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RESEARCH

Chemical Composition, Fatty Acid Profile, Phenolic Compounds, and Antioxidant Activity of Raw and Germinated Chia (*Salvia hispanica* L.) Seeds

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Abstract
The consumption of chia seeds has become popular due to their beneficial health properties and the germination of chia seeds seems to further enhance these properties. This study aimed to evaluate the changes in the nutritional composition of chia seeds after germination for 3 and 6 days. Chemical composition, fatty acid profile, phenolic content and antioxidant capacity were determined. The indices of lipid quality, atherogenicity, thrombogenicity, and the *n*-6/*n*-3 ratio were calculated. Chia sprouts presented a significant increase in minerals, proteins, and a reduction in total lipid content with maintenance of lipid quality. Total phenolic content decreased significantly as germination time increased, but there was a significant increase in the amount of rosmarinic acid. Chia sprouts showed a significant increase in antioxidant potential when compared to raw chia seeds. As a conclusion, the results of this study demonstrated that chia seed germination is a simple, economical, and short-term process capable of improving the nutritional composition of the seeds.

Keywords Germination · Sprout · Omega-3 fatty acids · Indexes of lipid quality · Rosmarinic acid

Introduction

Chia (*Salvia hispanica* L.) is an herbaceous plant, belonging to the Lamiaceae family, native to the mountainous regions of western and central Mexico and Guatemala. It was cultivated by the Mayas and Aztecs and had great importance because it was used as food, paints, handicrafts and even as medicine [1, 2]. Currently, the commercial cultivation of chia seeds is mainly concentrated in countries such as Mexico, Bolivia, Ecuador, Guatemala, Colombia, Peru, Argentina, and Paraguay [3].

Chia seed is a food that has a high content of antioxidant compounds, protein, dietary fiber and polyunsaturated fatty acids, especially α-linolenic acid, belonging to the omega-3 fatty acid family [4, 5]. In addition, its consumption is associated with increased satiety, regularization of bowel function, decreased blood cholesterol and triacylglycerol, decreased risk of chronic noncommunicable diseases, among others [6–8].

Due to growing concerns about health and the environment, the population is increasingly seeking a healthier lifestyle, opting for fresh foods, and adopting plant-based diets. Therefore, the demand for healthy foods, such as sprouts, grown in natural systems has been increasing [9, 10]. Consumption of sprouts is an ancient practice and their production is simple; it does not require large spaces or many resources for cultivation [11–13]. Sprouts can be defined as the product obtained from the germination of seeds in water or other media, harvested before the development of true leaves and intended to be eaten whole, including the seeds [14]. Germination is a good way to improve the nutritional value of foods that are frequently consumed, and it can be used in food products [8].

The consumption of sprouts has been increasing as a result of the search for balance in the diet, and although

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there are still few studies, the literature has reported that the germination of chia seeds results in increased protein content and quality, increased total phenolic compounds and antioxidant capacity [15–17]. Regarding the fatty acid profile, no studies associated with chia sprouts have been found so far. In a study by Cabrera-Santos et al. [18], the changes in fatty acids during hydration of chia seeds at 10, 20 and 30 °C was investigated to determine the correlation between fatty acids, temperature, and germination.

Chia seeds are used by the population because of their beneficial properties to health, and the germination process seems to improve these properties. Therefore, scientific research still needs to evaluate the changes that occur in the nutritional value of chia seeds after germination at different times, especially changes related to fatty acid profile. Therefore, this work aimed to investigate the nutritional changes that occur in chia seeds during the germination process.

Materials and Methods

The Materials and Methods section is presented as supplementary material.

Results and Discussion

Seed germination is a natural and generally simple process that can improve the nutritional value of food [9, 19]. Regarding chia seeds, its ability to form mucilage when in contact with water, can negatively affect the entry of water and oxygen into the seed and decrease sprout development [20]. In this regard, it is worth noting that the germination process of chia seeds was challenging.

Chemical Composition

The results of the chemical composition analysis are shown in Table 1. In the present study, there was a significant increase in mineral content, as measured by ash content, after 3 and 6 days of germination compared to ungerminated chia seeds. Increase in mineral content was also found in the study by Gomez-Favela et al. [17], in which germination of chia seeds under optimized conditions (21 °C, 156 h) resulted in more than 59.4% in mineral content. On the other hand, Beltran-Orozco et al. [16] found no increase in ash content after 3 days of germination of chia seeds.

