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Evaluation of Bioactive Components and Antioxidant Properties of Two Chia Seeds (*Salvia Hispanica L.*)

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Abstract

The aim of this investigation was to evaluate black chia seed (*Salvia hispanica L.*) as a source of bioactive components comparing with white chia seeds. Also, antioxidant activities, identification of phenolic and flavonoids compounds, fatty acids composition and amino acids of chia seeds content were evaluated. The results showed slight differences in proximate composition, raw black and white chia seeds to have high nutritional content in the form of protein (25.29 – 24.30%), fibre (32.94-31.75%), fat (32.35-30.54%) and ash content (6.05-6.93%), respectively. Concerning to germinated chia seeds, data showed that protein content of white chia seed increased significantly 26.94% compared with raw white chia seeds 24.30%. While, fat and fiber contents were found to be decreased significantly as a result of germination process. Fermented chia seeds showed higher protein content 28.10 and 27.55% for black and white chia seeds, relative to raw chia seeds. Total phenol content was increased a significantly in germinated black seeds (263.51±28.44 mg GAE/100g) relative to raw seeds (166.76±4.13mg GAE/100g). While, fermented seeds showed significant increment in total phenols content for both chia seeds the values were 216.25± 4.75and 200.74±3.64 mg GAE/100g when compared with raw sample. The antioxidant activity as determined by DPPH and ABTS methods was relatively higher in raw black chia seeds than raw white chia seeds. Besides, chia seeds oil was found a high content of linolenic acid (ω 3) and (ω 6)content with a value of 59.25- 58.54%and 20.50-20.17% in black and white chia seeds oil, respectively. Both chia seeds had nearly similar essential amino acids.

Keyword: Chia seeds, germination, fermentation, antioxidant activity, Fatty acids.

Introduction

In recent years, with increased health awareness globally as a result of an increase in the number of non-communicable diseases, the demand for a healthier lifestyle through the consumption of foods that prevent and control these non-communicable diseases has increased **(Berner *et al*., 2014; Chadare *et al*., 2019)**. While, all foods are functional with some properties, functional foods have become one of the fastest growing sectors of the food industry with the growing awareness of people about deadly diseases such as cancer, diabetes and heart disease **(Hasler and Brown. 2009)**.

Chia (***Salvia hispanica* L., family: *Lamiaceae*)**was an herbaceous plant used for commercial and medical purposes. Originally, it was grown in tropical and subtropical climates but at present, it is grown all over the world as important food supplements. Nowadays, dry chia seeds, as important edible seeds, are added to many food-stuffs like fruit juices, smoothies, milk, yogurt, salads, soups, and baked products due to their nutritional properties **(Dick *et al*.2015; Kulczynski *et al*., 2019)**. The dry chia seeds contain high contents of essential fatty acids, carbohydrates, minerals, proteins, dietary fibers, and bioactive phenolic compounds **(Oliveira-Alves *et al*., 2017)**.Chia seeds are generally very small, oval-shaped, 2 mm long, 1 to 1.5 mm wide, and less than 1 mm thick **(Mohd Ali *et al*., 2012; Das 2018; Grancieri *et al*.,2019**). The color of the seed varies from black, grey, or black spotted to white.Moreover, the plant itself can produce 500 to 600 kg seed/acre under appropriate agronomic conditions **(Ullah *et al*., 2016)**.

Some recent studies reported that the dietary intake of dry chia seeds could protect against obesity-related diseases, oxidative stress, and cardiovascular diseases **(Poudyal *et al*., 2012;Marineli *et al*, 2015)**.

Through a simple and inexpensive germination process several edible seeds could be grown in a short time with improved nutritional values and functional properties. During germination, the main macromolecules such as carbohydrates, proteins and fatty acids are degraded into simple/free forms and reduced the anti-nutritional and indigestible factors **(Shi *et al*., 2010; Aguilera*et al*., 2013)**. Furthermore, the germination process increased the bioactive-phenolic compounds and many bioactivities in some edible seeds **(Gan *et al*., 2016;Abdel-Aty *et al*., 2019).**

Knez-Hrnčič *et al*. (2018) reported, that a marginal difference between black and white chia seeds that most consider them equal. Nutritional values are similar protein content in black chia seeds was 26.9% and fiber content was 32.6%. In white chia seeds, the protein content was found to be 26.5% and the fiber content was 32.4%. A slight difference was found only in morphology white seeds were larger, thicker, and broader compared to black seeds.

