

## Evaluation of Chia (*Salvia Hispanica* L.) Seeds Meal as a Source of Bioactive Ingredients

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### ABSTRACT

The main aim of this investigation was to evaluate the meal of Egyptian chia seed (*Salvia hispanica* L.) as a source of bioactive components comparing with the USA imported one. The results showed slight differences in proximate composition, dietary fibers components, nutritional value, total and HPLC separated simple phenolic compounds especially, caffeic acid, gentisic, rosmarinic and catechin between meals of local and imported chia seeds. Both meals had nearly similar amino acids pattern, *in vitro* protein digestibility (IVPD) (66.5 - 66.9%), computed- protein efficiency ratio (C-PER) (1.1 - 1.3) and biological value (C-BV) (69.4 - 71.6%), high water (7.5-8.5 g water /g meal), oil (5.1-5.3 g oil / g meal) holding capacities, good emulsification capacity (52-55 ml oil/100 g), poor foaming capacity and stability, high total (55.8-57.7%), insoluble (48.2-53.3%), neutral detergent (43-47.2%) and acid detergent (34.2-36%) dietary fibers. The antioxidant activity as determined by DPPH and ABTS methods was relatively higher in meal of the local than imported chia seeds. Dry fractionation of chia seed meal through 100 mesh sieve gave two fractions, one rich in protein (41.5-45.5%) and others high in neutral detergent (86.5 - 90.7%), acid detergent (49.01-54%) and hemicellulose (36.7-37.49%) dietary fibers. Panelists accepted the physical and sensory properties of the biscuit products containing 5% of rich protein and high fiber chia seed meal fractions. Such results indicated the successful use of chia seeds meal as a source of bioactive ingredients in preparing functional food.

**Keywords:** Chia seeds meal, nutritional quality, phenolic compounds, functional properties, fractionation, biscuit.

### INTRODUCTION

Protein content in a chia seeds (19- 23%) is higher than wheat (~14%), corn (~14%), rice (~8.5%), oats (~15.3%), barley (~9.2%) and amaranth (~14.8%) (Bresson *et al.*, 2009, Sandoval-Oliveros and Paredes-Lopez, 2013). It has a good balance of essential and non-essential amino acids (Ullah *et al.*, 2016). Defatting and mucilage removing led to rise of chia seed protein up to 35.5%. This protein was fractionated according to its solubility into 17.3% albumins, 52% globulins, 12.7% prolamins and 9-14.5% glutelins. It is free from gluten and can be digested by celiac disease patients (Sandoval-Oliveros and Paredes-Lopez, 2013). The

storage protein of chia seed was globulin. It was composed of two fractions with a molecular size ranged from 15 to 50 kDa. The first was with 11S sedimentation coefficient and known as legumin like or 11S globulins, and the second was with 7S sedimentation coefficient and referred as a 7S globulin or vicilin-like. Also, there was other one found in less proportion and known as 2S-like protein (Ullah *et al.*, 2016). According to Segura-Campos *et al.* (2013) and Orona-Tamayo *et al.* (2015) chia seed globulin protein provides a high concentration of biologically active peptides with antihypertensive and other important function. The *in vitro* protein digestibility of the defatted chia flour was slightly lower (77.5-78.9%) than that of its protein globulin fraction (82.5%) and higher than that of sorghum (59.1%) and corn (68.6%) (Sandoval-Oliveros and Paredes-Lopez, 2013). High fiber content of chia seeds meal may be behind the low of its *in vitro* protein digestibility (Vázquez-Ovando *et al.*, 2010). Such fibers are low digestibility and low caloric content. It is not attacked by the stomach and small intestine enzymes and therefore reaches the colon undegraded (Prosky, 2001). Generally, there are no reports of anti-nutritive factors in chia seeds that could retard its *in vitro* digestibility such as presence of protease inhibitors.

Vázquez-Ovando *et al.* (2010) used screening process to fractionate chia seed protein meal into fiber- and protein- rich fractions. The fiber rich fraction had high insoluble dietary fiber content, good water, oil and organic molecule absorption capacities. The protein rich fraction had high essential sulfur amino acids and non-essential amino acids as well as limited amino acids such as tryptophane and lysine. Capitani *et al.* (2013) showed that the chia seed meals with mucilage exhibited a statistically higher water holding capacity (WHC) than that of the meal without mucilage. The WHC of both meals was higher than that observed for canola (3.90 g/g), soybean meals (3.28 g/g) and similar to that of linseed (6.03 g/g) meals. Also, the chia seed meal with mucilage showed a low absorption of organic molecules and oil-holding capacity than meal without mucilage. Results of Vázquez-Ovando *et al.* (2013) indicated that the dry fractionated chia seed meal flour rich in protein

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had high emulsification activity (50-56%) and can be considered a good emulsion stabilizer during preparing ice cream, dressing, chocolates, mayonnaise and other products. Foaming properties of the chia flour were poor and that may probably due to its an adequate unbalance between hydrophobicity and hydrophilicity and its low albumin relative proportion (10%).

The defatted chia seed flour had 40% total dietary fibers (TDF), 5-10% of which was soluble and formed parts of the mucilage (Ullah *et al.*, 2016). Insoluble dietary fibers (IDF) was the main fraction with lignin as a main component, 39-41% of TDF. The other components of IDF were cellulose and hemi-cellulose (Pizarro *et al.*, 2015).

