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Changes in the nutritional composition and antioxidant capacity of chia seeds (*Salvia hispanica* L.) during germination process

María del Carmen Beltrán-Orozco[1] · Alfonso Martínez-Olguín[1] · María del Carmen Robles-Ramírez[1]

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Abstract This study aimed to evaluate the influence of germination on the content of protein, lipids, fiber, ashes, tryptophan, vitamin C, total phenolic and total flavonoid compounds, as well as on the protein digestibility and antioxidant activity of chia seeds germinated for 1, 2, 3, and 4 days. The results showed that germination for 2 days augmented the protein content of chia seeds by 13% while fiber, tryptophan, total phenolic and flavonoid contents increased by 46%, 93%, 300%, and 197%, respectively, after 4 days of germination. Vitamin C was not detected in dry seeds but increased up to 2.33 mg/100 g at fourth day of germination. The antioxidant capacity increased approximately 100% but protein digestibility decreased by 14% at day 4 of germination. Germination can be a good method to increase the nutritional and nutraceutical potential of chia seed for its use in the design of functional foods.

Keywords Chia seed germination · Phenolic compounds · Antioxidant activity · Vitamin C · Protein digestibility

✉ María del Carmen Robles-Ramírez
mcroblesr@ipn.mx; maricarmenrobles58@yahoo.com.mx

María del Carmen Beltrán-Orozco
mariaencb@gmail.com

Alfonso Martínez-Olguín
alphos69@hotmail.com

[1] Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Calle Wilfrido Massieu esquina Cda, Manuel Stampa, C.P. 07738 Ciudad de México, Mexico

Introduction

Chia (*Salvia hispanica* L.) is a plant of the *Lamiaceae* or *Labiatae* family which can grow in mild, tropical and dry climates. It is native of Mesoamerica and was among the main crops of pre-Columbian people along with corn, beans and amaranth. Mayans and Aztecs used this seed (in fact a fruit) as a medicine and food supplement (Sandoval-Oliveros and Paredes, 2013). However, after the Spanish conquest the chia cultivation was forgotten because of its close relationship with indigenous religious cults (Ayerza and Coates, 2009). Recently, the interest for chia cultivation resurged due to several studies that have demonstrated its high nutritive and nutraceutical value. Chia seed is not only the major plant source of the α-linolenic fatty acid (approximately 60% of the oil), but also provides high levels of dietary fiber (18–30%), proteins (15–25%), vitamins, minerals, and antioxidants such as rosmarinic, caffeic and chlorogenic acids, kaempferol, and quercetin (Ayerza and Coates, 2009; Olivos-Lugo, et al., 2010). Several studies in both animals and humans have shown that chia consumption leads to health benefits such as decreased cholesterol and triglycerides in blood, appetite suppression, weight loss, blood glucose control, and intestinal regulation (Ullah et al., 2016). However, although chia seed has high protein content, it has low protein digestibility and is deficient in some essential amino acids, mainly tryptophan (Ayerza, 2013; Vázquez-Ovando et al., 2010). On the other hand, chia is not a good source of vitamin C since it contains less than 2 mg ascorbic acid/100 g (USDA, 2016).

It has been demonstrated that germination, a simple and economic process, can modify the nutritional and antioxidant profile of seeds by improving their vitamin content, amino acid profile, protein digestibility and antioxidant capacity (Fernandez-Orozco et al., 2008; Ghavidel and

Prakash, 2007). However, little is known about the effect of germination on the nutritional composition and antioxidant capacity of chia seeds. Therefore, the objective of this study was to evaluate the effect of germination on the content of protein, fat, fiber, ashes, tryptophan, vitamin C, total phenolic and total flavonoid compounds, as well as on the protein digestibility and antioxidant activity of chia seeds. Germination could improve the nutritional and nutraceutical potential of this ancient grain for its use in the design of functional foods.

Materials and methods

Reagents

Pancreatin from porcine pancreas (8xUSP, P-8445), pepsin from porcine stomach mucosa (3276 U/mg solid, P-6887), peptidase from porcine intestine mucosa (100 U/g solid, P-7500), 4-(p-dimetilaminobenzaldehyde), 2,6-dichlorophenol-indophenol, Folin-Ciocalteu reagent, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazil), TPTZ (2,4,6-tripyridyl-*s*-triazine) were purchased from Sigma-Aldrich (St. Louis, MO).

