analysis.

2.7.2. Radical removal activity (DPPH)

In a test tube, protected from light, 100 μL of the extract obtained in the previous step was added to 1.5 mL of methanolic DPPH solution (1.1-diphenyl-2-picrylhydrazyl) and stirred by vortex (3000 rpm) for 30 s. After 30 min of standing, the absorbance of the solution was read in a spectrophotometer (Thermo scientific, 606 Evolution, USA) at 517 nm. The analytical curve was constructed using a 50–100 μmol/L trolox solution. The antiradical activity (AAR) was expressed in a μmol trolox equivalent/g of the sample (μmol trolox/g) (Bloor, 2001).

2.8. Determination of the total phenolic compounds content

The total phenolic compounds content in chia were determined using the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Aliquots of 0.5 mL of extract obtained in item 2.5.1 were added to 0.5 mL of Folin-Ciocalteu reagent (20%). After homogenization, 0.5 mL of sodium carbonate (7.5%) was added. The reaction mixture was homogenized by vortex (2865 g, 10 s) and incubated at room temperature (30 min).

The reading of absorbance was performed in spectrophotometer (Thermo scientific, Evolution 606, USA) at 765 nm. Analytical curve of gallic acid (0,005–0,10 mg/mL) was used to quantify the compounds. The results were expressed in mg of gallic acid equivalents/g of chia flour (mg GAE/g).

2.9. Determination of phytic acid

The concentration of phytic acid was determined by ion exchange chromatography and spectrometry (Latta & Eskin, 1980) with modifications (Ellis & Morris, 1986). For the extraction of phytic acid, 0.1 g of chia flour was weighed and 5 ml of HCl 2.4% were added, remaining under horizontal shaking for 12 h at 250 rpm. The extract was centrifuged at 2865*g* (Hermle®, model Z216MK, Germany) for 15 min and the supernatant was filtered in a vacuum büchnner funnel and purified using ion-exchange column with the stationary phase constituted by Dowex-AGX-4 resin.

The column was preconditioned with NaCl 2 M and the obtained extract of the previous step was applied. Inorganic phosphors were eluted with NaCl 0.05 M, followed by elution of phytic acid retained with NaCl 2 M. Phytic acid was determined colorimetrically at 500 nm. An analytical curve of phytic acid (Sigma®) was prepared in concentrations of 10–100 $\mu L/mL^{-1}$, to express the concentration of phytic acid in g/100 g of chia flour

2.10. Determination of tannin

The tannin determination was performed by the reaction of vanillin/HCl (Burns, 1971) with modifications (Maxson & Rooney, 1972; Price, Scoyoc, & Butler, 1978). 200 mg of chia were weighed, and a solution of 10 mL 1% HCl in methanol was added. The tubes were placed in an automatic shaker (Marconi, MA093, Brazil) (80 g, 20 min, 30 °C) for extraction of tannin. Then, they were centrifuged (Hermle®, Z216MK model, Germany) at 2865*g* for 20 min.

Aliquots of 1 mL of supernatant were added to 2.5 mL of 1% solution of vanillin in methanol and 2.5 mL of 8% solution of HCl in methanol. The absorbance reading was held in spectrophotometer (MultiskanGo, Thermoscientific, USA) at 500 nm. The results were expressed as milligrams of catechin per gram of sample according to the analytical curve of catechin. For the construction of the curve, 200 mg of catechin were weighed in a volumetric flask (200 mL) and the volume was completed with methanol. Aliquots were withdrawn for 5, 10, 20, 25 and 50 mL of concentrated solution and the absorbance reading made at 500 nm.

2.11. Experimental design and statistical analysis

A completely randomized design was used. The data were analyzed using a *t*-test ($\alpha = 0.05$) for independent samples to verify difference between chia seeds grown in different places. All statistical analyzes were conducted using SPSS software, version 20.