Chia seeds are a promising source of natural antioxidants due to the presence of non hydrolyzed polyphenols, chlorogenic and caffeic acids and hydrolyzed polyphenols, myricetin, quercetin, kaempferol and flavonol glycosides. They are responsible of the antioxidant activity of chia (Ayerza *et al.*, 2002). The phenolic compounds content in chia seeds ranged from 7.6 to 8.8% as a gallic acid equivalent (GAE) (Reyes-Caudillo *et al.*, 2008). These phenolic compounds showed high antioxidant activity in the DPPH assay (85.3%). The phenolic extract was effective in neutralizing more than 70% of the free radicals. They act as a radical sequestrators and hydrogen donors. Therefore, they able to lower the incidence of cardiovascular disease and to prevent the oxidation of unsaturated fatty acids (Michele and Myriam, 2014 and Ullah *et al.*, 2016).

The main objectives of this study were; to characterize the Egyptian defatted chia seeds comparing with imported one, to detect its proximate composition, nutritional quality, functional properties, type and amount of its major antioxidant compounds using HPLC technique, in addition to its utilization in food purposes.

## MATERIALS AND METHODS

### Materials

**Chia (*salvia hispanica* L.) seeds meal:-** Two types of black chia seeds were used in this study. The first was imported from USA, 2016 harvest. It was purchased from one commercial retail at Alexandria city, Egypt in November 2016. The second was locally produced in one private farm at El-Haddadeen city, El-kalyobiya, Egypt, in November 2016. These seeds were locally produced by sowing the USA imported black chia, 2015 harvest, with 80% germination capacity at a seeding rate of 1.5 to 2 kg/acre. The seeds were grown in loamy soil in the period of November 2015 to April 2016. The seeds were not treated chemically prior to sowing and also with insecticides during growth. They were sown in

drills with 5-10 cm deep in soil which has been leveled before cultivation. Before sowing, the soil was treated with super nitrate (100 kg/acre) fertilizer. At sowing, the seeds were planted in rows with 10 m long. The crop was irrigated 4 times at a rate of 15000 m<sup>3</sup>/ acre. Rainfall was moderately occurred through end of December and first half of January and the mean of temperature was ranged from 18-24°C during growth of plant. All cultivation work was carried out manually. Seeds were manually harvested and the seed yield was 500 kg/acre. The harvested seeds were packed in polyethylene bags and stored under hygienic conditions. The cleaned dried chia seeds were ground in Wiley mill to pass through 60 mesh sieve before defatting with petroleum ether (b.p.40-60°C) in Soxhlet apparatus for 16 h. as mentioned by Amato *et al.* (2015). The defatted meal was desolventized at 60°C in an air draft oven until solvent removal, 50 min.

**Food ingredients:-** The following food ingredients were obtained from local market at Alexandria city, Egypt; 72% extraction wheat flour, refined sunflower oil, margarine, sugar, common salt, sodium bicarbonate and ammonium bicarbonate.

**Chemicals:-** Most of the chemicals used in this investigation were purchased from El-Gomhouria Pharmaceutical Company and El-Nasr Pharmaceutical Chemical Company, Egypt. The used reagents, solvents and phenolic standard compounds for HPLC analysis were bought from Sigma-Aldrich company (St. Louis, Missouri, USA).

**Packing materials:-** Aluminum foil, trays and polyethylene bags were purchased from local markets at Alexandria city, Egypt.

### Methods

**Physical methods:-** Triplicate random samples of biscuit were weighed using Mettler Toledo AB 204 digital balance ( $\pm 0.01$  g sensitivity). The average of these triplicates was reported as biscuit weight. 10 biscuit pieces samples were used to measure their thickness, length and width by a digital micrometer with an 0.01 mm accuracy (Khattab, 2004).

**Chemical methods:-** The defatted chia seeds were ground in a willy mill to pass through 60 mesh sieve (250 microns), filled in glass kilner jars and stored at 4°C until analysis.

**Proximate composition:-** The chemical analysis of chia seeds meal was run in triplicate. Moisture content was determined by drying the samples at 130°C for 4 h. as described by Guiotto *et al.* (2011). Total nitrogen was determined using the Kjeldahl method and crude protein content was calculated by multiplying the nitrogen content by factor of 6.25 (Nielson, 2010). Ash content

was determined after pre-ashing in muffle furnace (Vulcan A-550) at 550°C until getting light gray ash and a constant weight was resulted. Crude fiber content was determined according to A.O.A.C. (2003) method via filter bags technology using Fiber analyzer (Ankom 200. Model No. A220, USA). Nitrogen free extract of chia seeds was calculated by difference.

**Chia seed meal fractionation:-** After extraction of oil, chia seed meal was milled to pass through 0.5 mm screen before fractionated by sifting for 20 min. using Tyler 100 mesh (150 microns) screen and a Ro-Tap1 agitation system according to Vázquez-Ovando *et al.* (2013) into two fractions; protein-rich fraction which passed through the 100 mesh and fiber rich fraction which retained in the 100 mesh. Each fraction was packed in glass jar and stored at 4°C until analysis.