Seeds

Chia seeds (*Salvia hispanica* L.) were harvested in the municipality of Acatzingo, Puebla, Mexico (18° 60′ N, 98° 13′ W).

Germination

Germination conditions were established on the basis of preliminary assays in our laboratory. Seeds (10 g) were soaked in 250 mL water for 10 min for the complete hydration of the mucilage and then washed with 0.03% liquid detergent followed by distilled water. Afterward, seeds were placed inside plastic trays containing a sheet of filter paper as substrate, and were then allowed to germinate at 30 °C in the dark. The sprouts were harvested at different intervals (0, 1, 2, 3, and 4 days), dried at 60 °C and ground. The percentage of germinated seeds was evaluated every 24 h.

Proximate analysis

Moisture, protein, fat, ash, and crude fiber contents were determined following the methods described by the AOAC (1995).

Determination of tryptophan

The method of Spies and Chambers (1948) was used to determine tryptophan in raw and germinated seeds. Dry powdered defatted sample (10 mg) was mixed with 2.5 mL of p-dimetilaminobenzaldehyde (0.5% in 1 N HCl) and 12 mL of 60% H_2SO_4; it is formed a condensation product that is oxidized with 0.1 mL of 0.05% $NaNO_2$ to form a blue color which is measured at 590 nm in a spectrophotometer Genesys 10S UV–Vis (Thermo Fisher Scientific, Waltham, MA, USA).

Determination of ascorbic acid

The method of Ranganna (1986) with modifications was used. Ascorbic acid of samples (5 g) was extracted with 5 mL of 3% metaphosphoric acid and centrifuged at 10,000 × *g* for 15 min. An aliquot of 1 mL of supernatant was mixed with 1 mL of acetate buffer (pH 4), 1 mL of 0.7 mM 2,6-dichlorophenol-indophenol and 7.5 mL of xylene. The absorbance of the organic phase was measured at 520 nm. The absorbance values were referred to the corresponding concentration in a calibration curve (0–100 μg/mL ascorbic acid).

In vitro protein digestibility

In vitro digestibility of chia seeds protein was determined using the method of Hsu et al. (1977). To a dry sample containing 6.25 mg of protein/mL, 50 mL of distilled water was added and then adjusted to pH 8.0 at 37 °C. An aliquot of 5 mL of a multienzyme solution containing 1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase per milliliter (adjusted at pH 8.0) was added to the sample suspension, stirred, and the pH of the mixture measured in a pH meter HI 223 (Hanna Instruments, CDMX, Mexico) at exactly 10 min. In vitro protein digestibility was calculated using the following equation: % digestibility = 210.464 − 18.103 X, where X represents the pH after 10 min incubation.

Extraction of antioxidant compounds

Ground samples (2 g) were extracted in a shaker with 20 mL of 80% ethanol at room temperature overnight. Afterward, the extracts were recovered by centrifugation at 6000 × *g* at 4 °C for 15 min. Phenolic compounds and antioxidant activity were determined in the extracts.

Determination of total phenolic content

The content of total phenolic compounds was determined using the Folin-Ciocalteu reagent (Singleton et al., 1999).

An aliquot of 20 μL of the ethanolic extract of sample or standard gallic acid solution (100–500 μg/mL) was mixed with 1.58 mL of distilled water and 100 μL of Folin–Ciocalteu reagent (diluted 1:1 with distilled water). After 3 min, 300 μL of sodium carbonate solution (10% w/v) was added. The mixture was allowed to stand at room temperature for 60 min and the absorbance of the mixture measured at 765 nm. The content of total phenolic compounds was expressed as mg gallic acid equivalent (GAE)/100 g of dry sample.

