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ASSESSMENT OF NUTRITIONAL COMPOSITION, BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF CHIA SEEDS GROWN IN BANGLADESH

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Abstract

The present study was conducted to determine the proximate, fatty acids, minerals and antioxidants content in chia seeds. The results showed that fat ranged from 28.85-29.53%, protein 18.63-18.71%, moisture 5.27-5.28 %, ash 4.45-4.47 %, total carbohydrate 25.21-27.86 %, fiber 17.35-19.12% and energy obtained 428.11-436.70 kcal/100g. Fatty acid profile indicated the presence of linolenic (56.76-57.65 %), linoleic (20.49-21.47 %), palmitic (9.38-11.34 %) and oleic (7.47-7.99 %) were abundant, while less amount of saturated fatty acids myristic (0.09-0.15 %), lauric (0.11-0.33 %) and stearic (2.52-3.13 %) were found all the tested samples. Mineral content in all the tested samples showed that high amount of calcium (578.62-589.71 mg/100g), potassium (518.95-535.42 mg/100g), phosphorous (668.62-697.61 mg/100g), magnesium (334.24-344.51 mg/100g), iron (7.13-7.56 mg/100g), zinc (4.23-4.57), manganese (2.19-2.95 mg/100g), and copper (1.59 -1.83 mg/100g) were found. The total phenolic (1.71-1.92 mg GAE/g), total flavonoids (1.07-1.16 mg QE/g), DPPH (2,2-diphenyl-1-picrylhydrazyl) (2.51-2.81 mg GAE/g), and Ferric-reducing Antioxidant Power, FRAP (1.86-2.22 mg TE/g) were found in the seeds.

Keywords: Antioxidant capacity; Bioactive compound; Chia seeds; Minerals; Fatty acid profile; Phenolic compound; Proximate composition.

Introduction

Chia (*Salvia hispanica* L.) is a tropical and subtropical climates herbaceous plant that belongs to the family *Lamiceae*, originated from Mexico and Guatemala. Chia is oilseed crop oval in shape, smooth and shiny with black, brown, gray, black-spotted or white in colour. Chia seed contains high amount of omega-3 fatty acids, high quality protein, high amount of dietary fibres, minerals, vitamins and also contains wide range of polyphenolic antioxidants which protect the chia seeds from chemical and microbial oxidation (Cahill, 2003). The chia seed also contains mucilage inside the epidermal cells of mature chia seeds. On the other hand, chia seeds play an important role as a functional food and nutritional supplement.

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However, its nutritional composition and concentration of bioactive compounds such as high level of poly unsaturated fatty acid or high amount of the essential fatty acid alpha-linolenic acid (ALA $C_{18:3}$ (n-3), which is required for maintaining certain physiological functions (Meester *et al.* 2008). Moreover, another nutrient protein of chia seeds are promising food additives which can help in improving food quality and extend the shelf-life of food products (Valdivia-Lopez and Tecante, 2015). The dietary fibers present in chia seeds and mainly in whole grains is an important bioactive component because it has potential health benefits. Chia seeds are rich in vitamins such as niacin (8.83 mg/100 g), thiamine (0.62mg/100 g), riboflavin (0.17 mg/100 g) and vitamin C (Munoz *et al*. 2012). Chia seeds are also high in concentrations of phosphorus, iron, calcium, magnesium, zinc and potassium. Chia seeds and its oil is widely used in bread such as pasta, breakfast cereals, fruit juices, yoghurt, biscuits, snacks, cake, and cereal bars in the food industry of various countries around the world. According to recent research chia seeds contain high antioxidants (phenolic compounds) and these compounds may reduce the growth of cancer cells and improve the health (Valdivia and Tecante, 2015). Chia is a new crop in Bangladesh. However, so far know no study their nutritional and chemical composition and bioactive compound of chia seeds has yet been done. This study was therefore aimed at determining the proximal chemical composition, fatty acids, nutritional quality, antioxidant capacity and functional properties of chia seeds.

Materials and Methods

This study was implemented in the Central laboratory, Bangladesh Agricultural Research Institute (BARI), Gazipur. Chia seed line-1 was collected from Agronomy Division, BARI, Gazipur and another Chia line-2 was purchased from one commercial retail shop at Gazipur city, Bangladesh in October 2022. The seeds of chia was taken and ground to powdered form by using a grinder.