**Total (TDF), soluble (SDF), insoluble (IDF), acid detergent (ADF) and neutral detergent (NDF) dietary fibers:-** They were determined in fat extracted chia seed flour and its fractions as mentioned in A.O.A.C. (2000). The sample was treated with heat stable amylase, amyloglucosidase and protease in an autoclave. An enzymatically undigested fiber (insoluble residue) was filtered, dried, weighted, ashed and reweighted to give the amount of insoluble dietary fiber (IDF). The digested fraction of sample was precipitated with ethanol, treated with amylase from porcine pancreas to remove soluble carbohydrates and protein then filtered and the resulted residue was dried, weighted, ashed and reweighted to give soluble dietary fiber (SDF). Total dietary fiber (TDF) was calculated as sum. of the IDF and SDF. Determination of NDF was based on refluxing the samples with hot solution of neutral detergent to measure the total cell wall materials. Meanwhile, estimation of ADF was based on refluxing of sample with hot sulfuric acid solution containing detergent to get a good measure of the cellulose and lignin.

**Total phenolic content:** - It was determined according to the procedure of Zilic *et al.* (2012) using Folin-Ciocalteu reagent and gallic acid as standard. One g of defatted sample was extracted with 10 ml methanol and shaken for 3h. in dark at room temperature (25± 2°C). 100 µl of the methanol extract was transferred into a test tube and the volume adjusted to 3.5 ml with distilled water before adding 250 µl of Folin-Ciocalteu reagent. The reaction mixture was kept in dark for 2 h. before measuring the absorbance at 765 nm wave length using UV-Vis spectrophotometer, Laxco-Alpha1102, Suite.

**Separation and determination of simple phenolic compounds by HPLC:-** This was carried out according to Kim *et al.* (2006) with some modifications.

**1)Extraction of phenolic compounds:-** Extraction of phenolic compounds was done by weighing one gram of defatted chia seed flour in quick fit conical flask, adding 20 ml of 2 M NaOH, flushing the flask with N<sub>2</sub> gas, replacing the stopper flask, shaking for 4 h. at 1200 rpm at room temperature (23± 2°C), adjusting the pH to 2 with 6 N HCl and centrifugation at 5000 rpm for 10 min. The phenolic compounds in the obtained supernatant were extracted twice with 50 ml of 1:1 (v/v) mixture of ethyl ether and ethyl acetate. The organic phase was separated in separated funnel, concentrated under vacuum at 45°C by a rotary evaporator, re-dissolved in 2 ml ethanol, filtered through 4.5 µm milli pore filter (Gelman, Laboratory, MI).

**2)Separation and determination:-** 50 µl of the resulted filtrate was injected in HPLC. Agilent type 11100 series (Agilent. Technologies; CA, USA). The apparatus equipped with solvent delivery system, photodiode array detector (CDAD, series G1315D) interfaced with an AC/DC convertor, an auto sampler (series GI239A) and HP chem. station (rev. B03.01) software package, a Rheodyne 7125 six ways injector with 10 ml sample loop and Agilent Zorbax eclipse XXDB reversed phase C18 (150 X 4.6 mm, particle size 5 µm) analytical guard column (Agilent, USA). HPLC elution was done at room temperature (23± 2°C) by solvent A (acetonitrile) and solvent B (acetic acid and water 2:98 v/v ratio) at a flow rate of 0.8 ml/min. with the following linear grading program, 100% B to 85% B for 30 min., 85% B to 50% B for 20 min., 50% to 0.0% B for 10 min. and 0.0% to 100% A for 10 min. The chromatogram was simultaneously monitored at 280 and 230 nm. (with 2 nm. band width). The spectra was taken continuously through the elution. Calculation of the concentration was based on the external standard method of an aqueous solution containing the following phenolic standards; gallic, protochatchuic, gentisic, chlorogenic, caffeic, syngic, vanillic, ferulic, sinapic, ellagic, rosemarinic, cinnamic acid, catachine and chyrsin to fit a standard curve (peak area versus concentration in µg/g) with lineary regression for each individual compound.

#### **Antioxidant activity:-**

It was determined by two methods.

**1) 1,1diphenyl 2-picrylhydrazine (DPPH) radical scavenging assay:-** It was used according to Hwang and Do-Thi (2014). This method based on the evaluation of the free radicals scavenging effect on DPPH. The extracted sample was diluted with extraction buffer and 400 µl aliquot was mixed with

200 µl of 0.5 mM DPPH in methanol and the absorbance was measured at 517 nm. against a blank of pure methanol after 60 min. of incubation in a dark condition using UV-VIS spectrophotometer (Laxco-Alpha-1102, suit).

**2) Radical ABTS\* scavenging activity:-** Antioxidant activity was quantified by dyeing assay of the radical cation 2,2-Azino-Bis-3-Ethylbenzothiazoline-6 Sulfonic Acid (ABTS\*) according to Hwang and Do-Thi (2014). Equal quantities of a 7 mM aqueous solution of ABTS\* was mixed with 2.45 mM potassium persulfate for 16 h. at room temperature (23 ± 2°C) in the dark to prepare stock solution of ABTS\*. One ml of the stock solution was diluted with 60 ml of ethanol: water (50:50, v/v) to obtain an absorbance of 1.0 ± 0.02 at 734 nm using UV-VIS spectrophotometer (Laxco-Alpha-1102, suit). To 50 µl of the sample ethanol extract, 4.95 ml of the ABTS\* solution was added and left for 1 hour in a dark. Then the absorbance was measured at 734 nm. using the above spectrophotometer.

