PROTEIN DIGESTIBILITY OF CHIA SEED *Salvia hispanica L*

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Introduction

Alternatives sources of high quality protein are required by the accelerated increase of population, to exploited alternative food sources and also to obtain and additional benefice from its source. Chia, native to southern Mexico, was use by ancient Aztecs, Mexicans and habitants of Southern California and Arizona as food supplement for energy, endurance and strength needed under extreme conditions such as heat, dryness, short-term food, water deficiency and for medicine (1). About this crop the plant explorer Edward Palmer wrote (1871): "In preparing chia for use the seeds are roasted and ground, and the addition of water makes a mucilaginous mass several times the original bulk, sugar to taste is added, and the result is the much prized semi-fluid pinole of Indians and others—to me one of the best and most nutritive foods while traveling over the deserts (2)".

Chia is a cereal composed of several species: Salvia polystachya, Hyptis suaveolens and Salvia hispanica L (3), the last one, currently harvested in a small scale in few Mexico´s states like Morelos, Puebla, Guerrero and Jalisco (4).

Chia seed has a large potential to be exploited. Traditional use in Mexico involve beverage and flour production, also its oils is employed to increase quality of paint production. It have been claimed that chia seed could help to control excess weight; users report that a glass full of orange juice with a teaspoon of presoaked seeds leaves one feeling full and without hunger until noon (5).

Extraction of polysaccharide polymer can be employed to obtain laundry-cleaning ingredient (6). Chia seed has been described for controlling, in one embodiment reducing, blood glucose levels, preferably post-prandial blood glucose levels, also improving endothelial function, coagulation, fibrinolysis and iron status (7). By that reason chia seed is present as un active agent in the treatment and/or management of diabetes and/or the treatment and management of diabetes associated conditions or risk factors, such as one or more of the following: blood pressure and blood glucose levels, post-prandial glycemia, inflammatory factors (C-reactive protein), coagulation (fibrinogen, factor VIII, von Willenbrand factor), and fibronolytic factors (such as t-PA), iron status and endothelial function, (such as increase in nitric oxide generation) (8).

Among several alternatives crops, chia was selected for production as an industrial crop by the Northwestern Argentina Regional Project (8; 9), focusing mainly in oil extraction. But also, excellent levels of omega-3 fatty acid, anti oxidants, dietary fiber (22.1%) and proteins (10, 11, 12, 13). Chia is consumed as a soaked beverage called “Agua de Chia”, this preparation could be a good source of protein, in this point, chia seeds are higher in protein content and amino acid content than most traditionally utilized grains. For example, chia seeds contain approximately 19-23% protein, which is higher that the protein concentration in wheat (14%), corn (14%), rice (8.5%), oats (15.3%) and barley (9.2%) (14,15, 16).

Ayerza, 2002 (17) published reported beneficial effects of chia seeds on growth, body mass, fat content in animals fed. According to these studies, 6.2% reduction in weight was observed in animals with a diet supplemented with 20% of chia seeds.

It have been demonstrated that chia seed proteins contain a good essential amino acid balance, but one of the most important qualities properties that edible protein must have is a good digestibility (18, 19). According to literature (20) in vivo protein digestibility is classified in: high (93-100%), been represented by animal origin

protein (meat, fish, milk, egg) and some of vegetable origin like: wheat flour low in fiber, soy protein isolated; intermediate (86-92%), here we can count, integral flours (corn, soy bean, wheat, oat); low, (70-85%) in this category are found cereals processed for direct consumption (corn, wheat, oat, etc) (21). Digestibility parameter is determinant in quality protein, since its biological value could be inferred, a good digestibility might mean that a protein is adsorbed and finally employed by the organism (22).

Differences in digestibility are due to intrinsic characteristics like, configuration, susceptibility to proteases, fiber content, tannins and phytates, and structural modification due to process, like temperature, grinding and soak (23-29).

In the current manuscript, Chia seeds were processed to obtain a traditionally Mexican beverage (Agua de Chia), protein digestibility was assayed from this and others treatments.

Materials and Methods

Chia seed

Chia seed (Salvia hispanica L) was obtained from local market in León, Guanajuato State, Mexico.

Treatments

Chia seeds were subjected to different treatments: toasted, raw and toasted whole flour, soaked, and without treatment.
Toasted. Chia seeds were toasted in an aluminum container directly to fire in a domestic stove (SupermaticÔ) for 7 min at 120±10°C.
Blended. Road and toasted chia seed were blended in a PhilipsÔ (Model HR2810/A in order to obtain whole flour.
Soaked (Agua de chía). Agua de chía, is a traditional preparation obtained by soaking chia seed in lemon juice. In order to determinate protein quality of this source, Agua de Chia was prepared as follow: Chia seed were soaked for 15 minutes in lemon juice (90 mL of lemon juice in 2 L of water plus 40 g of sacarose). During throw the process pH was of 3.0, temperature 21 °C and soluble solid concentration was 30 mg/dL (Portable refractometer, American Optic). Lemon juice density was 1.090 Kg/L. Refractometer was adjusted to cero with deionized water.