3. Results

High concentrations of dietary fiber (35.3%, on average), lipids (31.2%, on average) and proteins (18,9%, on average) were found in chia Brazilian seeds. Chia grown in the state of RS showed higher ($p < 0.05$) content of moisture, fatty acids (saturated, monounsaturated and polyunsaturated) and carbohydrates compared to the chia grown in MT (Table 2). Both chia seeds showed high concentration of polyunsaturated fatty acids, standing out n-3.

Among the minerals presented in chia seeds, iron, calcium, phosphorus and potassium were highlights. Chia seed grown in RS presented higher content of iron, manganese, boron, lead, aluminum, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur and copper. The concentrations of zinc, cadmium and sodium were similar in both seeds (Table 3).

Chia seeds grown in RS ad MT showed high vitamin E concentration. γ-tocopherol was the main component found (on average, 90.9% and 91.5%, in chia grown in RS and MT, respectively). The occurrence of carotenoids in chia grown in MT was not observed as well as vitamin C and 3-deoxyanthocianidins in both chia seeds (Fig. 1). The concentration of flavones and flavanones in chia seeds grown in MT was higher than that grown in RS (Table 4).

Similar concentrations of phenolic compounds were observed (0.97 ± 0.01 mg GAE/g sample and 0.99 ± 0.02 mg GAE/g sample

Table 2
Nutritional composition of chia seeds grown in different places, Brazil, 2015 (g.100 g^{-1}).

Variables*	Mean** ± SD*** RS Chia flour	Mean** ± SD*** MT Chia flour
Moisture	7.14 ± 0.26[a]	5.62 ± 0.03[b]
Ash	4.56 ± 0.04[b]	5.07 ± 0.07[a]
Lipids	32.16 ± 0.29[a]	30.17 ± 0.22[b]
16:0	1.82 ± 0.12[a]	1.85 ± 0.10[a]
18:0	0.90 ± 0.07[a]	1.03 ± 0.10[b]
18:1 (n-9)	1.43 ± 0.10[a]	1.67 ± 0.09[b]
18:1 (n-7)	0.26 ± 0.02[a]	0.23 ± 0.03[a]
18:2 (n-6)	5.69 ± 0.42[a]	5.09 ± 0.05[b]
18:3 (n-3)	20.37 ± 1.38[a]	18.74 ± 1.29[b]
Saturated	2.88 ± 0.18[a]	2.73 ± 0.03[b]
Monounsaturated	1.89 ± 0.12[a]	1.69 ± 0.06[b]
Polyunsaturated	27.75 ± 1.80[a]	25.73 ± 1.32[b]
Protein	18.18 ± 1.20[a]	19.72 ± 3.09[a]
Food Total fiber	33.37 ± 0.26[b]	37.18 ± 0.21[a]
Soluble fiber	2.89 ± 0.09[b]	3.88 ± 0.68[a]
Insoluble fiber	30.47 ± 0.35[b]	33.30 ± 0.46[a]
Carbohydrates	4.59 ± 0.34[a]	2.23 ± 0.56[b]
Total energy value (kcal·100 g^{-1})	380.52 ± 1.83[a]	359.33 ± 3.87[b]

* Values expressed in dry matter.
** Mean of three replicates.
*** Standard deviation; same letters on the line do not differ by *t* test at 5% probability, RS: Rio Grande do Sul, MT: Mato Grosso.

in chia seeds grown in RS and MT, respectively). The chia seed grown in RS presented higher ($p < 0.05$) antioxidant activity (478.2 ± 0.02 μmol TEAC/g sample) when compared to grown in MT (466.3 ± 0.06 μmol TEAC/g sample). The concentration of phytic acid in the seed grown in RS and MT were similar ($p > 0.05$) (0.96 and 1.16 g.100 g^{-1}, respectively) and the concentration of tannins were higher ($p < 0.05$) in chia seed grown in Mato Grosso (19.08 ± 1.08 eq.catechin/g sample) than chia seed grown in Rio Grande do Sul (14.93 ± 0.24 eq. catechin/g sample).