Determination of proximate composition of chia seed:

The proximate composition of chia seeds were determined according to the Association of Official Analytical Chemists (AOAC, 1995) methodology: crude oil/fat (AOAC, 920.39c), crude protein (AOAC, 928.08), moisture (AOAC, 950.46), carbohydrate (AOAC, 935.08), fibre (AOAC, 921.13), and ashes (AOAC, 923.03).

Energy Value: The energy values (Kcal/100 g samples) of chia seeds were estimated using the factors for protein (4 Kcal/g), fat (9 Kcal/g) and carbohydrate (4 Kcal/g). The equation is

Food energy = (% Crude protein × 4) + (% Fat content × 9) + (% Carbohydrate × 4).

Mineral determination:

Mineral contents of chia seeds were determined by spectrophotometer and atomic absorption spectrometer. The each sample was dissolved in 100 ml 1N nitric acid, the solution mixture filtered through Whatman paper no. 42 and stored in 150 ml plastic bottles with tight lids. All minerals determination was done in triplicate. Calcium, magnesium, potassium, iron, zinc, manganese and copper were analyzed by atomic absorption spectrophotometer (AAS) with appropriate standards (Garcia, R. and P. Baez, 2012). Total phosphorus was determined by a pH adjustment method (ammonium molybdate/ ammonium vanadate mixed reagent) (Okalebo *et al.* 1993).

Chromatographic determination of fatty acid composition by gas chromatography (GC):

Fatty acid composition in chia seeds was determined by colorometric method. One gram chia seeds were crashed and were taken in a 15 ml tube. Then 5 ml of ethylate reagent (sodium hydroxide, ethanol and petroleum ether mixed) was added and vortex the sample tube 1 min. The plastic tubes were put in 10-12 h overnight at room temperature. Then 5 ml salt solution (sodium hydrogen sulphate and sodium chloride mixed) was added in each tube and shaken after 30 second. After then the ether content was evaporated and remaining oily surface was injected into gas chromatography for fatty acid profile. Chromatographic analysis of the fatty acid methyl esters (FAMEs) from the seeds extracted from the samples was conducted using a gas chromatograph (Thermo Scientific Company) apparatus equipped with an auto sampler, flame–ionization detector (FID) and supelco wax column (30 m x 0.25 μm film coating). The samples (1 μl) were injected with helium (1.2 mL/min) as a carrier gas onto the column, which was programmed for operating conditions such as column oven temperature 160°C @ with subsequent increase of 3°C/min until 180°C. The column oven temperature was increased from 180°C to 220°C @ 1°C/min and was held for 7.5 min at 220°C. Split ratio was 50% with injector 240°C and detector 250°C temperatures. The peak areas and total fatty acids composition were calculated for each sample by retention time using Shimadzu Chemical Station software. The standards of fatty acids methyl esters purchased from Sigma-Aldrich were also run under the same conditions for comparison with experimental samples. FAMEs were identified by comparing their retention times with a standard retention time supelco 37 component FAME Mix (Sigma-Aldrich, MO, USA). For calculation, the fatty acids were normalized to 100%, considering the composition (moles %) from fatty acid composition data (area %) and was expressed as percentage of total fatty acid methyl ester in the oil. Each of the experiment was repeated three times.

Determination of total DPPH-RSA (2, 2-diphenyl-1-picrylhydrazyl) content:

The total DPPH content was determined by a colorimetric method as in (Ao *et al.* 2008) with some modifications. The defatted 0.5 g of chia flour was soaked in 10 ml of 80% methanol and shaken in room temperature. The extracts were centrifuged for 15 min and supernatant were collected. The extract was mixed with methanol and DPPH (100 µmol/L in ethanol) and shaken vigorously. The mixture was incubated for 30 min at room temperature in the dark. The absorbance was read at 515 nm in a spectrophotometer (Shimadzu UV-2401PC, Japan). The total DPPH content are expressed as mg gallic acid equivalent (GAE)/g DW.

Determination of Total Phenolic content (TPC):

The total phenolic content was measured the Folin-Ciocalteau method (Velioglu, *et al.* 1998) with some modifications. The defatted 0.5 g of chia flour was soaked in 10 mL of 80% methanol and shaken in room temperature. The extracts were centrifuged for 15 min and supernatant/methanol extracts were collected. The methanol extract (50 µL) was mixed with Folin-Ciocalteu reagent, distilled water and incubated at room temperature for 5 min. Then, 7.5% of sodium carbonate solution was added to the mixture and incubated for 20 min at room temperature. Absorbance was read at 750 nm in a spectrophotometer. The total phenolic content is expressed as mg gallic acid equivalent (GAE)/g DW.