Analytical determination

Seed composition determination. In order to determinate chia seed contents of protein, fat, ash and fiber, humidity, the Official Methods of Analysis of AOAC International (30) were followed, carbohydrates were determinate by differences. These analyses were performances only in raw seeds.

In vitro protein digestibility. In this study protein digestibility was determinated according to Official Mexican Norm NMX-Y-85-2002-SCFI (31), in this protocol pepsin in vitro reaction is performance. Briefly, samples were ground and then passed through over a U.S. Standard No. 20 sieve. After that 1 gram of sample was defatted with acetone. After defatted, samples were load in an Erlenmeyer flask and 150 ml of pepsin (1:10000 in 0.075M HCl). Mixture was incubated for 16 h at 45 °C at 150 rpm. After that no digested protein was recovered by filtration, followed of three washes with 15 ml of acetone, and then residues were transfer to Kjeldahl flask for protein determination. Digestibility was reported as percent of digestible protein

$$\% \text{ Digestible protein} = 100 - \frac{\text{Protein in residue X } 100}{\text{Total protein}}$$

In this study, digestibility was defined in three levels according to FAO/WHO (32) in: low digestibility for 70 – 85%, intermediate digestibility for 86 – 92% and high for 93 – 100%. As a standard soybean flour (Maxilu, México) was employed.

Statistics analiysis. All experiments were done twice and ANOVA was performance.

Results and Discusion

Chia seed proximate composition: Seed composition was assessment for not treated seeds (Table 1). Chia seed contains 18% of protein content, a level markedly greater than other nutritional grains such as wheat (14%), corn (14%), rice (8.5%), oats (15.3%) and barley (9.2%) (33; 34, 35). This content is higher than previously reports; this could be due to environment conditions were chia seeds were obtained (36). Besides, having in consideration that chia seeds have a good balance of amino acid this represent an excellent alternative source of protein for human consumption.

Table 1.- Chia seed proximate composition

Component	%
Water	6.25
Protein (Nx6.25)	18.65
Oil	33.00
Crude Fiber	28.38
Minerals	4.35
Carbohydrate (by differences)	9.37

Oil content (33%) was determinated, this value was in concordance with previously reports, it is important to state that chia seed is a valuable source of omega-3 fatty acids, of this up to 57% is the n-3 fatty acid a-linoleic acid (37). Some authors consider chia seed as the highest vegetable edible source of omega-3 fatty acid, flaxseed have a highest level, but trials have shown the negative effects that the antinutritional factors in flax have over animal and human health (38). These facts increase the value of this ancient Mexican crop. Crude fiber was of 28.38%, this value was similar to other reports, carbohydrates, obtained by differences, was of 9.37%, mineral content was found in a 4.35% and humidity was of 6.25%.

Protein content in chia samples: Crude protein contents after processing were from ca. 16.91 up to ca. 19.77% as could be see in Table 2. Protein content of seed (19.77%), toasted seed (19.72%) and toasted flour (19.33%) were significant different from flour (18.64%) and soaked seeds (16.91%) ($p=0.5$). In the first cases, moisture could be lost by toasted process, resulting in an increase of protein content. In the other hand, seed protein could be lost during soaking process; water acquired during soaking might have a dilution effect over seed components, besides protein could be lost by lixiviation.

In vitro protein digestibility: Low digestibility values were observed in all treatments (Table 2), but significant differences could be detected. The lowest values correspond to observed for toasted seed; in these cases 10.87% of digestibility was obtained. Carbohydrates and proteins present in seed could have reacted avoiding enzymes actions. López et al (39) reported that toasted treatment improve protein digestibility, and in general this behavior have been observed in vegetables. Our results could be due to temperature and exposition time of toasted. This to parameters should be analyzed in order to determinate optimal conditions in futures projects.

When soaked seeds were analyzed, a low value was obtained (24.30% of digestibility), we were expecting a highest value. It have been reported that acidic environments like employed in soaked treatment (pH 3.0) increase digestibility susceptibility, like in artichoke products where pH 4.0 induce increase in this parameter (40). But, hydration of soluble fiber induces a gel formation, which might affect in an adverse fashion enzymatic activity which in this case might be observed in low digestibility activity (41).