Table 1 Basic composition of ungerminated chia seeds, 3-day sprouts and 6-day sprouts *per* 100 g (dw)

Component (g/100 g)	Ungerminated chia seeds	3-day sprouts	6-day sprouts
Protein	19.05 ± 1.11[c]	21.22 ± 0.74[b]	24.55 ± 0.15[a]
Lipids	40.11 ± 0.55[a]	34.79 ± 0.23[b]	28.59 ± 0.36[c]
Carbohydrates	36.47	39.12	41.68
Ash	4.37 ± 0.01[c]	4.87 ± 0.04[b]	5.18 ± 0.06[a]

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

In the present study, there was a significant increase in protein content during chia seed germination, as the 3-day sprouts showed significantly higher values than those found in chia seeds, and the 6-day sprouts showed higher values than the 3-day sprouts. Gomez-Favela et al. [17], when comparing chia seed flour with sprouted chia flour, found an increase in protein, as in our study, after 156 h (6.5 days) of germination. The increase in protein content during germination has also been reported in other studies with Australian sweet lupin [21], chickpea, lentil, and yellow pea [22]. The metabolism of non-protein components, such as the reduction of lipids, may have contributed to the proportional increase in protein content [16].

During germination of chia seeds, there was a significant decrease in lipid content compared to raw chia seeds in the present study. This reduction also occurred in other studies that investigated the effect of germination on chia sprouts. For example, Gomez-Favela et al. [17] reported a 55.3% reduction in lipid content in chia flour germinated at 21 °C after 6.5 days when compared to chia flour. Beltrán-Orozco et al. [16] found a significant increase in lipid content in the first 48 h of germination (from 37.98 to 42.16 g/100 g); however, lipid content reduced after 72 h (38.4%) and 96 h (31%). Other studies conducted on sprouts of Australian sweet lupin [21], wheat (*Triticum aestivum* L.), alfalfa (*Medicago sativa* L.), radish (*Raphanus sativus* L.) and lentil (*Lens culinaris* L.) [23] also reported a reduction in total lipid content during the germination process. This effect can be attributed to increased lipolytic activity, which results in the breakdown of triacylglycerols into fatty acids and glycerol, used as an energy source for seed germination [24, 25].

Fatty acid Profile

Table 2 shows the fatty acid composition of the ungerminated chia seeds and the 3- and 6-day-old sprouts. The lipids present in ungerminated chia seeds are mostly composed of polyunsaturated fatty acids, such as α-linolenic acid and linoleic acid [4]. Therefore, it was found that the content of total unsaturated fatty acids, especially polyunsaturated fatty acids, was predominant in all the study samples. For both the ungerminated chia seeds and the 3- and 6-day-old sprouts, α-Linolenic acid (C18:3) was predominant, followed by linoleic acid (C18:2), palmitic acid (C16:0), oleic acid (C18:1), and stearic acid (C18:0). In a study by Imran et al. [26], as in the present study, it was shown that fatty acids present in the highest amount in crude chia oil

Table 2 Percentage of major fatty acids found in ungerminated and germinated chia seeds

Fatty acid (%)	Ungerminated chia seeds	Sprout 3 days	Sprout 6 days
Palmitic acid (C16:0)	7.69 ± 0.15^a	8.59 ± 0.73^a	9.27 ± 0.91^a
Stearic acid (C18:0)	3.68 ± 0.21^a	4.04 ± 0.24^a	4.11 ± 0.09^a
Oleic acid (C18:1)	6.14 ± 0.04^a	6.39 ± 0.64^a	6.59 ± 1.02^a
Linoleic acid LA (C18:2)	18.77 ± 0.35^a	19.10 ± 0.40^a	19.22 ± 0.20^a
α-Linoleic acid ALA (C18:3)	62.66 ± 0.15^a	60.51 ± 2.17^a	59.10 ± 2.60^a
Unsaturated fatty acids	87.91 ± 0.42^a	86.44 ± 1.06^a	85.51 ± 1.11^a
Monounsaturated fatty acids	6.37 ± 0.06^a	6.70 ± 0.71^a	7.02 ± 1.23^a
Polyunsaturated fatty acids	81.55 ± 0.48^a	79.74 ± 1.77^a	78.49 ± 2.34^a
Saturated fatty acids	12.09 ± 0.42^a	13.56 ± 1.06^a	14.49 ± 1.11^a
Omega-3	62.68 ± 0.19^a	60.57 ± 2.17^a	59.19 ± 2.60^a
Omega-6	18.79 ± 0.35^a	19.13 ± 0.40^a	19.26 ± 0.26^a
Omega-9	6.30 ± 0.08^a	6.59 ± 0.68^a	6.77 ± 1.11^a
n6/n3 ratio	0.3	0.3	0.3
index of atherogenicity	0.09	0.10	0.11
index of thrombogenicity	0.06	0.06	0.07