Measurement of total flavonoids content

The total flavonoid content was determined by a colorimetric method as in Barreira *et al.* (2010) with some modifications. The defatted 0.5 g of chia flour was soaked in 10 mL of 80% methanol and was shacked in room temperature. The extracts were centrifuged for 15 min and supernatant/methanol extracts were collected. The methanol extract (400 µL) was mixed with 1600 µL distilled water and 150 µL $NaNO_2$ (5%) solution and the mixture was incubated for 5 min. At the end of the reaction, 75 µL of $AlCl_3.6H_2O$ (10%) solution were added, and the mixture was allowed to stand for 5 min. Finally, 0.5 mL NaOH (1 M) was added to the reaction mixture and absorbance was read at 510 nm in a spectrophotometer. The total flavonoid content is expressed as mg catechin equivalent (CE)/g DW.

Determination of Ferric-reducing Antioxidant Power (FRAP):

In order to determine the FRAP of the extracts, the method described by Benzie and Strain (1996) was used. The FRAP reagent (Fe (III) solution-TPTZ) was obtained from the combination of 25 mL of 0.3 M acetate buffer, 2.5 ml of a solution TPTZ (tri-piridiltriazina) 10 mM and 2.5 ml of a 20 mM aqueous solution of ferric chloride. In a test tube was added 200 µL of pre-diluted sample and 1.8 mL of the FRAP reagent and kept in a water bath at 37^0C for 30 min. Afterwards, the absorbance of the colored complex formed with Fe^{2+} and TPTZ was measured

at 593 nm in a spectrophotometer. FRAP reagent was used as a blank. The compound trolox was used as standard for the calibration curve and expressed in milimol the trolox equivalents per kilogram of dry sample (mmol/kg ET dry sample).

Results and Discussions

Proximate composition of chia genotypes

The average oil/fat, protein, fiber, moisture, ash, carbohydrate and energy in Chia Line-1 and Chia Line-2 ranged from 28.85-29.53%, 18.63-18.71%, 17.35-19.12%, 5.27-5.28%, 4.45-4.47%, 25.21-27.86% and 428.11-436.70 kcal/100g, respectively (Table 1). As shown in the Table 1, proximate composition of chia seeds such as fat, fibers, ash, carbohydrate data, there is no difference between Chia Line-1 and Chia Line-2. The crude fat represents the major component in the chia seed, followed by crude protein, fiber and total carbohydrate. The moisture, ash and protein contents were close to 7.86%, 3.63% and 21.52%, respectively as reported by Sargi *et al.* (2013). Chia seed is a good source of fibers. Ullah *et al.* (2016) showed that defatted chia seed contains from 34-40% of dietary fiber. This amount is equal to 100% of the daily recommendations for adults to decrease the risk of coronary heart disease, diabetics' mellitus type 2 and several types of cancer (Amato *et al.* 2015). Carbohydrate that was obtained by a difference ranged from 25.21-25.86% and corroborates the findings as reported by Marineli *et al.* (2014) and Owaga *et al.* (2018). However, many factors that can affect its nutritional composition and concentration of bioactive compounds, such as climatic conditions, soil type, geographical area, agricultural practices and to some extent the extraction methods (Suri *et al.* 2016). Indeed, results on proximate composition support other findings that chia seeds are rich in proteins, fats and fiber particularly insoluble fiber Sargi *et al.* (2013) and Ullah *et al.* (2016).

Table 1. Proximate composition of chia seeds

Nutrients	% of seeds	
	Chia Line-1	Chia Line-2
Crude Oil/Fat	29.53± 0.93	28.85 ± 0.59
Crude Protein	18.71 ± 1.08	18.63 ± 0.71
Crude Fibre	17.35 ± 1.59	19.12 ± 1.23
Carbohydrate	25.86 ± 1.25	25.21 ± 1.02
Ash	4.47 ± 0.42	4.45 ± 0.59
Moisture	5.27 ± 0.65	5.28 ± 0.46
Energy	436.70 ± 3.99 Kcal/100g	428.11 ± 3.08 Kcal/100g

Values are means SD ± (standard deviation) of triplicate determinations (n = 3).

