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Characterization of chia seeds

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Abstract
Chia seeds are of great interest due to its ability to improve consumer health. In this study, chia seeds have been characterized, and they were observed to be rich in fiber, and in phenolic compounds. Furthermore, chia seeds had high antioxidant activity.

Keywords: chia, seeds, composition, activity

Introduction
Seed from *Salvia hispanica* L. or more commonly known as chia is a traditional food in central and southern America. Currently, it is widely consumed for various health benefits, especially in maintaining healthy serum lipid level. This effect is enhanced by the presence of phenolic acid and omega 3/6 oil in the chia seed (Citelli *et al*., 2016) [1]. Chia also promotes glycemic and weight loss due tothe high dietary fiber content (Jenkins *et al*., 2016) [2], with minimal adverse effects. Furthermore, Chia seeds do not contain toxic compounds and gluten, thus, making seeds a safe ingredient also for gluten free diets (Menga *et al*., 2017) [3]. Human dietary chia is usually consumed raw in salads, as sprouts or seeds, and added to beverages (Jin *et al*., 2010; Mohd Ali *et al*., 2012) [4,5]. Other important applications of chia include its use as animal feed supplement to raise omega 3 content in milk (Ayerza and Coates, 2006) [6]. Recently, it was used as an ingredient in some foods such as cookies, bread, snacks, cake and ice cream (Iglesias-Puig and Haros, 2013; Inglett *et al*., 2014; Coelho and Salas-Mellado, 2015) [7-9]. Adding chia to a food product does not only improve nutritional and healthy properties of a product and its components, it also confer technological properties like high water-holding capacity, water absorption capacity, emulsifying activity or gelling capacity (Coorey *et al*., 2014)[10]. The use of chia as an ingredient in the processing of widely consumed foods appears to be a promising approach. Therefore, the goal of this study is to characterize chia seeds which could incorporate in foods.

Materials and Methods
Characterization of Chia seeds
Dark chia seeds were provided by commercial supplier. Before analysis, they were tried and dried in a vacuum oven at 60°C for 24 hours. All samples were analyzed in three replications.

Proximate composition
Moisture was measured in an oven at 105°C, according to AOAC (1995)[11]. Ash was quantified after combustion of samples overnight at 600°C (AOAC, 2016)[12].Total nitrogen was determined by Kjeldahl Method. The protein content of seeds was determined by multiplying the TN by the factor 6.25 (AOAC, 2000) [13]. Total lipids were obtained from a one hour methanol/chloroforme (1/1) extraction (Wang *et al*. 2016) [14]. Carbohydrates were calculated as follow: Carbohydrate = 100 - (Moisture+ ash+ protein+ lipids) (AOAC, 2016) [12].

Phytochemical analysis of fibre
The contents of hemicellulose, cellulose, neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were determined in the chia seeds by the detergent method (Van Soest *et al*., 1991) [15] using Fibertec system 2010 (Foods, Sweden).

Extractable phenolics and antioxidant activity
To measure total phenolic, flavonoid and tannin content, aqueous extract of chia seed was obtained. 1g of powder was macerated in 20 ml of water at room temperature for 24 h. Then, filtrate was dried at 50°C, weighed and solubilized in water.

Determination of total phenolics contents
The total phenolics content was determined by the Folin–Ciocalteu spectrophotometric method (Singleton *et al*., 1999) [16]. 0.5 ml of aqueous extract was mixed with 0.5 ml of Foline–Ciocalteu's phenol reagent and with 1 ml of 7.5% sodium carbonate solution (w/v). After 1 h of reaction at ambient temperature, the absorbance at 760 nm was measured by a Spectrophotometer (Jenway 6300, France). Measurements were calibrated to a standard curve of prepared gallic acid solution. Total phenolic content of chia seeds powder was expressed as mg gallic acid equivalents per gram of dry weight matter (mg GAE/g DW). All samples were analyzed in three replications.

Determination of total flavonoid content
For the determination of total flavonoid contents, 1ml of the aqueous extract was mixed with 1 ml $AlCl_3$ (2%) (Yi *et al*., 2007)[17]. After 15 min, the absorbance of the mixture was determined at 430 nm. Total flavonoid content of chia seeds powder was expressed as mg quercetin equivalents per gram of dry weight matter. The calibration curve range was 10-150 mg ml^{-1}.

Determination of condensed tannins

In presence of concentrated H_2SO_4, condensed tannins were transformed by the reaction with vanillin to anthocyanidols (Sun *et al*., 1998)[18]. 50 µl of the aqueous extract was mixed with 3 ml of 4% methanol vanillin solution and 1.5 ml of H_2SO_4. After 15 min, the absorbance of the mixture was determined at 500 nm. Condensed tannin contents of seeds were expressed as mg catechin equivalents per gram of dry weight through the calibration curve with catechin. The calibration curve range was 50–500 mg ml^{-1}.

DPPH radical scavenging activity

The DPPH scavenging activity was estimated according to Hanato *et al*. (1988)[19]. Briefly, 1 ml of aqueous extract at different concentrations ranging from 1 to 200 µg ml^{-1} were added to 1 ml of a 0.06 mM DPPH ethanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark, and then the absorbance was measured at 517 nm. For each dilution of the extract, the DPPH scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = ((A0 - A1)/A0) \times 100$$

Where A0 is the absorbance of the control at 30 min, and A1 is the absorbance of the sample at 30 min. The antiradical activity was finally expressed as IC50 (µg ml^{-1}). A lower IC50 value corresponds to a higher antioxidant activity of the plant extract. BHT was used as positive control.