#### Nutritional quality:-

**1) Amino acid composition:-** Fifty milligrams of the chia seed meal were mixed with 10 ml of 6 N hydrochloric acid containing 50 µl mercapto ethanol in heat-resistant tube. The tubes were sealed, heated in oven at 110°C for 24 h., then cooled to room temperature and filtered through Whatman No. 1 filter paper. Both tube and the precipitate were washed with distilled water. The washed water was added to the previous filtrate then completed to 25 ml in a volumetric flask. Five ml of the filtrate were transformed to 25 ml beaker and placed in a vacuum desiccator until dryness in presence of potassium hydroxide. The dried residue was dissolved in one ml of sodium citrate buffer (pH 2.2) and analyzed by the Bechman Amino acid analyzer model 119CL (Duranti and Cerletti, 1979).

**2) In-vitro protein digestibility (IVPD):-** Pepsin followed by pancreatin digestion procedure was used as described by Ali (1999). Two hundred and fifty mg of the defatted ground chia seed flour were suspended in a conical flask containing 15 ml of 0.1 N hydrochloric acid in which 1.5 mg of pepsin was dissolved. The mixture was gently shaken then incubated at 37°C for 3 h. in an incubator shaker. The incubated solution was neutralized with 0.5 N sodium hydroxide and treated with 4 mg of pancreatin dissolved in 7.5 ml of 0.2 M phosphate buffer (pH 0.8) containing 0.05 M sodium azide as antimicrobial. The resulted mixture was gently shaken and incubated for 24 h. at 37°C. At the end of incubation period, 4 ml 30% trichloroacetic acid

(TCA) was added to stop the reaction. The digest mixture was kept for 1 h. at room temperature (23 ± 2°C) to precipitate the undigested protein, then centrifuged at 6000 rpm for 20 min. Soluble protein content was determined by micro-Kjeldahl method and the IVPD was calculated from the following equation;

$$\% \text{ IVPD} = \frac{\text{Protein content in the supernatant}}{\text{protein content in the sample}} \times 100$$

#### 3) Computed-protein efficiency ratio (C-PER):-

Computed-protein efficiency ratio was calculated by the procedure of Khattab (2004). C-PER was calculated according to the following regression equations:

$$\text{PER}_A = -0.684 + 0.456 \text{ Leu} - 0.047 \text{ Pro.}$$

$$\text{PER}_B = -0.468 + 0.454 \text{ Leu} + 0.105 \text{ Tyr.}$$

$$\text{PER}_C = -1.816 + 0.435 \text{ Met} + 0.780 \text{ Leu} + 0.211 \text{ His} - 0.944 \text{ Tyr.}$$

All amino acids used were g per 100 g protein and the mean of the three equations was taken as the C-PER value of the sample.

#### 4) Computed-biological value of protein (C-BV):-

Biological value of chia seed protein was calculated according to Khattab (2004). Using the following regression equation:

$$\text{BV} (\%) = 39.55 + 8.89 \times \text{lysine (g/100 g protein)}$$

**Functional properties:-** Water and oil holding capacities were determined according to the method of Capitani *et al.* (2013) using one gram of defatted chia seed flour, 10 ml distilled water or 10 ml refined corn oil. The emulsification capacity was determined at room temperature as mentioned by Attia (1987) using one gram sample, 12 ml of distilled water, refined corn oil and blending until the emulsion break point was reached (phase separation into 2 layers visually). Emulsification capacity is expressed as ml oil emulsified by 100 g sample. The foam capacity and stability were determined by the procedure of Ali (1999) using 3 grams of samples, 100 ml distilled water and blending for 5 min. at room temperature (23 ± 2°C). The suspension along with foam was poured into 200 ml graduated measuring cylinder and the total volume was recorded after 30 sec. The volume of the foam capacity was calculated after 30 sec. and recorded as foam capacity (FC) according to the following formula;

$$\% \text{ Volume increase or foam capacity} = \frac{\text{Total volume} - \text{Initial volume}}{\text{Initial volume}} \times 100$$

The foam volume was recorded after 0, 5, 10, 15, 30, 45 and 60 min. of standing at room temperature (23 ± 2°C) in the cylinders as foam stability according to the following equation;

Foam stability (ml) = Total volume – Liquid volume

#### Technological methods:-

**Biscuits preparation:-** Both control and chia-enriched biscuits were prepared as mentioned by Mesías *et al.* (2016). Control biscuit was formulated with wheat flour (130 g), sucrose (35 g), distilled water (30 g), sunflower oil (26 g), sodium bicarbonate (0.8 g), ammonium bicarbonate (0.4 g) and salt (1 g). Also, biscuits were formulated after replacing wheat flour with 5% of either defatted high protein fraction and/or high dietary fiber fraction chia seed flour. Accordingly, 3 types of biscuits were prepared; (A) control, free from chia seed meal flour, (B) containing 5% high protein meal fraction and (C) containing 5% high fiber meal fraction. The prepared biscuits dough was left for 60 min. in polyethylene bags before forming into bars and baking at 210°C for 8-10 min. in an electric oven (G.E.C. QA50QV I5A35OV). After cooling biscuit was packed in polyethylene bags and after 24 h. of baking, biscuits weight, thickness, length and wide were measured as mentioned above.