Determination of total flavonoid content

The flavonoid content of the samples was carried out according to the method described by Ebrahimzadeh et al. (2008). An aliquot of 0.5 mL of the extract or standard quercetin solution (10–100 μg/mL) was mixed with 1.5 mL of ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was left 30 min at room temperature and the absorbance was read at 415 nm. The total flavonoid content was expressed as mg quercetin equivalent (QE) per 100 g of dry sample.

ABTS radical-scavenging activity

The ABTS radical-scavenging activity of raw and germinated seeds was determined in the ethanolic extracts following the method described by Dudonné et al. (2009). The $ABTS^{\bullet+}$ stock solution was prepared by mixing 7 mM of ABTS with 2.45 mM of potassium persulfate, and allowed to react at room temperature in the dark for 16 h. The stock solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Extracts or Trolox standard solutions (20 μL) were allowed to react with 1980 μL of $ABTS^{\cdot+}$ for 7 min, and then the absorbance was measured at 734 nm. Ethanol (80%) was used as blank. The percentage inhibition of absorbance due to the presence of antioxidants was calculated. A calibration curve was obtained by plotting the percentage of inhibition against concentration of standard antioxidant Trolox (300–1500 μM). The antioxidant activity of samples was determined from such curve and expressed as μmol of Trolox equivalents (TE) per gram dry weight.

DPPH radical-scavenging activity

The DPPH radical-scavenging activity was determined following the method described by Dudonné et al. (2009). A volume of 100 μL of the extracts or of the Trolox standard solution was mixed with 2 mL of 0.06 mM DPPH ethanol solution and the absorbance was read at 515 nm after incubation at room temperature for 30 min. Then, the percentage of inhibition of absorbance was determined. A calibration curve was obtained by plotting the percentage of inhibition against concentration of standard antioxidant Trolox (150–750 μM). The antioxidant activity of samples was determined from such curve and expressed as μmol TE/g dry weight.

Ferric reducing antioxidant potential (FRAP)

The ferric reducing power of chia samples was carried out according to the method reported by Dudonné et al. (2009) with slight modifications. The working solution was prepared by mixing 1 volume of 10 mM TPTZ in 40 mM HCl with 1 volume of 20 mM $FeCl_3.4\ H_2O$ and 10 volumes of 300 mM acetate buffer, pH 3.6. A volume of 900 μL of the working solution was mixed with 90 μL of distilled water and 30 μL of the sample extract. The mixture was maintained at 37 °C for 30 min and the absorbance was read at 595 nm. A standard curve was prepared by plotting the absorbance against concentration of Trolox (300–1500 μM). The results were expressed in μmol TE/g dw.

Statistical analyses

Values are expressed as the mean ± SD of three replicates for all determinations. Data were analyzed by one-way ANOVA and Tukey post hoc test. Pearson correlation analysis was carried out to identify relationships between antioxidant compounds and antioxidant capacity measurements. All statistical analyses were done using SigmaPlot 12.3 (Systat Software Inc., San José, CA, USA). Differences were considered statistically significant if $p < 0.05$.

Results and discussion

The percentage of germination and the radicle length of *Salvia hispanica* germinated for 1, 2, 3, and 4 days are shown in Table 1. The maximum percentage of

Table 1 Seed germination characteristics of *Salvia hispanica* L

Germination time (days)	Germinated seeds (%)	Radicle length (cm)
1	52 ± 2a	0.08 ± 0.03a
2	70 ± 4b	1.25 ± 0.35b
3	82 ± 3c	4.00 ± 1.41c
4	91 ± 2d	5.50 ± 0.71d

Values are mean ± standard deviation. Different letters in the same column mean significant differences ($p < 0.05$)

germination (91%) was achieved on the fourth day, in which the length of the radicle reached an average of 5.5 cm. These results differ from those obtained by Pereira et al. (2016) who found a lower percentage of germination (55%) and higher radicle length (7.94 cm) than those obtained in this work under similar conditions, maybe due to the different variety and origin of chia seed used.