Fatty Acid Profile of chia seeds

Results on fatty acid composition indicated the presence of lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic (LA), and linolenic (ALA) in all the tested samples (Table 2). However, linolenic (56.76-57.65 %), linoleic (20.49-21.47 %), palmitic (9.38-11.34 %) and oleic (7.47-7.99 %) were abundant, while low content of saturated fatty acids myristic (0.09-0.15 %), lauric (0.11-0.33 %) and stearic (2.52-3.13 %) were found in Chia Line-1 and Chia Line-2. As shown in the Table 2, fatty acid composition of chia seeds, there is no difference between Chia Line-1 and Chia Line-2. The amount of ALA (ω-3, 56.76-57.65%) was similar to that reported by Ding *et al.* (2018). Furthermore, the amount of LA (ω-6) and ALA (ω-3) in chia seeds was close to 17-26% and 50-57% respectively (Fig. 1 and Fig. 2) as cited by Saphier *et al.* (2017). Chia seeds had the highest amount of ALA 57.65 % but this was lower (56.76 %) reported by Owaga *et al.* (2018). High levels of ALA implies the beneficial effects of chia seeds have on human health, for example, the anti-hyperglycemia, anti-hyperlipidemia, anti-hypercholesterolemia, anti-inflmatory and anti-cancer properties (de Ramzi and Sharada, 2014). On the other hand, the average ratios of ω-6 PUFA to ω-3 PUFA were 1:3 (Fig. 1 and Fig. 2). This observation is an important hint towards lowering the risk of coronary heart disease (CHD) as cited by Mozaffarian *et al.* (2011).

Table 2. Fatty acid composition of chia seeds

Fatty acids	Chia Line-1	Chia Line-2
Unsaturated fatty acids	**(%)**	
Lenolenic/ω-3 ($C_{18:2}$)	56.76 ± 0.48	57.65 ± 0.32
Lenoleic/ω-6 ($C_{18:2}$)	20.49 ± 0.40	21.47 ± 0.08
Oleic/ω-9 ($C_{18:1}$)	7.84 ± 0.56	7.99 ± 0.48
Palmitolic ($C_{16:1}$)	0.16 ± 0.01	0.20 ± 0.04
TSFA	14.31 ± 0.56	13.94 ± 0.49
MUFA	8.56 ± 0.57	8.29 ± 0.66
PUFA	77.25 ± 0.88	79.05 ± 0.88
ω-3/ ω-6	2.77 ±1.19	2.68 ±1.66
ω-6 /ω-3	0.36 ± 0.84	0.36 ± 0.33
Saturated fatty acids		
Lauric ($C_{12:0}$)	0.32 ± 0.11	0.33 ± 0.02
Myristic ($C_{14:0}$)	0.15 ± 0. 02	0.11 ± 0.02
Palmitic ($C_{16:0}$)	11.34 ± 0.36	10.76 ± 0.24
Stearic ($C_{18:0}$)	2.52 ± 0.11	2.74 ± 0.22

** TSFA = Total saturated fatty acid; TUFA = Total unsaturated fatty acid; PUFA = Poly unsaturated fatty acid; MUFA = Mono unsaturated fatty acid. Values are means SD ± (standard deviation) of triplicate determinations.