**Sensory evaluation:-** The prepared biscuits were subjected for sensory evaluation, using ten panelists of the Food Science and Technology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Each panelist was asked to evaluate the biscuit properties according to descriptive sheets described by Arbeitsgemeinschaft Getreideforschung (1978) method.

**Statistical analysis:-** Results were analyzed for statistical significance at  $P \leq 0.05$  by student's t-test using statistical package for social sciences software (SPSS) version 22 (2018).

## RESULTS AND DISCUSSION

### Defatted chia seed flour

**A. Proximate composition:-** Data in Table (1) reveal that, extraction of oil caused an increase in protein, ash, crude fibers and nitrogen free extract from 26.1 to 41.14, 5.15 to 8.27, 22.22 to 35.03 and 9.96 to 15.56%, respectively in local, from 27.89 to 44.41, 4.8 to 7.64, 24.31 to 38.86 and 5.8 to 9.09%, respectively in imported chia meals. This means that protein and crude fibers are the main components of chia seed meal. They represent more than 75% of the meal components. The ratio between both components was ranged from 1.1-1.2 protein to 1 crude fiber. Therefore, chia seed meal flour is a good source of protein and fibers. Ullah *et al.* (2016) showed that defatted chia seed flour contains from 34 to 40% of dietary fiber. This amount is equal to 100% of the daily recommendations for adults to decrease the risk of coronary heart disease, diabetes

mellitus type 2 and several types of cancer (Amato *et al.*, 2015).

**B. Dietary fibers :-** As shown from the results in Table (1), defatted chia seed flour contained high level of total dietary fiber. This component was 57.7% in local and 55.8% in imported defatted chia seed. It was composed of 53.3 and 48.2% insoluble dietary fiber (IDF), 4.4 and 7.6% soluble dietary fiber (SDF) in local and imported defatted chia seed flour, respectively. This amount and types of dietary fiber are higher than those mentioned for flaxseed (27.3% TDF), amaranth (6.7% TDF), quinoa (7% TDF), peanut (8.8% TDF) and soybean (9.6% TDF). Generally, SDF forms part of chia seed mucilage.

The IDF to SDF ratio is an important index for certain food application. This ratio (IDF/SDF) is nearly 12.11:1 and 6.34:1 in defatted local and imported defatted chia seeds flour, respectively. According to Spiler (1986) IDF/SDF ratio from 1:2.2 is the most advantageous factor for the beneficial physiological effects. Generally, the high of IDF in defatted chia seed meal suggests possible application in dietetic physiological products. Intake this type of fiber is linked to a sensation of satiety, since as the fiber absorbs water, swells and fills stomach and reduces from the consumed food. It is also, increased the volume and weight of the fecal mass, improved digestive process and prevented constipation and colon cancer (Alfredo *et al.*, 2009).

Chia seed meal contained high amount of neutral detergent fiber (NDF), 43-47.2% and low level of acid detergent fiber (ADF), 34-36%. This means that dietary fiber of chia seed had more cell wall materials including pectin (IDF) than cellulose and lignin. The difference between NDF and ADF gives the hemicellulose (Dick *et al.*, 2015).

**Table 1. Proximate composition and dietary fiber of chia (*Salvia hispanica L.*) seeds defatted flour**

Component (% on dry weight)	Local chia	Imported chia
Crude protein	41.14	44.41
Ash	8.27	7.64
Crude fiber	35.03	38.86
Nitrogen free extract (NFE*)	15.56	9.09
Soluble dietary fiber (SDF)	4.40	7.60
Insoluble dietary fiber (IDF)	53.30	48.20
Total dietary fiber (TDF)	57.70	55.80
Neutral detergent fiber (NDF)	47.20	43.00
Acid detergent fiber (ADF)	36.00	34.20

\* NFE was calculated by difference.

**C. Nutritional quality:-**

**1) Amino acids:-** The amino acid pattern of local and imported defatted whole chia seed flour in Table (2) was nearly similar. Both local and imported chia meal had eight essential amino acids. These acids can be arranged according to their concentration in the following decreasing order, leucine, phenylalanine, lysine, valine, sulfur containing amino acids, isoleucine, threonine and histidine, respectively. Among non-essential amino acids, glutamic acid found at high concentration followed by arginine, aspartic acid, alanine, glycine, tyrosine, serine and proline. 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).

**2) Protein digestibility:-** The *in vitro* protein digestibility (IVPD) of the defatted chia seed flour was low, (Table 2). It was very close in both local (66.5%) and imported (66.97%) chia seed defatted flour. This may be due to their high fiber content in addition to presence of other antinutritional factors such as polyphenols. Sandoval-Oliveros and Paredes-Lopez (2013) found that IVPD of defatted chia seed flour ranged from 77.5% to 78.9%. In

contrast, Timilsena *et al.* (2016) reported that IVPD of chia seeds ranged from 49.4% to 78.9%.