Several studies have been performed on the usage of chia seeds in the food industry can be used in different shapes: whole, ground, in the form of flour, oil, and gel. Also, can be added or mixed into biscuits, pasta, cereals, snacks, and cakes as supplements**(Das 2018)**. Chia gel may be used as substitutes for oil or eggs in baked products. It was shown that chia oil can replace 25% of the egg in cakes **(Kulczyński *et al*., 2019)**. The nutritional value of butter can be increased by mixing it with chia oil in a proportion from 6.5% to 25%, when the concentration of ω-3 fatty acid in chia fortified butter increases from 4.17% to 16.74% (**Ullah *et al*., 2016)**.The aim of this work is to evaluate the chemical composition, phenolic and flavonoidscomponents, fatty acids and amino acids contents of two chia seeds genotypes (black and white).

Material and Methods

Chia seeds (*Salvia hispanica*) used in this study were purchased from the local market.All the utilized chemical materials (solvents, mineral salts, *etc.*…) were purchased from El Gomhoryia, El Allamyia and El Nasr Chemical companies, Egypt and the solvents were purified before using. Chemicals, solvents and all standard materials which were used for fractionation and identification by HPLC, purchased from Sigma/Aldrich Chemical Company, USA.2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS)and Folin-Ciocalteu reagent were obtained from LOBA chemie, from Win. Lab. Laboratory chemicals reagents. All other chemicals used were of analytical reagent grade.

Preparation of raw chia seedsmaterials

Raw chia seeds weremilledin a Laboratory mill (IKA-Laboratechnic, Janke and Kunkel Type: MFC, Germany) to obtain a whole meal flour and kept at -20ºC until analysis.

Germination of chia seeds

Germination conditions were established on the basis of preliminary assaysaccording to the method described by**(Beltrán-Orozco *et al*,2020)**.

Fermentation of chia seeds

Fermentation condition were established on the basis of preliminary assays according to the method described by**(Tamime et al. 1997).**

Proximal composition

Using de-fatted chia flour, crude protein was calculated by the micro/Kjeldahel method, and moisture, ash and fiber contents were determined by using standard methods **(AOAC, 2000)**. The Soxhlet extraction method was used to measure total fat content. Carbohydrate content was calculated by differenceas follows equation: -

Total carbohydrate =100 - (Moisture + Ash + Crude protein + Crude fat) according to **A.O.A.C (2016)**.

Determination of minerals

Perkin Elmer (Model 3300, USA) Atomic Absorption Spectrophotometer was used to determine these minerals. Such as zinc, iron, calcium, manganese, cupper, potassium, phosphors, sodium and magnesium contents by using methods according to **A.O.A.C (2016).**

Determination of total phenolic and total flavonoids contents

Total phenolics were determined as described by **Singleton and Rossi (1965)**. Gallic acid was chosen as a standard to prepare the standard curve. Total phenolics was expressed as mg gallic acid equivelant/100g sample dry weight basis. While, total flavonoids were determined according to the method of **Zhishen *et al.* (1999).** Catechin served as standard compound was used for preparing the calibration curve. Total flavonoids were calculated as mg catechin equivalent (CAE)/100g on dry weight basis.

Identification of phenolic acids and flavonoids compounds by HPLC

The phenolic acids and flavonoids compounds of the samples under investigation were determined according to the method described by **Hakkinen *et al.* (1998) and Mattila *et al*. (2000).**

Determination of antioxidant activity

The antioxidant activity of chia seeds methanol extracts was determined based on the radical scavenging ability in reacting with a stable DPPH free radical according to **Brand-Williams *et al.* (1995)**. While, theABTS assay was carried out according to **Re *et al.* (1999)**.

Fatty acid compositions and Identification of the amino acid content

The fatty acid compositions of chia seeds oil were analyzed by Agilent HP 6890 capillary gas chromatography according to the method described by **Glass (1971)**. While, amino acids content of the sample were determined using amino acid analyzer (Biochrom 30) according to **Duranti and Cerletti, (1979).**Amino acids were expressed as g /100g protein on dry weight basis.

Results and Discussion

The chemical compositions ofraw, germinated and fermented chia seeds genotypes (black and white) are measured the obtained results are tabulated in Table (1). From these data showed that raw black seeds had a higher level of protein 25.29% and fat 32.35 compared with raw white chia seeds 24.30 and 30.54%,respectively. In addition, crude fiber and ash contents were also high, they showed nearly the same levels for both chia seeds genotypes (32.94 and 6.05%) for black chia seeds and (31.75 and 6.93%) for raw white chia seeds. The predominant carbohydrate portion (30-34%) of chia seeds is dietary fibre, which cover 100% of the adult population's daily requirements (**Motyka *et al*., 2021)**

Concerning to germinated chia seeds, data showed that protein content of white chia seed increased significantly 26.94% compared with raw white chia seeds 24.30%. While, fat and fiber contents were found to be decreased significantly as a result of germination process.The reason for the decreased fat content is due to its consumption in the germination process to obtain energy. Slight changes occurred in ash content but fiber content decreased in both chia seed genotypes. At the same time total carbohydrates increased significantly by in germinated seeds to12.70 and 17.04% for germinated black and white chia seeds, respectively.

