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Characterization of phenolic compounds in chia (*Salvia hispanica* L.) seeds, fiber flour and oil

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ABSTRACT

The consumption of chia seeds products has increased recently and it has been suggested that the inclusion of this functional food in a daily human diet could contribute to improve consumers' health. However, a better knowledge about the composition of these products is mandatory. In this work, the phenolic compounds from commercial samples of chia seed, fiber flour and oil were extracted using an ultrasound-assisted methodology and were separated and identified by high-performance liquid chromatography coupled to a mass spectrometer. Methanol:water extracts were prepared and submitted to an acidic hydrolysis. Crude and hydrolyzed extracts were analyzed and phenolic compounds found were mainly caffeic acid and danshensu and its derivatives, such as rosmarinic and salvianolic acids. TPC was higher in the hydrolyzed extracts. These results supply new information about the main phenolic compounds presents in chia, which are important dietary sources of natural antioxidants for prevention of diseases caused by oxidative stress.

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1. Introduction

Chia (*Salvia hispanica* L.) is an annual herbaceous plant that belongs to the *Lamiaceae* family, native from southern Mexico and northern Guatemala. More recently chia has been cultivated for commercial purposes in Argentina, Colombia, Ecuador, Peru, Bolivia, Paraguay and Australia (Busilacchi et al., 2013).

Different parts of this plant are now commercially available for human consumption all over the world, as food supplements. The chia seeds are usually consumed grounded or as whole grain in fruit juices, with milk, in refreshing drinks and salads. More recently, the chia fiber flour started to be consumed as an ingredient in bakery products and in beverage industries due to its nutritional and functional properties, that include fat-binding and gel-forming (Coorey, Tjoe, & Jayasena, 2014).

The increasing interest in the study of chia seed is due to their nutritional and health promoting properties that have been recognized in some of their components, namely it's high content in essential fatty acids in the oil (21.4–32.6 g/100 g), which contains higher polyunsaturated fatty acids, mainly α-linolenic acid (59.9–63.2 g/100 g) and a low percentage of saturated fatty acids (Porras-Loaiza, Jiménez-Munguía, Sosa-Morales, Palou, & Lopez-Malo, 2014). Also the high content in minerals, proteins, dietary fiber and other bioactive components such as tocopherols and phenolic compounds (Capitani, Spotorno, Nolasco, & Tomás, 2012; Marineli et al., 2014; Porras-Loaiza et al., 2014) contribute to the interest of the scientific community and consumers in this product.

Recent studies show that the dietary intake of bioactive components as phenolic compounds from chia seeds are related to a reduced risk of cardiovascular disease and hepatoprotective effect (Poudyal, Panchal, Waanders, Ward, & Brown, 2012) and a protective effect against plasma oxidative stress and obesity related disease (Marineli, Lenquiste, Moraes, & Maróstica, 2015).

Caffeic and rosmarinic acids (Capitani et al., 2012; Martínez-Cruz & Paredes-López, 2014; Reyes-Caudillo, Tecante, &

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Valdivia-López, 2008) are among the phenolic compounds already identified in chia products, and they play an important role in the prevention and management of different neurological disorders, such as epilepsy (Coelho et al., 2015). Caffeic acid is known to exhibit various properties, such as a hypoglycemic activity and memory protective effect (Chang, Kuo, Chen, Wu, & Shen, 2015). The rosmarinic acid has been reported as a major phenolic compound in *Salvia* species (Cvetkovikj et al., 2013) and has diverse immunoregulatory functions including antimicrobial, antioxidant and anti-inflammatory activities, antidiabetic effect (Jayanthy & Subramanian, 2014) and inhibition of the inflammatory process associated with hepatic ischaemia reperfusion (Rocha et al., 2015).

Yang, Hong, Lee, Kim, and Lee (2013) showed that when both caffeic and rosmarinic acid were present, there was an increase in the hepatoprotective effect, related to increased levels of endogenous antioxidant enzymes and glutathione (GSH) and decreased lipid peroxidation in liver. Chlorogenic (Reyes-Caudillo et al., 2008) and protocatechuic acids (Martínez-Cruz & Paredes-López, 2014) were identified in chia seeds as well as some flavonoids like quercetin and kaempferol (Capitani et al., 2012; Reyes-Caudillo et al., 2008). Caffeic and chlorogenic acids, together with myricetin, quercitin and kaempferol were also detected in chia oil (Ixtaina et al., 2011).

Different methods have been used for the identification of phenolic compounds, but high-performance liquid chromatography (HPLC) with photodiode array (PDA) and/or mass spectrometry (MS) detection are usually chosen. The lack of reference substances to be used for identification purposes is compensated by the advances in mass spectrometry methodologies using exact mass equipment, with an increase in sensitivity and fast screening capabilities, which have contributed to assign more peaks in complex chromatograms. Other detectors, as electrochemical detector (ED), have also been used, as they are sensitive, selective and can elucidate about sample components that contribute to antioxidant properties (Dobes et al., 2013).