**3) Computed protein efficiency ratio (C-PER) and computed biological value of protein (C-BV):-** C-PER and C-BV of the defatted local and imported chia seed flour were varied from 1.1 to 1.3 and 69.4 to 71.6, respectively, (Table 2). Both of these nutritional parameters were based on the amino acids content of chia seeds. The low value of these parameters due to the limiting amino acids in chia seeds threonine, methionine, lysine, leucine and histidine (Weber *et al.*, 1991).

**D. Phenolic content and antioxidant activity:**

**1) Total phenolic content:-** Many of polyphenolic compounds exert a marked protective effect on plant lipids especially that containing high concentration of polyunsaturated fatty acids (PUFA) such as chia seeds. As shown from Table (3) the total phenolic content (mg/g) as gallic acid equivalent (GAE), was higher in local (1.42 mg/g) than imported (1.1 mg/g) chia seed defatted flour. These components are responsible to prevent the chia seed lipids oxidation. Reyes-Caudillo *et al.* (2008) found that the total phenolic content in chia seeds ethanol extract was 8.8% on dry weight basis.

**Table 2. Nutritional quality of chia (*Salvia hispanica* L.) seeds defatted flour**

Parameter	Local Chia	Imported Chia
<b>1-Amino acids (g/100 g protein)</b>		
<b>a-Essential amino acid</b>		
Leucine	4.34	4.82
Phenylalanine	3.46	3.84
Lysine	3.36	3.60
Valine	2.97	3.68
Sulfur containing amino acids	2.75	2.36
Isoleucine	2.35	2.63
Threonine	2.20	2.46
Histidine	2.04	2.15
<b>b-Non-essential amino acid</b>		
Glutamic acid	12.36	13.03
Arginine	7.20	7.42
Aspartic acid	5.74	6.40
Alanine	3.33	4.39
Glycine	2.92	3.39
Serine	2.84	2.94
Proline	2.82	2.89
Tyrosine	2.70	3.06
<b>2-In vitro protein digestibility (IVPD) (%)</b>	66.50	66.97
<b>3- Computed- protein efficiency ratio (C-PER)</b>	1.30	1.10
<b>4- Computed- biological values (C-BV) (%)</b>	69.40	71.60

Taga *et al.* (1984) showed that the presence of polyphenols indicates that chia seeds possess natural antioxidants in appreciable quantity. Viladomiu *et al.* (2013) mentioned that phenolic compounds in plants have several functions such as attracting insects, protection against UV-radiation, regulating osmotic pressure and exhibiting an astringent effect. It has also an advantageous effect on human health, as inhibiting enzymes involved in oxidation reaction or scavenging free radicals. According to Hernandez (2012) chia seed had high values of phenolic compounds.

**2) Identification and quantification of simple phenolic compounds:-** Table (3) gives the concentration (ug/g) of the individual phenolic compounds identified in local and imported defatted chia seeds flour. The levels reported in this work represent only the free forms of phenolic compounds since no hydrolysis was carried out to the sample before HPLC analysis. These compounds were found in defatted flour of both local and imported chia seeds. They can be classified into 3 groups according to their concentrations. Group one includes the major components which found up to 100 ug/g. This group

formed from caffeic acid, gentisic, rosmarinic, catechin and rutin. Second group contains the compounds with the levels ranges from 20 to 100 ug/g. It is included ferulic acid and gallic acid. The last group includes the other phenolic compounds less than 20 ug/g. Crosby (2005) found that chia seed contained caffeic acid, chlorogenic acid, quercetin, phenolic glycoside K and glycoside Q in appreciable amounts. Reyes-Caudillo *et al.* (2008) showed that chia seed with a wide range of antioxidant compounds can be regarded as a great source of antioxidant. Ullah *et al.* (2016) found that addition of 750 ppm chia seed methanol extract had significant effect to prolong the shelf stability of winterized cotton seed oil at ambient temperature.

**3) Antioxidant activity:-** The results of the antioxidant activity measured by two methods, namely DPPH and ABTS of the local and imported defatted chia seed flour presented in Table (3). Generally, the antioxidant activity of the ethanolic extract of defatted local chia seed flour was relatively higher by the previous two mentioned methods than imported one. The same observation was reported above for total phenolic compounds of both seeds.

**Table 3. Total phenolic content, simple phenolic compounds and antioxidant activity of chia (*Salvia hispanica L.*) seeds defatted flour**

Parameter	Local chia	Imported chia
<b>1- Total phenols as GAE (mg/g)</b>	1.42	1.01
<b>2- Phenolic compound (ug/g)</b>		
Caffeic acid	252.39	284.04
Gentisic	209.95	220.67
Rosmarinic	138.12	134.63
Catechin	136.79	92.37
Rutin	92.58	217.70
Ferulic acid	24.96	46.69
Gallic acid	21.90	38.71
Vanillic acid	15.86	11.37
Cinnamic acid	12.42	7.00
P-hydroxybenzoic	5.91	12.65
Apigenin	4.97	5.30
P-coumaric acid	4.27	12.33
Apigenin-7-glucoside	2.87	1.62
Chrysin	1.44	0.94
Sinapic acid	0.67	0.82
Syringic acid	0.53	0.72
Kaempferol	0.39	2.08
Quercetin	0.27	1.25
Protocatechuic acid	0.00	3.26
<b>3- Antioxidant activity TE* mg/g</b>		
DPPH	1.85	1.68
ABTS	1.75	1.45

\* (TE) Trolox equivalent.