4. Discussion

The present study focused on the characterization of macronutrients and micronutrients of Brazilian chia seeds grown in two different places. Differences in the chemical composition of the seeds grown in the two Brazilian states may have been affected by growing location, soil conditions, temperature, humidity, light and growing conditions.

Brazilian chia seeds presented high concentrations of dietary fiber, lipids and proteins. The concentration of dietary fiber ranged from 30 to 38%, and about 6% of this amount comprises soluble fiber. Values similar to those observed in our study are reported in the literature for chia grown in other countries (Capitani et al., 2012; Olivos-Lugo, Valdivia-López, & Tecante, 2010; Reyes-Caudillo et al., 2008; Vázquez-Ovando et al., 2009). The concentration of fiber in chia was higher than in other cereals and grains such as corn (13.4%), soy (15%), wheat (12.6%), linseed (22.3%) and sesame (7.79%) (Dhingra, Michael, Rajput, & Patil, 2012).

The protein concentration observed in the chia seed was high (approximately 19%, on average), being this value is similar to those observed by other authors for chia seeds grown in other countries (Sandoval-Oliveros & Paredes-López, 2013). The protein content of the seed tends to decrease with increasing temperature at the place of cultivation (Ayerza & Coates, 2011). However, in our study, we found that chia grown in the state of Mato Grosso, which presents high temperature, showed the same protein concentration when compared to that grown in Rio Grande do Sul, with a lower temperature. The same protein concentration was observed in study that determined the location effect on the seed's protein content (Ayerza & Coates, 2009).

Chia seeds analyzed in our study were composed, on average, of 31% lipids, which may be noted in other studies for seeds grown in other countries (Ayerza & Coates, 2009; Monroy-torres, Mancilla-escobar, Gallaga-solórzano, & Santiago-garcía, 2008). The weather influenced the concentration of lipids present in chia, since seeds grown at high temperatures (MT) had lower ($p \leqslant 0.05$) lipid content when compared to those grown at lower temperatures (RS). Our data corroborate other studies that show that low temperatures generally increased the level of unsaturation of chia fatty acids (Ayerza & Coates, 2001).

The main type of fatty acid found in chia is polyunsaturated, mainly n-3 fatty acids. Chia essential oil has significantly higher content of α-linolenic and linoleic acids (Álvarez-Chávez, Valdivia-López, Aburto-Juarez, & Tecante, 2008) than linseed, canola and soybean oils (Gunstone & Padley, 1997). Polyunsaturated fatty acids have been associated with improved lipid profile, attenuating cardiometabolic risk and lowering the inflammation (Lesna, Suchanek, & Brabcova, 2013). The ratio n-6/n-3 observed in our study in chia seed was 1:3.6. The high concentration of n-3 is associated with reduction in the risk of coronary artery disease, hypertension, type 2 diabetes, rheumatoid arthritis, autoimmune disorders, and cancer (Connor, 2000).

The Brazilian chia seed was a highlight due to its concentration of iron, zinc, calcium, manganese, potassium and phosphorus. The differences among Brazilian chia seeds can be attributed to the geographic location, type of soil, climate, humidity and growing conditions in which they are grown. Values similar to the present study are noted wherein the minerals calcium (631 mg.100 g^{-1}), potassium (407 mg.100 g^{-1}), magnesium (335 mg.100 g^{-1}), iron (7.72 mg.100 g^{-1}) and zinc (4.58 mg.100 g^{-1}) are the most significant in chia (USDA., 2015). The concentration of calcium in chia seed was observed to be six times higher than that of milk, whereas the iron concentration was observed to be 2.4–6 times higher than the other sources of this mineral, as meat (USDA, 2015).

Table 3
Mineral composition in chia seeds grown in different places, Brazil, 2015.