Previous studies on characterization of chia seed used aqueous and organic solvents (Martínez-Cruz & Paredes-López, 2014) to perform extraction of compounds during sample preparation step. Ultrasound-assisted extraction associated with acid hydrolysis has also been used to leach and hydrolyze phenolic compounds, in a faster way than traditional methods, since the surface contact area between the solid and liquid phases is increased by particle disruption (Acosta-Estrada, Gutiérrez-Uribe, & Serna-Saldívar, 2014).

In the present work, an ultrasound liquid–liquid extraction methodology (USE) was used for the extraction of phenolic compounds from different fractions of chia as seeds, fiber flour and oil. An acidic hydrolysis procedure was also performed in the extracts. The characterization was done by liquid chromatography, using different detection modes and results were compared between different fractions of chia analysed using data from chromatogram profiles. The total phenolic content and antioxidant activity were also measured.

2. Materials and methods

2.1. Chia seeds, fiber flour and oil

Fresh seed, fiber flour (powder-435) and oil from chia (*Salvia hispanica* L.) commercially available from PFD S.A.-Benexia, (Santa Cruz, Bolivia and Santiago, Chile) were purchased at R&S Blumus Comercial de Produtos Alimentícios Ltda, Brazil. According to the information from the producer (FPT S.A Santiago, Chile), chia oil was obtained from the chia seed after a cold pressing process and the residual material was grounded at less than 435 μm to obtain the fiber flour powder-435. The oil was stored at 2–8 °C in amber glass bottles without head space, until it was used for analysis.

2.2. Reagents

The formic acid (HCOOH, 98% p.a.) was purchased from Merck®. Acetonitrile (CH_3CN, 99.9% LC–MS) and methanol (MeOH, 99.8% LC–MS) were purchased from Fisher Scientific. The Ultra-pure water (18.2 MΩ.cm) was obtained from a Millipore-Direct Q3 UV system (Millipore, USA). Trolox (6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid), AAPH (2,2′-azobis(2-methylpropionamidine)dihydrochloride), TPTZ(2,4,6-tris(2-pyridyl)-s-triazine and Folin-Ciocalteu's reagent were all obtained from Sigma-Aldrich (Sigma Co., St. Louis, MO. USA). Fluorescein sodium salt was purchased from Vetec Química (São Paulo, Brazil).

2.3. Extraction of phenolic compounds

2.3.1. Crude methanolic flour extracts

The ultrasound liquid–liquid extraction method (USE) was used for the extraction of the phenolic fraction (Pizarro, Becerra, Sayago, Beltrán, & Beltrán, 2013). Previously, the chia seeds were grounded in the laboratory (mill A11 basic BS32 IKA Mill, São Paulo) and 2 g of ground chia seed and fiber flour were extracted with 10 mL of methanol:water (80:20, v/v) solution. After shaking in a vortex for 10 s, samples were placed immediately in an ultrasonic water bath (Model B12, Onic 12Ultrasonics, Lisbon, Portugal). Extractions were performed at 48 kHz of ultrasound frequency and 55 W of power for 60 min at 25 ± 3 °C. After, samples were centrifuged at 4000 rpm for 30 min and the supernatant removed. The supernatant was evaporated to dryness at ±40 °C under reduced pressure. The dry residue was dissolved in 2 mL of a methanol:water (80:20, v/v) solution, filtered through a 0.22 μm PVDF membrane (Millipore, USA) and stored at −18 °C until analysis. All samples were extracted in duplicate.

2.3.2. Crude methanolic oil extracts

The procedure described in Section 2.3.1. was used with some modifications. Briefly: 2 g of chia oil were weighed and followed by the addition of 5 mL of hexane and 5 mL of methanol/water (80:20 v/v) solution. After shaking in a vortex for 10 s, the tube was placed immediately into an ultrasonic water bath (Model B12, Onic 12 Ultrasonics, Lisbon, Portugal). Extractions were performed at 48 kHz of ultrasound frequency and 55 W of power for 60 min at 25 ± 3 °C. This mixture was centrifuged at 4000 rpm for 10 min and the polar fraction (bottom phase) was separated. The polar fraction was evaporated to dryness at ±40 °C under reduced pressure. The dry residue was dissolved in 2 mL of methanol:water (80:20, v/v,) solution, filtered through a 0.22 μm PVDF membrane (Millipore, USA) and stored at −18 °C until analysis. All samples were extracted in duplicate.

2.3.3. Preparation of hydrolyzed extracts from flour and oil

Acidic hydrolysis of the extracts was performed according to Tsimidou, Nenadis, and Mastralexi (2014). Briefly, an aliquot (1 mL) of the crude methanolic extracts from ground chia seed, fiber flour and oil were mixed with 1 mL of a 1 M H_2SO_4 solution and kept in a water bath at 80 °C for 2 h. Each extract was filtered through a 0.22 μm PVDF membrane (Millipore, USA) and stored at −18 °C until analysis.

2.4. Chromatography equipment and conditions

2.4.1. HPLC-DAD-ESI-MS/MS

The phenolic compounds in the methanolic extracts were analysed in a Waters® Alliance 2695 (Waters, Ireland) equipped with a

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