On the other hand, fermented chia seeds showed higher protein content 28.10 and 27.55% for black and white chia seeds, relative to raw chia seeds. Same trend was observed in fiber content. It recorded 32.67 and 33.55% for fermented black and white chia seeds. From the above-mentioned, the data showed that total carbohydrate slightly decreased compared with germinated seeds. In general germination and fermentation treatments improved the nutritional value of the chia seeds.

These results are in agreement with **Suri *et al.* (2016);Ullah *et* a*l*., (2016);Knez-Hrnčič*et* a*l*., (2018)and Otondi *et al*., (2020)**they found that Chia seeds had a moisture 6.35%, protein 19.96%, carbohydrate 32.53%, fat 36.61% and ash 4.98%.,reported that protein content ranged 15–25% form chia seeds, our value is between this limit. Content of fat of chia seed was of 32.35 and 30.54 for black and white chia seeds.The difference of protein content and oil content could be connected to the difference in climatic conditions, agronomic practices, fertilization regimes, irrigation practices, etc. **(Ayerza, 2011)**.

Table 1. Chemical composition of chia seeds components (%) g/100g dry weight

Samples	%Moisture	%Protein	%Fat	%Fiber	%Ash	%T.C
BR	7.64±0.21[a]	25.29±0.42[c]	32.35±0.66[a]	32.94±0.22[ab]	6.05±0.11[b]	3.12±1.09[c]
WR	6.95±0.30[a]	24.30±0.23[d]	30.54±0.69[a]	31.75±1.01[b]	6.93±0.34[a]	6.03±1.12[c]
BG	6.32±0.37[b]	27.38±0.23[ab]	23.04±1.28[c]	29.61±0.32[c]	6.42±0.24[ab]	12.70±1.98[b]
WG	4.18±0.05[c]	26.94±0.23[b]	19.44±0.45[d]	28.56±0.27[c]	7.28±0.42[a]	17.04±0.05[a]
BF	3.88±0.61[c]	28.10±0.31[a]	25.88±0.47[b]	32.67±0.11[ab]	6.00±0.02[b]	10.98±0.03[b]
WF	3.54±0.02[c]	27.55±0.20[ab]	24.05±0.86[bc]	33.55±0.36[a]	6.74±0.57[ab]	11.32±1.49[b]
L.S.D at5%	0.80	0.83	1.92	1.17	0.83	2.92

TC= total carbohydrates, BR= raw black seeds, WR= raw white seeds, BG= germinated black seeds, WG= germinated white seeds, BF= fermented black seeds and WF= fermented white seeds.

The minerals content of chia seeds (raw, germinated and fermented) are measured and the results are presented in Table (4). The obtained results showed that chia seeds contained appreciable quantities of minerals. They considered a good source of minerals containing calcium, potassium, phosphorus, magnesium, zinc, iron and selenium in relatively higher amounts while, manganese, sodium and cupper in smaller quantities. Potassium was found to be abundant mineral in the both raw chia seeds genotypes (black and white) 1667.5 and 1537.5mg/100g, respectively. While, calcium was found to be the second element in chia seeds, white chia seeds had the highest amountwas 879.0mg/100g compared with 609.7 mg/100g for raw black chia seeds. Magnesium element content was 350.85 and 353.1mg/100g in raw black and white chia seeds respectively. Data revealed that both black and white chia seeds had a higher iron level 11.2 and 10.55mg/100g for raw black and white seeds. Also, chia seeds had high zinc level 7.9 and 6.45 mg/100g in black and white seeds. Data revealed that there were increment in calcium, zinc, iron and cupper for germinated and fermented samples.

Ding *et al*., (2018) reported that the main minerals in the chia seed are magnesium, calcium and potassium as well as iron, zinc, manganese and selenium. **da Silva *et al*., (2017)**reported the chia seeds was a highlight due to its concentration of iron, zinc, calcium, manganese, potassium and phosphorus are noted wherein the minerals, calcium (631 mg.100 g^{-1}), potassium (407 mg.100 g^{-1}), magnesium (335 mg.100 g^{-1}), iron (7.72 mg.100 g^{-1}) and zinc (4.58 mg.100 g^{-1}). These results are in agreement with those reported by **Ullah *et a*l., (2016).Kulczyński et al., (2019)**they mentioned that chia seeds supply many minerals, with phosphorus of (860–919 mg/100 g), calcium (456–631 mg/100 g), potassium (407–726 mg/100g) and magnesium (335–449 mg/100 g) found in greatest amounts.