Different letters in the same row mean significant differences ($p < 0.05$)

on day 0 of storage were α-linolenic (60.56 ± 1.22%), linoleic (12.14 ± 0.22%), oleic (8.34 ± 0.19%) and palmitic (6.76 ± 0.15%) acids.

As with flaxseed, chia is an important source of the essential fatty acids α-linolenic (omega-3) and linoleic (omega-6); after vegetable oils, it is the main plant sources of omega-3 fatty acid. It is noteworthy that essential fatty acids are those polyunsaturated fatty acids that must be supplied by food, since the animal organism is not able to synthesize them. The essential fatty acids α-linolenic and linoleic are precursors of other long-chain polyunsaturated fatty acids, such as the omega-3 fatty acids EPA and DHA and the omega-6 arachidonic fatty acid, all of which have important biological activities, *e.g.*, the synthesis of inflammation mediators with anti-inflammatory (omega-3) and pro-inflammatory (omega-6) action [1, 27, 28].

The results found in this study indicated that there was no significant reduction in the percentage of fatty acids after germination, and that chia sprouts represent an important source of essential fatty acids, especially omega-3, as shown in Table 2. A 50 g (1 ½ cup) serving of chia sprouts (1.97 g on a dry basis) already meets 100% of the daily value of adequate intake for α-linolenic acid (adequate intake for α-linolenic acid: 1.35 g *per* day) [29]. A study conducted in Turkey by Ghafoor et al. [30] compared the lipid profile of ungerminated and 4-day germinated chia and found a similar lipid profile to the present study. However, the authors found an increase in saturated fat content and a reduction in omega -3 after germination. This may have occurred due to the difference in seed origin of the studies. In a study conducted by Mattioli et al. [31], flaxseed sprouts (germinated for 3 days) showed a fatty acid profile similar to that of chia sprouts found in the present study.

It is worth noting that studies have shown that omega-3 consumption can reduce the risk of cardiovascular diseases by reducing blood pressure, lowering triacylglycerol levels, improving endothelial function, and reducing inflammation, in addition to having beneficial effects on the prevention and control of neurodegenerative diseases, such as Alzheimer's disease, and on mental health, such as depression and anxiety [32–34].

In this context, chia sprouts can significantly contribute to the improvement of important dietary lipid quality indices: omega-6:omega-3 ratio (n6/n3), index of atherogenicity (IA), and index of thrombogenicity (IT), as discussed below.

Both α-linolenic acid and linoleic acid are metabolized into their corresponding products by common enzymatic systems, i.e., very high amounts of linoleic acid can inhibit the conversion reactions of α-linolenic acid (competitive inhibitory effect), predisposing the body to a proinflammatory state [28, 35]. In this sense, n6/n3 of foods, and consequently of the diet, has great importance for the balance in the metabolism of these fatty acids. Taking into account the value recommended by the Institute of Medicine [29] (n6/n3 = 10) and values found in population studies with different dietary models (n6/n3 = 1.5 for Japanese and n6/n3 = 18 for North Americans), the literature points out values of n6/n3 ratio in the diet between 1.5 and 3 as adequate [29, 36, 37].

In the present study, the n6/n3 ratio of raw chia seeds and chia sprouts was 0.3 (Table 2). When chia is consumed frequently within a proper diet, this ratio can be considered important for maintaining the proper balance between omega-6 and omega-3 and preventing inflammatory processes, which are related to increased risk for several noncommunicable chronic diseases [38–40]. This is due to the fact that omega-6 fatty acid is more abundant in foods than omega-3, favoring an imbalance between the n6/n3 ratio [40].