Germination induced several changes in nutrient content of chia seeds. The changes that occurred during chia seed germination in the contents of protein, fat, fiber, vitamin C, tryptophan and protein digestibility are shown in Table 2. The initial protein content of chia seed (20.64%) was similar to that found by other researchers who have reported values between 18.6 and 24.4% for chia grown in different climate conditions (Ayerza and Coates, 2009; Gómez-Favela et al., 2017; Sandoval-Oliveros and Paredes-Lopez, 2013). In the present work, the concentration of protein augmented in approximately 13% during the first 48 h of germination and then decreased. Gómez-Favela et al. (2017) observed an increase of 20.9% in chia protein content after germination under optimized conditions (21 °C, 157 h) and it was demonstrated that the time and temperature at which this bioprocess occurs influence the nutrimental characteristics of the final product. The increase in protein content during germination has also been reported in other studies with legumes such as lupine (Rumiyati et al., 2012), lentils, beans, peas, and chickpea (Camacho et al., 1992). The metabolism of the non protein components, or their loss during the imbibition of the seed, could contribute to the proportional increase in the protein content. The subsequent decline of protein after 2 days of germination could be due to its use to obtain energy or to synthesize other components necessary for the growth of the new seedling.

The lipid content (37.98%) of ungerminated chia seeds was in the range reported by other researchers for different varieties of chia which were in the range of 26–39% (Ayerza et al., 2009; Ullah et al., 2016). Lipid content increased by 10% in the first 2 days of germination and then decreased giving a total diminution of 21% at fourth day (Table 2). This result was probably due to the use of lipids as energy source. Gómez-Favela et al. (2017) found a decrease of 55.31% in the lipid content of chia seeds after 157 h of germination at 21 °C. The reduction of lipids during germination has also been reported in soybean (Dikshit and Ghadle, 2003), lupine (Rumiyati et al., 2012), lentils, beans, peas, and chickpeas (Camacho et al., 1992).

Chia is an important source of dietary fiber with values ranging between 18 and 44% (Gómez-Favela et al., 2017; Olivos-Lugo et al., 2010; Reyes-Caudillo, et al., 2008). Chia seeds analyzed in the present work showed relatively low fiber content (16.6%) but germination promoted an augment of approximately 46% after 4 days (Table 2). This is an important fact, since the intake of dietary fiber reduces the risk of developing diseases such as diabetes, obesity, coronary heart disease, hypertension, stroke, and gastrointestinal disorders (Anderson et al., 2009). The augment of fiber during germination was also reported in other seeds such as broccoli (Taraseviciene et al., 2009), lupine (Rumiyati et al., 2012), peas (Martin-Cabrejas et al., 2003), and non-conventional legumes (Benítez et al., 2013). This increment has been attributed to changes in the polysaccharides found in the cell wall due to an augment in the cellular structure of the plant during germination (Martin-Cabrejas et al., 2003).

There was not a significant change in ash content during germination. The results obtained in the present work on ash content (5.06% dw) for raw chia was in agreement with those reported in other researches (Gómez-Favela et al., 2017; Vázquez-Ovando et al., 2010). However, Gómez-Favela et al. (2017) found that germination increased the mineral content by 59.4% under optimized conditions (21 °C, 156 h).

Table 2 Effect of germination on the proximate composition, vitamin C, tryptophan, and protein digestibility of Chia seeds

Determination	Germination time (h)				
	0	24	48	72	96
CP (g/100 g)	20.66 ± 0.10a	22.10 ± 0.47a	23.24 ± 0.07b	22.16 ± 0.10a	21.24 ± 0.02a
L (g/100 g)	37.98 ± 2.79b	41.65 ± 0.88c	42.16 ± 0.31d	38.44 ± 0.79b	30.98 ± 0.04a
CF (g/100 g)	16.60 ± 0.36a	18.66 ± 0.02b	21.14 ± 0.52c	22.30 ± 0.7 cd	24.25 ± 0.09d
Ash (g/100 g)	5.06 ± 0.02a	5.01 ± 0.01a	5.02 ± 0.03a	5.04 ± 0.04a	5.12 ± 0.04a
Vit C (mg/100 g)	ND	ND	0.43 ± 0.06a	1.24 ± 0.11b	2.33 ± 0.06c
Tryp (g/100 g)	2.51 ± 0.14a	3.60 ± 0.26b	3.99 ± 0.14c	4.12 ± 0.25c	4.84 ± 0.08d
PD (%)	79.6 ± 0.69c	71.9 ± 1.22b	71.0 ± 0.63b	71.7 ± 0.24b	69.7 ± 0.20a