Table 2. Content of individual macroelements and microelements (mg/100 g dry seeds) in the chia seeds (*S. hispanica*)

	Minerals content mg/100g dry seeds									
Samples	**P**	**Ca**	**K**	**Mg**	**Na**	**Fe**	**Mn**	**Zn**	**Cu**	**Se**
BR	160.25	609.7	1667.5	350.85	26.15	11.2	5.05	7.9	0.7	21.65
WR	192.2	879.8	1537.5	353.1	36.7	10.55	7.15	6.45	1.3	20.6
BG	168.65	722.4	2402.5	353.2	91.85	11.1	5.4	9	1.25	20.55
WG	190.2	842.85	1040	356.6	101.4	10.5	6.65	6.75	1.5	18.6
BF	167.25	744.2	817.5	348	520	12.8	4.8	8.55	2.3	15.75
WF	186.35	888.9	1215	355.65	543.55	12.25	6.45	7.05	2.35	14.05

BR= raw black seeds, WR= raw white seeds, BG= germinated black seeds, WG= germinated white seeds, BF= fermented black seeds and WF= fermented white seeds.

Total phenols, total flavonoids and their antioxidant activities: -

Total phenols, flavonoids content and antioxidant activitywere measuredin raw, germinated and fermented chia seeds and the obtained data are presented in Table (3). Results cleared that total phenols of raw black and white chia seeds were found to be166.76±4.13and 182.31±13.35mg GAE/100g,respectively. Total phenol content was increased a significantly in germinated black seeds (263.51±28.44 mg GAE/100g) relative to raw seeds. While, fermented seeds showed significant increment in total phenols content for both chia seeds the values were 216.25±4.75and 200.74±3.64 mg GAE/100g when compared with raw sample.

In the same Table (3) data showed that total flavonoids in raw black and white chia seeds was 66.85±5.35 and 56.96±5.93mgCE/100g, respectively. On the other hand, germinated chia seeds, total flavonoids wereincreased in both genotypes of chia seeds and the values were 70.29±5 and 62.67±6.78mg CE/100g whencompared with raw seeds. Germination probably induced the synthesis of phenolic compounds and also caused the release of these compounds from the food matrix of chia seeds. While, fermented seeds showed decreased significantly in total flavonoids content for black and white chia seeds the amount were 56.56±5.11and 54.44±2.06 mg CE/100g were compared with raw sample.

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, respectively, whereas **Martínez-Cruz and Paredes-López (2014)** found an average of 164 mg GAE/100 g in seeds from Colima. 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℃.Also, **Beltrán-Orozco *et al*. (2020)** reported the amount of phenolic compounds was 97.7 mg GAE/100 g in ungerminated seeds, but this value increased 3-fold after 4 days of germination.

The antioxidant activities of chia seeds were investigated using the DPPH and ABTS assays and the obtained values were found to be 86.23and 77.58 % for raw black chia seeds, respectively. While, raw white chia seeds recorded 85.69 and 76.94 %, respectively. There were non-significant changes between the two chia seeds genotypes. The DPPH values of black germinated and fermented seeds showed non-significant changes relative to raw samples. Same trend was observed in ABTS values for black seeds. While, germinated and fermented whiteseeds showed a significant decrement in DPPH and ABTS, the values were 59.84 and 52.13%, respectively.

The same results were obtained by other authors such as **Alacantara *et al*. (2019)** investigated antioxidant activity by the DPPH method it was 10.10 to 380.53 µmol TE/g extract. **Grancieri *et al*. (2019)** stated in their research that to investigate the specific antioxidant activity and **Beltrán-Orozco *et al*. (2020)**they found that the initial antioxidant activities of chia seeds were 77.7and 41.1 mmol ET/g (dw) determined by ABTS and DPPH methods, respectively. These values increased by 105.1%, 101.6%, and 87.7%, respectively, after 4 days of germination. Also, **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 at21 °C. 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. 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. They also found germination has also proved to increase the polyphenol content and the antioxidant capacity of different grains **(Álvarez-Jubete *et al*., 2010) and (Kim *et al*., 2012).**

Table 3. Total phenols, total flavonoids, DPPH and ABTS in raw, germinated and fermented chia seeds.