Regarding IA and IT, lower values are related to better lipid quality of the food. In present study, IA and IT were

0.09 and 0.06 for the ungerminated chia seeds, 0.10 and 0.06 for the 3-day-old sprouts, and 0.11 and 0.07 for the 6-day-old sprouts (Table 2), respectively, demonstrating that germination maintained the lipid quality of chia. These values were lower than the ones found by Molska et al. [41], who investigated the changes in fatty acid profile and lipid quality indices in conventional and probiotic added buckwheat sprouts. The authors reported values of 0.17 for IA and 0.32 for IT for both sprouts [41]. As the calculation of these indices is based on the content of omega-6 and omega-3 fatty acids, including the ratio between them, especially in the IT, the lower values found in the present study are supported by the high content of omega-3 and the low n6/n3 ratio.

Phenolic Compounds

Table 3 shows the results of total phenolic compound content found in the present study. There was a significant decrease in the levels of total phenolic compounds over the time of chia sprout growth, and the average content of total phenolic compounds in the 6-day chia sprout was significantly lower compared to the 3-day chia sprout. Previous studies have corroborated these findings, such as the study by Miyahira et al. [42], which evaluated the concentration of phenolic compounds in lentil sprouts and found a decrease in phenolic compounds over growth time. Similarly, Liu et al. [43] found a decrease in phenolic compound content after 6 days of the alfalfa germination process. The germination process results in the formation of new structures in plants, and during this process, soluble phenolic compounds tend to bind to carbohydrates and proteins to form new cell walls [44]. In addition, phenolic compounds are easily lost during seed soaking, as they are slightly water soluble [43, 45]. This may account for a reduced amount of these compounds in the soluble fraction [44].

However, other studies have shown different results for the levels of phenolic compounds in chia sprouts. Abdel-Aty et al. [15] reported higher levels of total phenolic compounds in the sprouts compared to the raw seeds. The authors found a significant increase of 4.7-fold in 3 days and 5.8-fold in 6 days, and these values decreased after the seventh day of germination. According to the authors, the increase in the first days of germination may be due to the degradation of phenolic compounds conjugated into soluble phenolic compounds, and with longer germination time, a decrease in free phenolic compounds may occur, as they conjugate with the cell wall again. The authors further argued that the differences in phenolic contents may be mainly due to the type of chia seed cultivar, which probably has more influence than germination conditions, such as temperature, humidity, light, and germination time [15]. Other studies also found increased content of total phenolic compounds in germinated chia sprouts when compared to raw seeds [16, 17].

The extracts of samples of raw and germinated chia seeds for 3 and 6 days showed many substances according to chromatographic analysis by HPLC/DAD and HPLC/MS (Fig. 1 supplementary), with two predominant signals – 10.421 min (1) and 11.133 min (2). By co-injection with standard and comparison of ultraviolet and mass spectra, it is suggested that the (2) signal at 11.133 min is from rosmarinic acid. Signal (1) also shows a UV spectrum similar to that of rosmarinic acid, but its mass spectrum shows a molecule with a molecular mass of 522 (detected m/z of 521) suggesting a heteroside of rosmarinic acid. As glycoside is the most abundant heteroside in nature, it is suggested that it is rosmarinic acid 3-O-glycoside.

The final concentrations of rosmarinic acid and its heterosides of the chia extracts in methanol were determined by HPLC-DAD (Table 3). The analyses showed that with increasing time of chia seed germination, there was a significant increase in the concentrations of rosmarinic acid, and a reduction in the concentration of heterosides. The presence of rosmarinic acid in chia seeds and chia sprouts had been previously reported by Motyka et al. [46], who investigated the presence of rosmarinic acid in different parts of chia plants, including seeds and sprouts. The results of the study showed that both chia seeds and sprouts contain rosmarinic acid, with concentrations

Table 3 Total Phenolic compounds, Rosmarinic acid, Rosmarinic acid 3-O- glycoside and antioxidant activity of the chia seeds, 3-day sprouts and 6-day sprouts, using the ABTS, DPPH and FRAP assays

Component	Ungerminated chia seeds	3-day sprouts	6-day sprouts
Total phenolics (mg GAE/g)	2.58 ± 0.17[a]	2.24 ± 0.12[b]	1.76 ± 0.07[c]
Rosmarinic acid (mg/g)	0.39 ± 0.004[a]	1.04 ± 0.004[b]	1.23 ± 0.004[c]
Rosmarinic acid 3-O- glycoside (mg/g)	0.07 ± 0.004[a]	0.01 ± 0.004[b]	0.008 ± 0.004[c]
Antioxidant activity			
ABTS (.M Trolox/g)	113.98 ± 0.11[a]	115.29 ± 0.16[b]	115.92 ± 0.19[c]
DPPH (%AA)	59.83 ± 1.92[a]	90.22 ± 0.19[b]	91.47 ± 0.19[b]
FRAP (.M Trolox/g)	2.11 ± 0.22[a]	3.37 ± 0.38[b]	4.74 ± 0.47[c]