Values are mean ± standard deviation. Different letters in the same row mean significant differences ($p < 0.05$)

ND not detected, *CP* crude protein, *L* lipids, *CF* crude fiber, *Vit C* vitamin C, *Tryp* tryptophan, *PD* protein digestibility

Chia is a good source of B vitamins and vitamin A but is deficient in vitamin C (USDA, 2016). In this study ungerminated chia seed had no detectable ascorbic acid but germination produced the increase of this vitamin from the second day and it continued to increase significantly over the next 2 days (Table 2). The increment of vitamin C during germination has been reported in various studies with different seeds such as soybean (Xu et al., 2005), mungbean (Fernández-Orozco et al., 2008), and lupine (Frias et al., 2005), indicating the involvement of this vitamin in the modulation of plant growth. It has been demonstrated that the activity of GLDH (L-galactono-γ-lactone dehydrogenase), a key enzyme in the biosynthesis of ascorbic acid, increases significantly during germination, reactivating the vitamin C biosynthesis (Xu et al., 2005).

Different studies have found that germination induces the increment in the essential amino acid ratio with respect to non-essential amino acids (Martínez-Villaluenga et al., 2008; Taraseviciene et al., 2009). Chia protein is deficient in some amino acids mainly tryptophan which diminishes the quality of its protein (Ayerza, 2013; Vázquez-Ovando et al., 2010). That is why in the present study the effect of germination on the tryptophan content was evaluated. Germination caused a rise of tryptophan content of about 100% after 4 days. However, besides the good essential amino acid balance, protein quality depends on another important factor, the protein digestibility. Different studies reported values of protein digestibility of chia seed ranging from 77.5 to 79.8% (Sandoval-Oliveros and Paredes, 2013; Vázquez-Ovando et al., 2010). These values are similar to that obtained in the present study in ungerminated chia seed (79.6%). However, the in vitro digestibility of chia protein decreased as the germination progressed (Table 2). This behavior differs from that observed by other researchers in chia and other seeds. Gómez-Favela et al. (2017) found an increase of 4.8% in the in vitro protein digestibility of chia germinated for 157 h at 21 °C. Dikshit and Ghadle (2003) found an increase of 33% in protein digestibility of soybean germinated for 72 h. Ghavidel and Prakash (2007) reported that germination for 24 h increased the digestibility of protein of green gram, cowpea, lentil, and chickpea by 14–18%, whereas Urbano et al. (2005) did not observed changes in the in vivo protein digestibility of peas when were germinated for 2, 4, and 6 days. The decrease of protein digestibility during germination of chia, observed in the present work, was likely due to the parallel increase in fiber (Table 2) and phenolic compounds (Table 3). It is known that these constituents of the seed can interact with digestive enzymes affecting their activity. On the other hand, viscous soluble fiber may hinder the access of digestive enzymes to their substrates (Ghavidel and Prakash, 2007).

The total phenolic and flavonoid contents, as well as the antioxidant capacity of chia seeds during germination for 1–4 days are shown in Table 3. The amount of phenolic compounds found in the present study was 97.7 mg GAE/100 g in ungerminated seeds, but this value increased 3-fold after 4 days of germination. It is possible that these compounds have a protective effect against pathogen organisms on the seedling development. The role of phenolic compounds against the attack of microorganisms, insects, and parasitic plants has been widely studied as well as their function as signal molecules to ensure the seedling survival (Ndakidemi and Dakora, 2003). Germination probably induced the synthesis of phenolic compounds and also caused the release of these compounds from the food matrix of chia seeds. The values obtained in this study are similar to those found by other researchers. Reyes-Caudillo et al. (2008) reported an average amount of phenolic compounds of 75.7 and 88.1 mg GAE/100 g in chia seeds from Jalisco and Sinaloa (Mexico), respectively, whereas Martínez-Cruz and Paredes-López (2014) found an average of 164 mg GAE/100 g in seeds from Colima (Mexico). On the other hand, Gómez-Favela et al., (2017) reported an amount of 190.8 mg GAE/100 g in chia seeds and observed an increase of 47.4% in total phenolics after 156 h of germination at 21 °C.