Minerals* Mean** ± SD***	Fe (mg 100 g^{-1})	Zn (mg 100 g^{-1})	Mn (mg 100 g^{-1})	Na (mg 100 g^{-1})	B (mg 100 g^{-1})	Pb (mg 100 g^{-1})	Cd (mg 100 g^{-1})	Al (mg 100 g^{-1})
RS Chia Flour	9.39 ± 052 [a]	3.65 ± 0.09 [a]	4.05 ± 0.12[a]	150 ± 00.00[a]	1.12 ± 0.02[a]	0.06 ± 0.02[a]	0.08 ± 0.03[a]	1.32 ± 0.13[a]
MT Chia Flour	7.69 ± 0.33 [b]	3.76 ± 0.10 [a]	2.48 ± 0.05[b]	140 ± 13.42[a]	0.93 ± 0.04[b]	0.00 ± 0.00[b]	0.11 ± 0.04[a]	0.99 ± 0.25[b]
Minerals* Mean ± SD*** **	**N (mg 100 g^{-1})**	**P (mg 100 g^{-1})**	**K (mg 100 g^{-1})**	**Ca (mg 100 g^{-1})**	**Mg (mg 100 g^{-1})**	**S (mg 100 g^{-1})**	**Cu (mg 100 g^{-1})**	**Cr (mg 100 g^{-1})**
RS Chia Flour	3607.00 ± 22.68[a]	640.00 ± 4.85[a]	620.00 ± 17.45[a]	480.00 ± 21.00[a]	350.00 ± 4.09[a]	200.00 ± 10.25[a]	1.32 ± 0.03[a]	0.00 ± 0.00[a]
MT Chia Flour	3620.00 ± 20.36[b]	530.00 ± 6.52[b]	550.00 ± 6.71[b]	430.00 ± 19.88[b]	330.00 ± 13.437[b]	150.00 ± 5.81[b]	0.63 ± 0.01[b]	0.00 ± 0.00[a]

* Values expressed in dry matter.
** Mean of three replicates.
*** Standard deviation; same letters in the column do not differ by *t* test at 5% probability. Fe: iron; Zn: zinc; Mn: manganese; Na: sodium; B: boron; Pb: lead; Cd: cadmium; Al: aluminum; N: nitrogen; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; S: sulfur; Cu: copper; Cr: chrome, RS: Rio Grande do Sul, MT: Mato Grosso.