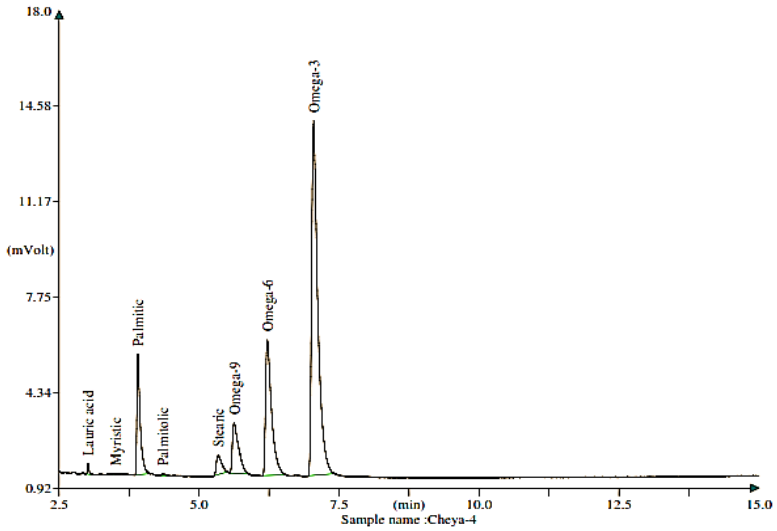

Fig. 1. Chromatogram of fatty acid composition of Chia line-1 seeds

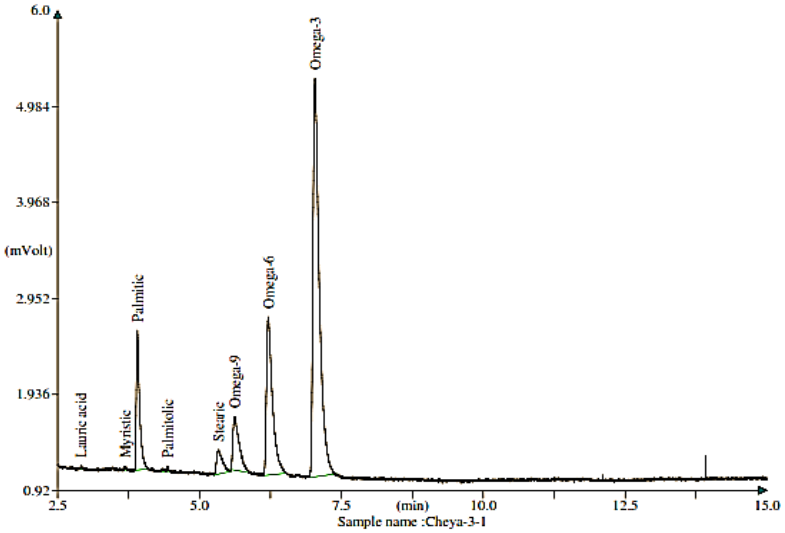

Fig. 2. Chromatogram of fatty acid composition of Chia line-2 seeds

Mineral Content of Chia seeds

Minerals found in chia seeds were high amount of calcium (578.62-589.71 mg/100g), potassium (518.95-535.42 mg/100g), phosphorous (668.62-697.61 mg/100g) and magnesium (334.24-344.51 mg/100g) in Chia Line-1 and Chia

Line-2 (Table 3). Furthermore, the amount of iron (7.13-7.56 mg/100g), zinc (4.23-4.57), manganese (2.19-2.95 mg/100g), and copper (1.59 -1.83 mg/100g) were also considerable (Table 3). The USDA, (2016) also reported that chia seed rich in calcium concentration (631 mg), phosphorus (860 mg), potassium (407 mg), magnesium (335 mg), iron (7.72 mg) and zinc (4.58 mg) per 100g chia. As shown in the Table 3, minerals composition of chia seeds, there is no difference between Chia Line-1 and Chia Line-2. The amount of phosphorus, calcium and potassium in chia seeds is 6-9 times higher than that contained in wheat, rice, oats and corn, while iron content is higher than that of spinach and lentils (Ullah *et al.* 2016). Kon *et al.* (2013) reported that mineral content of 100 g chia seed as calcium (536 mg), magnesium (350 mg), potassium (564 mg), phosphorus (751 mg), iron (6.3 mg), copper (1.4 mg), and zinc (4.4 mg). Mineral deficiency decreases enzyme activity affecting the human body and resulting in functional disorders of individual organs and the immune system thereby leading to frequent depressions. Munoz *et al.* (2012) analyzed chia seeds and contained six times more calcium, eleven times more phosphorus and four times more potassium than 100 ml of milk. Moreover, Beltran-Orozco and Romero, (2003) reported that chia contained 13 to 15 times more calcium, 6 to 9 times more phosphorus and 1.6 to 9 times more potassium than 100g of wheat, rice, oats and corn. The iron content of chia was also quite high compared to most other seeds. It contains six times more iron than spinach, 1.8 times more than lentils and 2.4 times more than liver.

Table 3. Mineral composition of chia seeds

Minerals	Amount in (mg/100g)	
	Chia Line-1	Chia Line-2
Macro-elements		
Calcium	589.71 ± 2.64	578.62 ± 2.08
Magnesium	344.51 ±2.16	334.24 ± 1.68
Phosphorus	697.61 ±2.02	668.62 ± 1.91
Potassium	535.42 ± 1.61	518.95 ± 2.15
Micro-elements		
Iron	7.56 ± 0.39	7.13 ± 0.19
Copper	1.59 ± 0.05	1.83 ± 0.13
Zinc	4.57 ± 0.51	4.23 ± 0.23
Manganese	2.19 ± 0.33	2.95 ± 0.71

Values are means SD ± (standard deviation) of triplicate determinations.