Among the phenolic compounds, flavonoid compounds stand out because of their potential health benefits that include antioxidant, anticancer, and anti-inflammatory activities; flavonoids such as quercetin, myrcetin, kaempferol, and daidzin have been identified in chia seeds (Martínez-Cruz and Paredes-López, 2014; Reyes-Caudillo et al., 2008). In this study, the content of total flavonoids represented more than 30% of the total phenolic content of chia seeds and its content augmented 197% after 4 days of germination.

The initial antioxidant activities of chia seeds were 77.7, 41.1, and 72.3 μmol ET/g (dw) determined by ABTS, DPPH, and FRAP methods, respectively. These values increased by 105.1%, 101.6%, and 87.7%, respectively, after 4 days of germination; this was probably due to the corresponding augment of antioxidant compounds observed in this study (vitamin C, total phenolic, and flavonoid compounds).

The high content of phenolic compounds of chia seeds was strongly related to their ABTS and DPPH radical scavenging activity as well as to their reducing power (Table 4). The antioxidant activity was also positively correlated with the content of total flavonoids and ascorbic acid which indicates that all these compounds contributed to the antioxidant capacity of chia seeds (Table 4). Gómez-Favela et al. (2017) observed an increase of 77.2% and 96.7% in phenolic compounds and antioxidant activity (ABTS method), respectively, after 157 h of germination at

Table 3 Effect of germination on the total phenolics, total flavonoids, and antioxidant activity of chia seeds

Determination	Germination time (h)				
	0	24	48	72	96
Total phenolics[1]	97.7 ± 2.8a	148.6 ± 0.7b	165.4 ± 7.33c	191.1 ± 3.5d	293.6 ± 1.3e
Total flavonoids[2]	35.8 ± 0.2a	43.0 ± 2.0b	62.4 ± 3.5c	82.3 ± 4.4d	106.0 ± 6.4e
Antioxidant activity[3]					
ABTS	77.7 ± 5.9a	98.3 ± 4.7b	120.5 ± 4.c	149.7 ± 3.4d	159.4 ± 1.4e
DPPH	41.1 ± 0.5a	49.2 ± 1.6b	61.9 ± 0.2c	75.6 ± 0.0d	82.9 ± 0.16e
FRAP	72.3 ± 1.7a	90.3 ± 0.5b	107.2 ± 0.1c	122.1 ± 1.9d	135.7 ± 0.1e

Values are mean ± standard deviation. Different letters in the same row mean significant differences ($p < 0.05$)

[1]mg gallic acid equivalents (GAE)/100 g sample (dw)

[2]mg quercetin equivalents (QE)/100 g sample (dw)

[3]μmol Trolox equivalents (TE)/g sample (dw)

Table 4 Correlation coefficients between antioxidant compounds and antioxidant capacity assays

Antioxidant compounds	ABTS	DPPH	FRAP
Total phenolic content	0.9034	0.9179	0.9344
Total flavonoid content	0.9723	0.9850	0.9760
Vitamin C	0.9126	0.9340	0.9163

21 °C. They also found a high correlation between total phenolics and antioxidant activity (r = 0.939). Germination has also proved to increase the polyphenol content and the antioxidant capacity of different grains such as amaranth, quinoa, buckwheat and wheat (Álvarez-Jubete et al., 2010), mungbean (Fernández-Orozco et al., 2008; Kim et al., 2012), soybeans (Fernández-Orozco et al., 2008), and lupine (Dueñas et al., 2009).

In conclusion, this study has shown that germination can be a good method to increase the nutritional and nutraceutical potential of chia seed. The germination of chia seeds produced maximum increments of protein, fiber, tryptophan, total flavonoid, and total phenolic contents of 13%, 46%, 93%, 197%, and 300%, respectively, while promoted the vitamin C synthesis. In addition, germination increased the antioxidant capacity of chia seeds by approximately 100%. However, there was a decrease of 12% in protein digestibility.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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