This activity of chia seed phenolic compounds is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators. Ullah *et al.* (2016) reported that the antioxidant activity of chia seed was greater than *Moringa olifera*, sesame cake extracts and similar to that high tannin sorghum varieties. It protects chia seeds from oxidative deterioration. Taga *et al.* (1984) attributed the high antioxidant activity of chia seed extract to its content of caffeic acid than other flavonols compounds such as myricetin and quercetin. Alfredo *et al.* (2009) showed that intake 7 g of the fiber rich fraction of chia seed meets the total need of dietary antioxidant activity level.

**E. Functional properties:**-The results of these properties were tabulated in Table (4).

- 1) **Water holding capacity (WHC):**- As shown from Table (4) both local and imported chia seed defatted flour are able to hold high amount of water. This may be due to the presence of SDF, mucilage and high protein level and its content of available polar amino acids which contain the primary sites for water binding. Vázquez-Ovando *et al.* (2013) reported that due to high water holding capacity of defatted chia seed flour, it is suitable to add in products requiring hydration, viscosity development and conservation of freshness such as baked products.
- 2) **Oil holding capacity (OHC):**- The oil absorption of chia seeds defatted flour was high. This is mainly due to the nonpolar sites of the amino acids in chia seed protein. Vázquez-Ovando *et al.* (2009) stated that small particle size of the flour have relatively more contact surface to hold more oil.
- 3) **Emulsification capacity (EC):**- Result in Table (4) indicates that the emulsification capacity of chia seed defatted flour was up to 50 ml/100 g. This is agreed with that mentioned by Alfredo *et al.* (2009). They found that emulsifying activity of chia seed defatted flour was 53.26 ml/100 g. Generally, this property is based on protein configuration, content and its hydrophobic groups. The increase of the exposed area of hydrophobic groups, required to bind oil, on the protein surface increases from the interacting with the oil phase.
- 4) **Foaming properties:**- Foaming capacity (FC) of chia seed defatted flour was low 18-20% in local and imported seeds, ( Table 4) . This may be due to weak an adequate balance between hydrophobicity and hydrophilicity, which is required to save the responsible amount of free energy of foam stability.

The foam stability (FS) was decreased slowly upon standing at room temperature Table (4). The instability of foams is due to the drainage of liquid from lamellae and the rupture in cell wall of the bubbles (Sosulski *et al.*, 1976).

**Table 4. Functional properties of chia (*Salvia hispanica* L.) seeds defatted flour**

Property	Local chia	Imported chia
WHC* (g water/g sample)	8.5 ± 0.02	7.5 ± 0.01
OHC* (g oil /g sample)	5.1 ± 0.01	5.3 ± 0.01
EC* (ml oil /100 g sample)	52 ± 0.05	55 ± 0.05
FC* (%)	20 ± 0.5	18 ± 0.5
FS* (%) after (min.)		
0	20	18
5	12	18
30	8	6
45	8	6
60	8	6

\* WHC: Water holding capacity, OHC: Oil holding capacity, EC: Emulsification capacity, FC: Foaming capacity and FS: Foam stability.

**F. Fractionation:**- Dry fractionation of the defatted chia seed flour by screening through 100 mesh sieve was done to obtain two fractions, one high in protein (down 100 mesh) and the other rich in fiber (up to 100 mesh) . Fig. (1) illustrates variation in the appearance and shape of the two fractions according to their particle size and content of other component than crude oil.

Results in Table (5) indicated that the neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose ranged from 86.5 to 90.7, 49.01 to 54 and 37.49 to 36.7%, respectively in high fiber fraction of local and imported chia seeds meal. In contrast, there is no much differences were observed in other determined components either in high protein and/or in high dietary fiber fractions. Vázquez-Ovando *et al.* (2013) fractionated chia seeds flour into fiber and protein rich fractions using dry fractionation process. The fiber rich fraction had high insoluble dietary fibers content (53.45%) and good functional properties particularly water and oil holding capacities. The protein rich fraction had high essential sulfur amino acids and non-essential amino acids as well as protein content (44.6%) with good functional properties similar to those of fiber rich fraction.

According to these results, chia seed meal fibrous fraction can use as an ingredient in dietetic product





**Fig.1. Appearance of high protein (HPF) and high dietary fiber (HDF) fractions of chia (*Salvia hispanica L.*) seeds meal.**

**Table 5. Protein and dietary fiber contents of high protein and high fiber fractions of defatted chia (*Salvia hispanica L.*) seeds flour**

Component %	Local chia		Imported chia	
	(A)	(B)	(A)	(B)
<b>Crude protein</b>	41.50	11.80	45.50	13.80
<b>Neutral detergent fiber</b>	51.20	86.50	53.70	90.70
<b>Acid detergent fiber</b>	25.66	49.01	28.00	54.00
<b>Hemicellulose</b>	25.54	37.49	25.70	36.70

(A) High protein fraction (HPF)

(B) High dietary fiber fraction (HDF)

such as baked and fried foods. Meanwhile, the protein rich fraction could be used as a potential food ingredient in emulsion foods.