FRAP Ferric-reducing antioxidant potential method, ABTS Trolox equivalent antioxidant capacity, DPPH 1,1-diphenyl-2-picrylhydrazyl free radical, GAE/g gallic acid equivalents *per* gram. Values are mean ± standard deviation. Different letters in the same row mean significant differences ($p < 0.05$)

of 1.27 ± 0.0003 and 1.34 ± 0.0004 mg/g, respectively. These findings are similar to the results of the present study, which also showed that the sprouts had a significantly higher concentration of rosmarinic acid compared to chia seeds. However, the present study showed higher values of rosmarinic acid in the sprouts.

Another study by Abdel-Aty et al. [15] showed that germination significantly increased the concentration of rosmarinic acid in seeds (0.32 ± 0.01 mg/g) after germinating chia for 7 days (0.60 ± 0.01 mg/g). This result is interesting since rosmarinic acid has antioxidant, anti-inflammatory, antimicrobial, and antitumor properties [47], and studies have proven that it may have benefits in treating conditions such as cancer, cardiovascular disease, diabetes, Alzheimer's disease, Parkinson's disease, osteoarthritis, asthma, and others [47–49].

Antioxidant Capacity

The results of the three methods for analysis of antioxidant activity (ABTS, DPPH and FRAP) (Table 3) show that chia sprouts had higher antioxidant activity compared to chia seeds. In addition, germination time had a positive effect on the antioxidant activity of the sprouts. These results suggest that chia sprouts can neutralize different types of free radicals, as observed in the ABTS and DPPH assays. In addition, chia sprouts also showed the ability to inhibit metal-mediated free radical formation, as evidenced by the FRAP assay.

According to Beltrán-Orozco et al. [16], who investigated the antioxidant activity of chia seeds from Mexico, there was a significant increase in antioxidant activity after four days of germination, as demonstrated by the ABTS, DPPH and FRAP methods. These findings indicate that the germination process exerts a positive effect on the antioxidant activity of chia seeds, which corroborates the results of the present study. The authors attributed the increased antioxidant activity to the increased concentrations of antioxidant compounds such as vitamin C, total phenolics and flavonoids, in addition to the production of new flavonoids and availability of soluble phenolic compounds.

Similar results have been reported by other authors, such as Abdel-Aty et al. [15], who investigated the antioxidant activity of chia and its sprouts using the ABTS and DPPH methods. In this study, the researchers found that chia sprouts exhibited higher antioxidant activity compared to chia seeds. A study conducted with the aim of obtaining functional flour from sprouted chia seeds under optimized conditions investigated the effect of sprouting on antioxidant activity and found that sprouted chia flour was able to neutralize ABTS + cation radicals about 96.68% more when compared to chia seed flour [17]. The consistency of these findings strengthens the evidence that chia sprouts may exhibit higher antioxidant activity than seeds, and this may play a key role in preventing free radical damage, which is associated with cellular aging and the development of chronic non-communicable diseases [50].

Conclusions

Germination of chia seeds can result in significant changes in the nutritional composition of the sprouts. During the germination process, there was an increase in mineral and protein content. In addition, chia sprouts are an important source of essential fatty acids, especially omega-3, which play essential roles in cardiovascular and brain health. Chia sprouts have a good omega-6:omega-3 ratio, which contributes to a better lipid quality of the diet. Chia sprouts not only demonstrated higher antioxidant activity compared to seeds, but also exhibited the ability to neutralize multiple types of free radicals and inhibit metal-mediated free radical formation, and there was significantly higher antioxidant activity in the 6-day-old sprouts. The results also suggest that the sprouts may be a potential source of rosmarinic acid. However, further studies are needed to evaluate the bioavailability and biological effects of rosmarinic acid present in chia sprouts and its derived products. Therefore, chia sprouts, especially 6-day-old, offer nutritional benefits, in terms of protein, rosmarinic acid, and essential fatty acids, which makes them a good choice for inclusion in one's diet. However, the chia seed germination process is not as simple as other seeds and the longer germination time can be more challenging.

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Data Availability Data is available upon request.

Declarations

Conflict of Interest The authors declare no conflict of interest.

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