Samples	Total Phenols mg GAE/100g	Total Flavonoids mg CE/100g	DPPH %	ABTS %
BR	166.76±4.13[d]	66.85±5.35[a]	87.17±1.24[a]	77.58± 0[a]
WR	182.31±13.35[cd]	56.96±5.93[b]	81.48±3.04 [a]	76.94±0.40[a]
BG	263.53±28.44[a]	70.29±5.00[a]	88.81±0.24 [a]	77.76±0.98[a]
WG	191.03±10.86[bcd]	62.67±6.78[ab]	66.82±9.00[b]	59.80±5.06[b]
BF	216.25±4.75[b]	56.56±5.11[b]	60.67±1.53 [b]	74.14±4.20[a]
WF	200.74±3.64[bc]	54.44±2.06[b]	64.07±0.78 [b]	52.13±1.67[c]
L.S.D at 5%	24.71	9.33	7.06	4.99

BR= raw black seeds, WR= raw white seeds, BG= germinated black seeds, WG= germinated white seeds, BF= fermented black seeds and WF= fermented white seeds.

Identification of phenolic acids and flavonoids compounds in chia seeds by HPLC: -

Phenolic compounds in chia seeds extracted was identified by HPLC and the obtained results are presented in Table (4). From these data the raw black chia seeds phenolic extract was contained eleven phenolic compounds with concentrations ranging from 0.07 to 6.32mg/100g. Ellagic acid concentration was 6.32mg/100g followed by catechin (5.36 mg/100g), chlorogenic (2.75mg/100g), caffeine (2.13mg/100g) and caffeic acid (1.52mg/100g), respectively. In the raw white phenolic extract, eight phenolic compounds were identified with concentrations ranging from 0.08 to 9.17mg/100g. the main phenolic compounds in both raw chia seeds were catechin and ellagic. New phenolic compound (ferulic) was recognized in black germinated seeds extract, while there new phenolic (chlorogenic, caffeine and ferulic) were recognized in germinated white seeds extract. Ellagic acid concentrations increased in germinated black and white seeds (20.57 and 12.46mg/100g) compared with raw seeds (6.32 and 5.33mg/100g), respectively. Ellagic acid concentrations increased in both fermented chia genotypes extracts. Black chia extract had higher concentration (32.4mg/100g) than that in fermented white chia seeds extract. In fermented black chia seeds, most of phenolic compounds increased pyrogallol, catechin, 4-amino benzoic, caffeic, vanillic, coumaric acid. Same trend was observed in fermented white chia seeds extract. In addition, coumarin considered new phenolic compound recognized in fermented white chia extract.

Table 4. HPLC of phenolic compounds in chia seeds (mg/100 g dry seeds)

Phenolic acids(mg/100g)	BR	WR	BG	WG	BF	WF
Gallic	0.15	0.23	0.32	0.21	2.75	0.22
pyrogallol	2.45	2.58	1.27	1.33	6.94	2.87
4-Amino-benzoic	0.07	0.08	0.29	0.17	0.6	0.24
Catechein	5.36	9.17	nd	1.88	11.43	2.82
Chlorogenic	2.75	nd	1.58	1.53	4.62	2.93
P-OH- benzoic	1.01	1.28	0.82	0.86	0.77	1.15
Caffeic	1.52	0.89	0.42	0.51	5.47	0.8
Vanillic	0.23	1.33	0.21	0.89	2.29	2.53
Caffeine	2.13	nd	1.46	1.03	2.62	1.17
Ferulic	nd	nd	21.44	10.93	nd	9.02
Ellagic	6.32	5.33	20.57	12.46	32.4	9.56
Coumarin	0.16	nd	1.07	0.6	0.85	0.46

BR= raw black seeds, WR= raw white seeds, BG= germinated black seeds, WG= germinated white seeds, BF= fermented black seeds and WF= fermented white seeds.

These results are slightly difference with those obtained by **Coelho and Salas-Mellado (2014)** mentioned, Chlorogenic acid (4.68 µg/g), Protocatechuic acid ethyl ester (0.74 µg/g), Ferulic acid trace and Quercetin (0.17 µg/g). While,**Abdel-Aty *et al.*, (2019)**mentionedthatPyrogallol, gallic acid, and coumaric acid were also raised 65, 62, and 36 times, respectively, while apigenin was only found in fermented garden cress seeds extract over unfermented extract**.** But,**Ritthibut *et al.*, (2021)** reported that ferulic and coumaric acids increased 63 and 20 times in fermented rice bran, respectively, compared to unfermented rice bran. Also, caffeic acid was found only in fermented rice bran and not in unfermented rice bran. Also, **Motyka *et al*., (2022)** reported rosmarinic acid is the dominant phenolic acid with the highest concentration (0.927 mg/g), followed by protocatechuic acid (0.747 mg/g), caffeic acid (0.027 mg/g), and gallic acid (0.012 mg/g).