**G. Utilization in food purposes:-** Three types of biscuits were prepared; (A) control made of 100% wheat flour; (B) product made of 95% wheat flour with 5% high protein chia seed meal fraction (high protein biscuit) and (C) biscuit prepared from 95% wheat flour with 5% high fiber chia seed meal fraction (high fiber biscuit). Data in Table (6) show the physical properties and sensory characteristics of these products. Length and weight of the biscuit products, containing 5% high protein and 5% high fiber chia seed meal were slightly reduced than control. The addition of both fractions caused a low reduction or dilution in wheat flour gluten. Presence of mucilage and fiber in both chia seed fractions help in keeping the dimension and weight of biscuits nearly close to the control product. Svec *et al.* (2016) found that addition of 10% chia seed flour to

wheat flour during bread production caused gluten protein dilution and a decrease in bread volume.

The panelists accepted the three types of biscuit. They observed that using chia seed at 5% level either as high protein and/or high fiber fractions caused changes in breakness from slightly tender to crispy, texture from fine to moderate, chewiness from excellent to good and taste from excellent to good. The control product was the superior and the other products were equal in the degree of acceptability. According to Mesías *et al.* (2016) adding chia seed flour in wheat based biscuit formulation enhanced the nutritional quality of this product, increasing protein, dietary fiber, antioxidants and polyunsaturated fatty acids. The latter component accelerates lipid oxidation in biscuits enriched with high amount of chia seed and that reducing its shelf life.

**Table 6. Physical and sensory characteristics of biscuits free and containing 5% of each of high protein and high fiber fractions of defatted chia seed flour.**

Biscuit type	Physical properties				Sensory characteristics						
	Length (cm)	Width (cm)	Thickness (cm)	Weight of 10 pieces (g)	General appearance	Surface and bottom characteristics	Leavening	Breakness	Texture	Chewiness	Taste
<b>100% wheat flour biscuit</b>	10	3	0.5	220	Excellent	Slightly bubbled	Very leavening	Slightly tender	Fine	Excellent	Excellent
<b>High protein biscuit</b>	9.8	3	0.5	218	Excellent	Slightly bubbled	Very leavening	Crispy	Moderate	Good	Good
<b>High fiber biscuit</b>	9.9	3	0.5	219	Excellent	Slightly bubbled	Very leavening	Crispy	Moderate	Good	Good

## CONCLUSION

The above results showed that chia seed (*Salvia hispanica* L.) meal as a source of protein, dietary fibers and polyphenols is promising as functional ingredients in preparing food products.

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## الملخص العربي

### تقييم جريش\* بذور الشيا (*Salvia hispanica L.*) كمصدر للمكونات النشطة حيويًا

أسامة بكير ربيع محمد ، منى حسن حسين بخيت ، أمل محمد عبد الرازق، يحيى جمال الدين محرم هدف البحث إلى تقييم جريش بذور الشيا المصرية كمصدر للمكونات النشطة الحيوية مقارنة مع مثيله المستورد الأمريكي. وأشارت النتائج إلى وجود اختلافات بسيطة في التركيب التقريبي والألياف الغذائية والقيمة التغذوية والمحتوى الكلي من المركبات الفينولية ومكوناتها المفصلة باستخدام جهاز كرماتوجرافيا السائل عالي الأداء (HPLC) خاصة حمض الكافيينك، الجيننتسيك، الروزمارينيك، والكتاكين بين جريش بذور الشيا المحلي والمستورد. وتشابه أيضا الجريشيين في محتوَاهما من الأحماض الأمينية والهضمية المعملية (٦٦,٥ - ٦٦,٩%) و نسبة كفاءة (١,١-١,٣) والقيمة الحيوية (٦٩,٤-٧١,٦%) للبروتين ،وقدرتها العالية على ربط الماء (٧,٥-٨,٥ جم ماء/ جم جريش) والزيت (٥,١-٥,٣ جم زيت / جم جريش) والسعة الاستحلابية (٥٢-٥٥ مل زيت / ١٠٠ جم) ، وانخفاض سعة وثبات الرغوة. وتميزا بارتفاع محتوَاهما من الألياف الغذائية الكلية (٥٥,٨ - ٥٧,٧%)

وغير الذائبة (٤٨,٢ - ٥٣,٣%) وفي المنظفات المتعادلة (٤٣ - ٤٧,٢%) والحامضية (٣٤ - ٣٦%). وكان النشاط المضاد للأكسدة المقدر بطريقتين (ABTS , DPPH) أعلى نسبيا في الجريش المحلي من المستورد . وأدى الفصل الجاف للجريش بمنخل ١٠٠ مش إلى الحصول على مكونين أحدهما غني في البروتين (٤١,٥-٤٥,٥%) والآخر عالي في كل من الألياف الذائبة في المنظفات المتعادلة (٨٦,٥-٩٠,٧%) والحامضية (٤٩,٠١-٥٤%) والهيميسليلوز (٣٦,٧-٣٧,٤٩%). وتقبل المحكمون كل من الخواص الفيزيائية والحسية لكل من البسكويت المحتوى على ٥% من مكون الجريش الغني في البروتين والعالي في الألياف. وأكدت النتائج نجاح استخدام جريش بذور الشيا ومكوناته كمصدر واعد لإعداد الأغذية الوظيفية.

\*جريش: (دقيق بذور الشيا المستخلص منها الزيت) .