Results and Discussion

Characterization of Chia seeds

Chia seeds components (Table 1) were in the range values quoted by Weber *et al*. (1991) [20], but lower than those cited by Grancieri *et al*. (2019) [21]. Indeed, chia composition depends on climatic conditions, geographic location, nutrients, soil conditions, production practice, and a year of cultivation (Ayerza, 2009) [6]. Furthermore, sample composition was close to the contents in Brazilian chia seeds (da Silva *et al*. 2017) [22].

Table 1: Proximate composition of Chia seeds

Components (%)	Chia seed
Moisture	9.61±0.2
Ash	5.52±0.1
Protein (Nx6.25)	15.05±0.12
Lipids	21.52±0.11
Carbohydrates	48.30±0.22
NDF[a]	39.7±0.2
Cellulose	7.15±0.1
Lignin	7.41±.16
Hemicellulose	25.21±0.18

NDF: neutral diatary fiber, a: on a dry weight basis, Data means ± standard deviation (n = 3).

Chia seeds had 15.05% ± 0.12 of protein, which has complete essential amino acids (FAO/WHO/UNU Expert Consultation, 1985) [23], in contrast with major plant proteins. Also, Segura-Campos *et al*. (2013) [24] cited that chia proteins could be a potential source of bioactive peptides, and its incorporation into human diets is recommended to produce a more balanced protein source.

Chia lipids content was 21.52%±0.11; thus, it is considered as an oilseed plant. Chia oil is rich in ω3 fatty acids (ALA) with a good ratio of ω3 and ω6 fatty acids (2.65) (Alvarez-Chavez *et al*., 2008) [25]. Wherefore, it was reported that chia can decrease serum triglyceride and increase high-density lipoprotein (Guevara-Cruz *et al*. 2012)[26]. It should also be good for the cardiovascular system in humans (Mohd Ali *et al*. 2012) [5].

Chia seeds were characterized by their high concentration of insoluble fiber (39.7 %) distributed as follow: 7.15% of cellulose, 25.21% of hemicellulose and 7.41% of lignin. These findings were in accordance with findings from other studies (Reyes-Caudillo *et al*., 2008) [27]. Meanwhile, they were markedly higher than whole grain cereals (Ragaee *et al*., 2006) [28]. The ratio between IDF and SDF gives important information on nutritional and physiological effects in consumers. The American Dietetic Association recommends fibre intakes of 25–30 g/day for adults with an IDF/SDF ratio of 3–1 (Borderıas Sanchez-Alonso and Perez-Mateos, 2005) [29]. Furthermore, dietary fibers play an essential role in intestinal health, and appear to be significantly associated with a lower risk of developing coronary heart disease, hypertension, diabetes and obesity (Willem van der Kamp *et al*., 2010) [30]. Furthermore, lignin can absorb bile acids and have a hypocholesterolemic effect. On the other hand, the fiber-rich fraction in chia has higher water holding, absorption, and organic-molecule absorption with high emulsifying activity and emulsion stability (Alfredo *et al*., 2009) [31]. These properties should be well applied in foods.

Total phenols of chia seeds were 2.6 mg GAE/g (Table 2). This result is in line with data already reported by Scapin *et al*. (2016) [32]. However, Reyes-Caudillo *et al*. (2008) [33] and Marineli *et al*. (2014) [34] found values between 0.66 to 1.63 mg GAE/g. These differences might be due to the extraction method (Chandrasekara and Shahidi 2010) [35], and/or to the genotype and agronomic conditions (Shahidi and Naczk, 1990) [36], Moreover, polyphenols amounts of chia seeds were higher than cereals, such as barley, oat, rice and corn (Irakli *et al*., 2012) [37]. There is need to note that chia seeds could be used with cereals or replaced in the human diet. Flavonoids in chia seeds were the 1.17 mg QE/g. It account for 45% of total phenols. Although, Rahman *et al*. (2017) [38] reported that flavonoids represent 60% dietary polyphenols in plant foods. This variation may be due to the same factors cited previously for total phenols. However, condensed tannins value (0.3 mg CE/g) is in accordance with results reported by Yi *et al*. (2018) [39]. These components are responsible for the characteristics of astringency of foods (Giada, 2013) [40].

Table 2: Total phenolics, Total flavonoids, Condensed tannins contents and DPPH antioxidant activity of Chia seeds extract

Parameters	Total phenolic[a]	Total flavonoids[b]	Condensed tannins[c]	DPPH IC_{50}[d]
Chia	2.6±0.21	1.17±0.18	0.3±0.08	81.80

a: mg GAE/100 g DW; b: mg quercenin equivalents /100 g DW; c:mg catechin equivalents/100g DW

d:The inhibitory concentration (µg/ml): amount of antioxidant needed to decrease the initial DPPH concentration by 50%.

Antioxidant activity of aqueous extract of chia seeds, expressed as IC50 was 85.8µg/ml; beside, IC50 for ascorbic acid, a reference antioxidant, is 61.3µg/ml. Hence, chia seeds had high antioxidant activity. This is due to several

reactions, including prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging protecting against oxidative damage. So adding chia seeds in foods could be beneficial to consumer health.

In conclusion, chia seeds are rich in antioxidant, fibers, lipids and proteins. Thus, they could be adding in food products to improve consumer health.

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