On the other hand,the flavonoids content (mg/100g) in raw, germinated and fermented black and white chia seeds are estimated and presented in Table (5). From these results flavonoids showed differences between chia seeds genotypes with different compounds (i.e., Rutin, Naringin, Quercitrin, Apigenin-7-glucose,Quercetin, Naringenin, Kaemp-3-(2-p-comaroyl) glucose and Kaempferol).Naringin was the major compound detected, with values of 10.76 and 17.95mg/100g in raw black and white chia seeds, respectively, followed by Rosmarinic acid (1.98 and 4.13mg/100g) and Rutin 1.28 and 2.1mg/100g, respectively. Apigenin was found in raw black chia seeds while in raw white seeds was not recognized. Flavonoids content increased in germinated chia seeds genotypes extract Rutin compound which disappeared in white chia seeds. Highly increment was observed in Naringin content (12.41mg/100g) for fermented black chia seeds extract compared with raw seeds (10.76mg/100g) while that compound disappeared in fermented white chia seeds extract. In addition, quercitrin increased in both fermented chia seeds genotypes (black and white). In white fermented chia seeds, Quercetin increased (1.96mg/100g) compared with raw seeds (0.8mg/100g). Also, Kaempferol increased in fermented black and white chia (1.87 and 1.57mg/100g), respectively. New flavonoids compound Apigenin was rerestarted in germinated and fermented white chia 0.45 and 0.44mg/100g, respectively.

Table 5. HPLC of total flavonoids compounds in chia seeds (mg/100 g dry seeds)

Flavonoids components	BR	WR	BG	WG	BF	WF
Rutin	1.28	2.10	19.53	nd	nd	6.49
Naringin	10.76	17.95	nd	6.35	12.41	nd
Rosmarinic	1.98	4.13	22.44	12.91	31.48	9.51
Quercetrin	0.23	0.20	0.2	1.67	4.92	0.91
Apigenin-7-glucose	0.09	0.15	0.15	1	nd	nd
Quercetin	0.80	0.73	0.73	0.85	nd	1.96
Naringenin	0.09	0.07	0.07	0.24	0.41	0.85
Kaemp.3-(2-p-comaroyl) glucose	0.80	0.86	0.86	2.75	12.23	9.97
Kampferol	0.20	0.14	0.14	0.48	1.87	1.57
Apigenin	0.13	nd	nd	0.45	0.33	0.44

BR= raw black seeds, WR= raw white seeds, BG= germinated black seeds, WG= germinated white seeds, BF= fermented black seeds and WF= fermented white seeds.

These values were lower than those reported by **Martínez-Cruz and Paredes-López (2014) and Rahman *et al.* (2017)** for non-defatted and defatted chia seeds. **Jin *et al*. (2012)** found Kaempferol 0.013 µg/g), Kaempferol 3-O-glucoside 0.029 µg/g), Epicatechin 0.029 µg/g), Rutin 0.22 µg/g), *p*-Coumaric acid 0.24 µg/g) and Apigenin 0.005 µg/g). **Pellegrini *et al*., (2018)** mentioned seven polyphenolic compounds were detected in both chia and defatted chia seed samples. Rosmarinic acid was the major compound detected and quantified, with values of 653.98 and 669.88 µg/g in non-defatted and defatted chia seeds, respectively, followed by quercetin.

Fatty acids content of chia seeds oil: -

Fatty acid content for black and white chia seeds are determined and the results are shown in Table (6). Data cleared that chia seeds have been considered a good source of healthy poly unsaturated fatty acids (PUFA). Linoleic acid C18:2 (ω6) content in black chia seeds was similar to white chia seeds (20.50 and 20.17%), respectively. Also, linolenic acid C18:3 (ω3) contentin black and white seeds was 59.25 and 58.54% of total fatty acids, respectively. Palmitic acid for black and white chia seeds was 7.5 and 7.77%. Stearic acid C18:0 in white chia seeds was higher than that in black chia seeds (4.05 and 2.83%), respectively. While, oleic acid content in white seed was higher than black seeds (7.98 and 6.71%), respectively.In addition, the n6: n3 ratio was 0.35. **Ixtaina *et al*., (2010)** reported that n-3: n-6 ratio was higher than 3.5 might reduce cholesterol levels and improve the plasma lipid. These results are in agreement with theirobtained (**Di Marco *et al*., 2020; Enes *et al*., 2020 and Ghafoor *et al*., 2020)**they reported that chia seeds oil has been contain an excessive number of fatty acids, especially polyunsaturated fatty acids (PUFA), which consist of more than 60% α-linoleic acid and more than 20% linoleic acid.