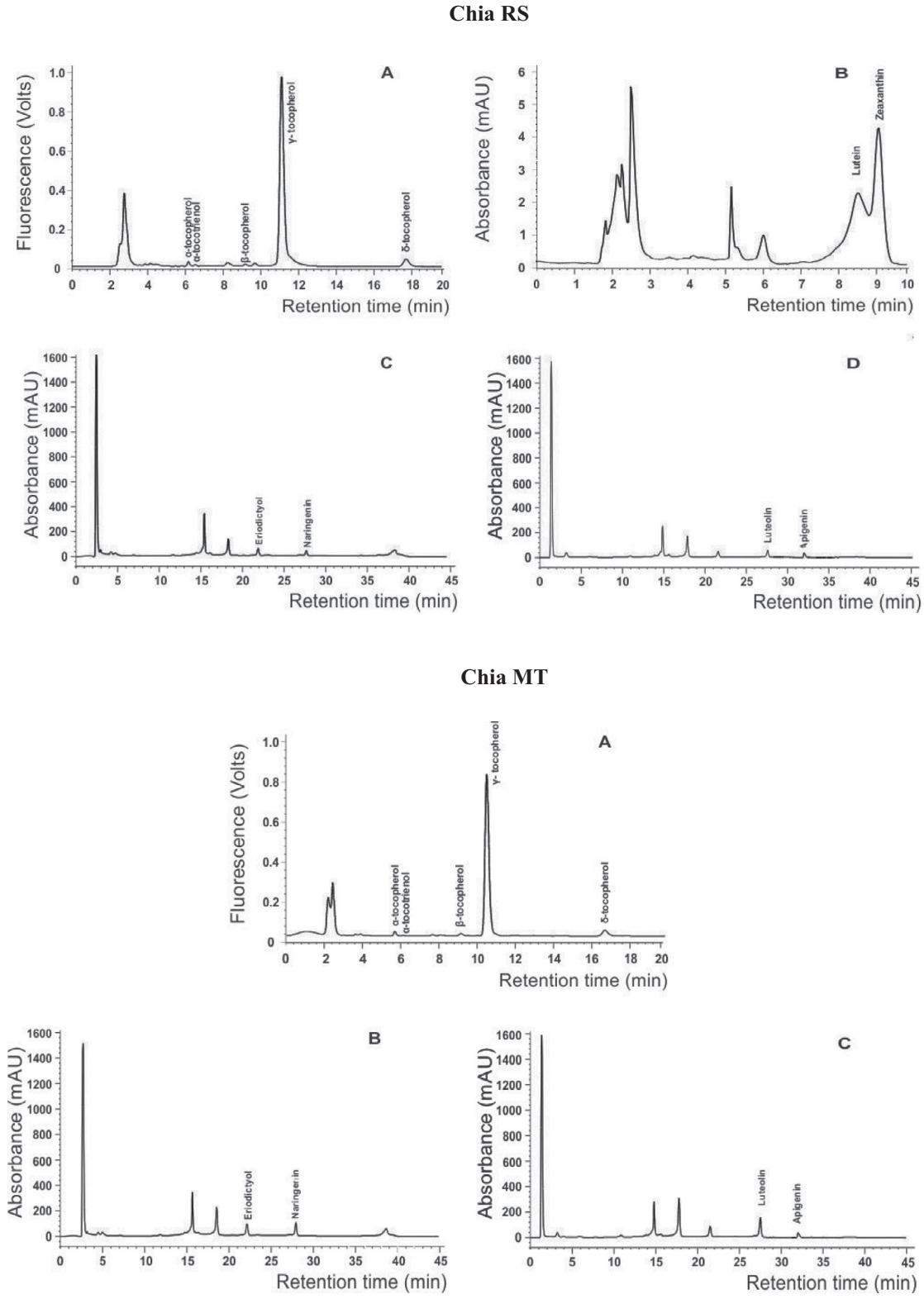

Fig. 1. HPLC analysis of vitamin E (A), carotenoids (B) and flavonoids (C and D) in chia seeds grown in Rio Grande do Sul (RS) and Mato Grosso (MT).

The average content of vitamin E in chia (8205.6 μg.100 g^{-1}, on average) was higher than that observed in cereals like wheat, oats, barley, rye and sorghum (de Morais Cardoso et al., 2015; Okarter, Liu, Sorrells, & Liu, 2010; Tiwari & Cummins, 2009). The γ-tocopherol was found in higher quantities in chia flour as observed by de Morais Cardoso (2014) in sorghum, followed by δ-tocopherol. A similar result was observed by Capitani et al. (2012) in chia grown in Argentina, wherein the component present in larger amounts in chia oil was γ-tocopherol. Controversial results were observed in other studies that used chia grown in Argentina and Guatemala (Capitani et al., 2012; Ixtaina et al., 2011) since the authors did not detect presence of β-tocopherol. In addition, α-tocopherol concentration of this study was equal to 190.7 and 202.4 μg.100 g^{-1} for chia grown in Rio Grande do Sul and Mato Grosso, respectively.

Table 4
Occurrence and concentration of carotenoids, vitamins and bioactive compounds in chia seeds grown in different places, Brazil, 2015.