Phenolic and flavonoid content

Total phenolic and flavonoid contents of the defatted ground chia seeds are given in Table 4. The total phenolic and flavonoid contents of Chia Line-1and Chia Line-2 seeds were found to be 1.92 ± 0.11, 1.71 ± 0.15 mg GAE/g mg GAE/g and 1.07

± 0.13, 1.16 ± 0.12 mg GAE/g, respectively. Phenolic and flavonoid content of chia seeds, there is no difference between Chia Line-1 and Chia Line-2. It was reported that the total phenolic content of Chilean chia seeds to be 0.94 ± 0.06 mg GAE/g sample (Marineli *et al.* 2014), and Mexican chia seeds to be 1.64 ± 0.08 mg GAE/g sample (Martinez-Cruz and Paredes-Lopez, 2014). The differences in total phenolic content between this study and the others could be due to two factors; i) the samples used in our study and in the others are harvested from different locations, and it has been proven that growing locations significantly impact the composition of chia seeds (Ayerza, 2013); ii) the methods used for the extraction of phenolic compounds in different studies vary, and different extraction methods have been shown to dramatically influence the total phenolic contents of chia seeds measured (Scapin *et al.* 2016). Beside, Lin and Tang (2007) reported that the content of total flavonoids was obtained 0.75 mg EQ/g for green peppers, 0.41 mg EQ/g for yellow pepper.

Table 4. Extractable phenolic, flavonoids and antioxidant capacity of chia seeds

Elements	Amount (mg g^{-1})	
	Chia Line-1	Chia Line-2
Phenolic compounds		
Total phenolic (mg GAE/g)	1.92 ± 0.11	1.71 ± 0.15
Total flavonoids (mg EQ/g)	1.07 ± 0.13	1.16 ± 0.12
Antioxidant capacity		
DPPH (mg GAE/g)	2.81 ± 0.20	2.51 ± 0.46
FRAP (mg TE/g)	2.22 ± 0.39	1.86 ± 0.16

Values are means SD ± (standard deviation) of triplicate determinations.

Antioxidant activity

Total antioxidant activities of chia seeds were determined by testing the DPPH free radical scavenging activity of the defatted extracts. DPPH radical scavenging activities and antioxidant power of iron reduction, FRAP of chia seed were found 2.81 ± 0.20, 2.51 ± 0.462.51 ± 0.46 mg GAE/g and 2.22 ± 0.39, 1.86 ± 0.16 mg TE/g, respectively (Table 4). The results are lower than those reported for defatted Chilean seed flour (4.26±9.7 mg TE/g) (Marineli *et al.* 2014), Argentinian chia fibrous fraction (4.46±19.8 mg TE/g) (Capitani *et al.* 2012), and Mexican chia fibrous fraction (4.88 TE/g) (Vazquez-Ovando *et al.* 2009). The study showed that chia seed has higher antioxidant activity than other highly edible cereals such as sorghum, millet, barley, rye, and wheat, which have 1.95 ± 8.82, 2.38 ± 0.67, 2.10 ± 0.83, 1.21 ± 0.50, and 4.33 ± 0.17 mgTE/g of sample, respectively (Ragaee *et al.* 2006). Jeong *et al.* (2004) reported that increased chia seed consumption the antioxidants influence oxidation of free radicals there by providing better health and preservation of food lipid systems in the body.

Conclusion

Chia is now a high value crop for its unique nutritional and potential medicinal properties. Chia seeds have high contents of dietary fibre and proteins, high contents of polyunsaturated fatty acids, mainly alfa-linolenic acid ($C_{18:2}$), belonging to the group of omega-3 fatty acids. These seeds are also a good source of many minerals and vitamins, as well as bioactive compounds of high antioxidant activity. The research confirmed the cardioprotective, antihypertensive, antidiabetic, antiatherosclerotic, nephroprotective, anti-inflammatory, as well as antioxidant properties and its valuable chemical composition and biological activity with their availability. The results from this study support other findings that Chia Line-1 is the best-known plant source with the highest content of quality fats, fibers, proteins, alfa-linolenic acid, ω-3 and ω-6, antioxidant capacity and functional properties of chia. Finally, chia seeds are the best source of nutrients and are suggested as super foods or novel foods (EU food safety authority) and can be widely used in the food industry.

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