While, **Carrillo *et al.* (2018)** reported the composition of omega 3 as 54.08%, the content of omega 6 as 18.69%, and omega 9 as 10.24%. (**Das,2018; Knez-Hrnčič and IvanovskI,2019 and Kuznetcova *et al*., 2020)**they found that theoil obtained from the seeds of S. hispanica accounts for 30–33% of fatty acids. Also, the ratio of omega-6 to omega-3 fatty acids in chia seeds is very favorable (around 0.30 –0.35)**.** Monounsaturated fatty acids, which belong to the group of omega-9 fatty acids, constitute about 10% of the fatty acid pool in chia seeds, with oleic acid being dominant. Saturated fatty acids constitute the remaining 10% of the fatty acid pool, and the dominant ones are palmitic and stearic acid **(Coelho and Salas-Mellado, 2014)**. **Rajaram (2014)** reported that α-linolenic acid, which is derived from plant origin, has the potential to reduce cardiovascular disease, fracture, and diabetes Type 2.

Table 6. Comparison of fatty acid contents profile of two chia seeds genotypes

Fatty acids (% of total fat content)	Black chia seeds	White chia seeds
palmitic acid C16:0	7.5	7.77
stearic acid C18:0	2.83	4.05
oleic acid C18:1	6.71	7.98
linoleic acid C18:2 n-6	20.5	20.17
linolenic acid C18:3 n-3	59.25	58.54
Saturated fatty acids (SFA)	10.33	11.82
polyunsaturated fatty acids (PUFA)	86.46	86.69
Fatty acids ratios		
n-6: n-3	0.35	0.35

Amino acids content of chia seeds: -

The amino acidscontent of black and white chia seedswere determined by using amino acid analyzer and the obtained results are presented in Table (7). From these data, the chia seeds contain 18 out of 22 amino acids, of which 10 are essential which contribute significantly to human health. These amino acids can be arranged according to their concentration in the following decreasing order, arginine, leucine, phenylalanine, valine, lysine, methionine, threonine, isoleucine, histidine and tryptophan, respectively. Among non-essential amino acids, glutamic acid found at high concentration followed by aspartic acid, alanine, glycine, serine, proline, tyrosine and cystine. Generally, glutamic acid considered an important amino acid in the diet. It is able to modulate imm-regulatory response and enhances athletic performance. Also, arginine plays role in preventing heart diseases **(Timilsena *et al*., 2017)**.

These results are increment with these reported by **(Olivos-Lugo *et al*., 2010)** found to originate from essential amino acids such as leucine, isoleucine and valine, and high amounts of glutamic acid (123 g/kg), arginine (80.6 g/kg) and aspartic acid (61.3 g/kg).While,**Ullah*et al*., (2016) and Ding *et al*., (2018)**they found that the content of amino acids was serine is (1.05 g/100 g), glutamic acid (3.50 g/100 g), glycine (0.95 g/100 g), alanine (1.05 g/100 g), lysine (0.97 g/100) g, and histidine (0.53 g/100 g).According to **USDA (2018)** the amino acids found in *S. hispanica* seeds, Glu is dominant (3.50 g/100 g dry seeds). Arg and Asp are also found in a high amount (2.14 and 1.69 g/100 g dry seeds, respectively), while others constitute less than 1 g/100 g dry seeds.

Table 7. Amino acids composition of proteins from black and white chia seedsgenotypes(g/100g)

Amino acids (g/100g)	Black chia seeds	White chia seeds
Essential amino acids		
Arginine	6.97	7.6
Leucine	4. 6	5.36
Lysine	3.37	4.08
Phenylalanine	3.6	3.8
Valine	3.44	3.52
Methionine	2.47	2.9
Threonine	2.44	2.77
Isoleucine	2.38	2.7

Histidine	1.93	2.27
Tryptophan	0.95	1.15
Total	27.55	36.15
Non-essential amino acids		
Glutamic	12.73	14.38
Aspartic	5.59	6.68
Alanine	4.31	4.26
Glycine	3.6	4.23
Serine	3.47	3.66
Proline	2.7	2.59
Tyrosine	2.51	2.84
Cystine	1.96	2.91
Total	36.87	41.55