Compounds*	Mean** ± SD*** RS Chia	Mean** ± SD*** MT Chia
Total Vitamin E (μg.100^{-1})	8237.64 ± 161.92^{a}	8169.50 ± 155.20^{a}
α-tocopherol	190.66 ± 12.24^{a}	202.40 ± 12.70^{a}
α-tocotrienol	46.50 ± 3.22^{a}	17.86 ± 1.61^{b}
β-tocopherol	94.45 ± 2.10^{a}	81.91 ± 2.80^{b}
β-tocotrienol	nd	nd
γ-tocopherol	7487.69 ± 121.30^{a}	7472.97 ± 138.20^{a}
γ-tocotrienol	nd	nd
δ-tocopherol	418.34 ± 27.70^{a}	393.53 ± 23.40^{a}
δ-tocotrienol	nd	nd
Total Carotenoids (μg.100^{-1})	57.01 ± 1.68^{a}	nd^{b}
Lutein	10.98 ± 0.61^{a}	nd^{b}
Zeaxantin	46.01 ± 1.33^{a}	nd^{b}
Vitamin C (μg.100^{-1})	nd	nd
Ascorbic acid	nd	nd
3-Deoxyanthocianidins (μg.100^{-1})	nd	nd
Luteolinidin	nd	nd
Apigeninidin	nd	nd
7-methoxy-apigeninidin	nd	nd
5-methoxy-luteliolinidin	nd	nd
Flavones (μ.g^{-1})	6.07 ± 0.03^{b}	16.03 ± 0.06^{a}
Luteolin	5.91 ± 0.05^{b}	15.79 ± 0.08^{a}
Apigenin	0.16 ± 0.01^{b}	0.35 ± 0.03^{a}
Flavanones (μ.g^{-1})	4.39 ± 0.15^{b}	9.34 ± 0.41^{a}
Naringenin	0.22 ± 0.04^{a}	0.39 ± 0.01^{a}
Eriodictyol	4.17 ± 0.43^{b}	8.95 ± 0.60^{a}

* Values expressed in dry matter.
** Mean of five replicates.
*** Standard deviation; nd: not detected; same letters on the line do not differ by *t* test at 5% probability; nd: not detected, RS: Rio Grande do Sul, MT: Mato Grosso.

In our study, lutein and zeaxanthin were verified only in chia grown in the RS. Zeaxanthin was the main carotenoid (80.7%) observed in the seed. The zeaxanthin content (46.01 μg.100 g^{-1}) was higher than in other cereals such as sorghum (15.48 μg.100 g^{-1}) (de Morais Cardoso et al., 2015).

The antioxidant activity in the samples was quite similar to that reported by Vázquez-Ovando et al. (2009) (488.8 TEAC, μmol/g) and Capitani et al. (2012) (446.4 TEAC, μmol/g) for a Mexican chia fibrous fraction. The high antioxidant activity of chia can be attributed to its high content of phenolic compounds, tocopherols and tocotrienols. Therefore, the consumption of chia seeds Brazilian can promote benefits to human health.

Although the content of phenolic compounds did not differ between Brazilian chia seeds (0.97 and 0.99 mg GAE/g of chia seeds grown in the RS and MT, respectively), the average value was higher than that reported in Mexican chia (Porras-Loaiza, Jiménez-Munguía, Sosa-Morales, Palou, & López-Malo, 2014; Reyes-Caudillo et al., 2008). The changes in total phenolic content between different studies can be attributed to factors such as cultivation techniques, weather conditions, as well as the methods used for the determination of phenolic compounds. The concentration of phytic acid present in chia was similar to that observed in another study (Ferreira, 2013). There are studies that evaluated the tannins concentration in chia seeds. However, it is known that, depending the concentration found in foods, these compounds are useful due to remarkable activity in cancer prevention and treatment.

5. Conclusion

The Brazilian chia seeds showed high concentrations of lipids, proteins, total dietary fiber, minerals and vitamin E. The chia grown in RS showed a higher concentration of lipids, minerals like iron, manganese, boron, lead, aluminum, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, copper and antioxidant capacity than chia grown in MT. The use of chia must be stimulated since this food presents a high nutritional value and bioactive compounds that are related to benefits to the human health.

Conflict of interest

The authors declare no conflict of interest.

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