Conclusions

Chia, *Salvia hispanica L.*, is a plant species used since ancient times for dietary and medical purposes. From the abovementionedresults it can be concluded that the chia seeds contain a high protein, high fat content, dietary fiber, carbohydrates, mineralsand antioxidants. Chiaseeds contain the flavonoids, rosmarinic acid, quercetin, chlorogenic acid, and caffeic acid, which are proven to have anti-cancerogenic, anti-hypertensive, and neuron protective effects. Furthermore, chia seeds are a rich source of nutrients such as polyunsaturated ω-3 and ω-6 fatty acids that protect from inflammation, and antioxidant compounds that reduce the risk of chronic diseases. Moreover, the high amount of fiber decreases the risk of coronary heart disease, the risk for diabetes type 2, and several types of cancer. Finally, chia seeds are already used in the food and pharmaceutical industry. Also, chia seeds contain 18 out of 22 amino acids, of which contribute significantly to human health.

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تقييم المكونات الحيوية النشطة والخواص المضادة للأكسدة لنوعين من بذور الشيا (*Salvia hispanica L.*)

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الهدف من هذا البحث هو تقييم بذور الشيا السوداء (*Salvia hispanica L.*)كمصدر للمكونات الحيوية النشطة مقارنة مع بذور الشيا البيضاء. وأيضا تقدير المكونات الكيميائية الحيوية وكذلك محتواها من المعادن ومحتواها من المواد المضادة للأكسدة، الفينولات والفلافونيدات وتفريدهاوالتعرف عليها بإستخدام جهاز التحليل الكروماتوجرافي العالي (HPLC) والأحماض الدهنية المشبعة وغير المشبعة والأحماض الأمينية ومعرفة الأساسية منها والغير أساسية.

أظهرت النتائج اختلافات طفيفة في التركيبالكيميائيين بذور الشيا الخام (السوداءوالبيضاء)وإرتفاع القيمة الغذائية لها متمثلة فمحتواها من البروتين(25.29 – 24.30٪) ، ألياف (32.94 – 31.75٪) ، دهون (32.35 – 30.54٪) ومحتوى رماد. (6.93-6.05٪) على التوالي. بالنسبة لبذور الشيا النابتة ، أظهرت البيانات أن محتوى البروتين في بذور الشيا البيضاء زاد معنويا 26.94٪ مقارنة ببذور الشيا البيضاء الخام 24.30٪. بينما وجد أن محتوى الدهون والألياف انخفض بشكل ملحوظ نتيجة لعملية الإنبات. أظهرت بذور الشيا المتخمرة محتوى بروتيني أعلى بنسبة 28.10 و 27.55٪ لبذور الشيا البيضاء والسوداء مقارنة ببذور الشيا الخام. تم تفريد المركبات الفينولية الكلية بواسطة جهازالتحليل الكروماتوجرافي العالي (HPLC)ومنها حمض الكافيك، الروزمارينيك وحمض إيلاجيك بين نوعي بذور الشيا. زاد محتوى الفينولات الكلية بشكل معنوي في البذور السوداء النابتة (263.51 ±28.44مللجم / 100جمجاليك اسيد)مقارنة بالبذور الخام(166.76 ± 4.13مللجم / 100جمجاليك اسيد)بينما أظهرت البذور المخمرة زيادة معنوية في محتوى الفينولات الكلية لكل من نوعي بذور الشيا حيث كانت القيم 216.25 ± 4.75 و 200.74 ± 3.64 مللجم / 100جمجاليك اسيد عند مقارنتها بالعينة الخام. تحتوي كلا من نوعى بذور الشيا على أحماض أمينية أساسية بنسب متقاربة. كان النشاط المضاد للأكسدة الأعلى نسبيًا في بذور الشيا السوداء الخام مقارنةببذور الشيا البيضاء الخام. إلى جانب ذلك ، يحتوي زيت الشيا على نسبة عالية من حمض اللينولينيك (ω 3)بقيمة 59.25 – 58.54٪ من إجمالي محتوى الأحماض الدهنية في زيت بذور الشيا السوداء والبيضاء على التوالي. محتوى حمض اللينوليك (6ω) كان من 20.50-20.17٪.وقد تبين من هذه الدراسة احتواء بذور الشيا علي العناصر الغذائية بنسب متوازنة وكذا تعد كمصدر غنى بالمعادن، مضادات الأكسدة، الأحماض الدهنية غير المشبعة، الأحماض الأمينية الأساسية والتي لها دور في الحماية من الالتهابات وتقليل مستوي الكوليسترول وتقليل خطر الإصابة بالنوبات القلبية والسرطان وتوفر الحماية ضد مرض السكري من النوع الثاني.