Table 2. Protein content and digestibility (%) of chia seed treatments and soybean flour

Samples	Treatment	% Crude protein (N X 6.25)	% Digestibility	Digestibility score
Raw seed	Without treatment	19.72^{B}	29.01^{CD}	NC
Toasted seed	Toasted	19.65^{B}	10.87^{E}	NC
Flour	Grind	18.65^{BC}	$*79.80^{B}$	LD
Toasted Flour	Toasted and Grind	19.34^{B}	34.18^{C}	NC
Soaked seed	Soaked	16.91^{C}	24.30^{D}	NC
Soybean flour	None	47.68^{A}	92.20^{A}	ID

Values in the same column with different roman superscripts are significantly different ($p < 0.05$). Digestibility classification: HD: High digestibility; ID: Intermediate digestibility; *LD: Low digestibility; NC: No Classified

Toasted flour and raw seed also showed low digestibility values (29.01% and 34.18% respectably) that could be related to high content of fiber, but less interference due to gel formation. The highest value of digestibility was observed in chia flour (79.80%), this correspond to low digestibility according to FAO/OMS (42) standards. It looks like grinding help protein digestion of raw seed. This treatment improve digestibility that could happen due that grinding divide and expose all seed component allowing enzymes actions. Finally, soybean flour shows an intermediate digestibility score, which is in concordance with previously a report.

Concluding Remarks

Chia seed looks like an excellent source of protein because of its excellent levels of nutraceutic fatty acid (omega-3 fatty acid), along with the benefices of the large amount of fiber and complex carbohydrates. This study shows that seed protein quality of this seed is poor, but through applications of biotechnology approaches, protein digestibility could be enhanced. Considering the large contents of raw fiber in chia seed, is necessary to continues study about dietetic fiber, amino acids and its effects over digestibility, principally in the peeled flour as well as the analysis in combination with another cereals (oat, corn, amaranth) and oil seed (soy bean).

Abstract

Chia seeds (Salvia hispanica L) have been consumed in Mexico since pre-hispanic time, recently this seed has been characterized as an excellent source of omega-3 fatty acid, as well of fiber, a show a good amino acid balance, but information regarding its protein quality is poor. Protein digestibility is a valuable parameter to establish protein quality, which could be classified in: high, intermediate, and low. The protein digestibility is influenced by amino acids composition, anti-physiological elements, fiber, pH, temperature, grinding and soaking. The overall aim of the project was to evaluate the in vitro protein digestibility of chia seed. Analytical study with four samples that received different treatments: toasting, grinding (flour), toasting plus grinding, a soaking in water; one was left without treatment. The proximate compositions were studied only to chia seed without treatment. For all samples, the protein digestibility was evaluated by the pepsin method. The sample of chia seed treated with grinding got a low digestibility score (79.8%). The rest of the samples did not get any digestibility classification. Only the grinding treatment improved the digestibility. The amount of fiber could have influenced the digestibility of the protein. It is necessary to continue studying the digestibility of chia seed in combination with other grains and leguminous, and the recommendations would be to eat the chia in combination to improve its digestibility.

Key words: Digestibility protein, chia seed Salvia hispanica L

Resumen

Las semillas de Chia (Salvia hispanica L) se ha consumido en México desde la época pre-hispanica, esta semilla se ha caracterizado recientemente como fuente excelente del ácido graso omega-3, como también de fibra, exhibe un buen equilibrio de aminoácidos, pero la información con respecto a su calidad de proteína es pobre. La digestibilidad de la proteína es un parámetro valioso para establecer la calidad de proteína, en la cual podría ser clasificado: alto, intermedio, y bajo. La digestibilidad de la proteína es influenciada por la composición de aminoácidos, elementos anti-fisiolo'gicos, fibra, pH, temperatura, molienda entre otros aspectos. El propósito del proyecto fue evaluar la digestibilidad in vitro de la proteína de la semilla del chia. El estudio analítico contemplo con cuatro muestras que recibieron diversos tratamientos: el tostar, moliendo (harina), tostando más moler, el empapar en agua; uno fue dejado sin el tratamiento. Para todas las muestras, la digestibilidad de la proteína fue evaluada por el método de la pepsina. La muestra de la semilla del chia que se trató con moler consiguió una cuenta baja de la digestibilidad (79.8%). El resto de las muestras no consiguió ninguna clasificación de la digestibilidad. Solamente el tratamiento que fue molida mejoró la digestibilidad. La cantidad de fibra habría podido influenciar la digestibilidad de la proteína. Es necesario continuar estudiando la digestibilidad de la semilla del chia conjuntamente con otros granos y leguminoso, y las recomendaciones serían comer el chia en la combinación para mejorar su digestibilidad.

Palabras clave: Digestibilidad de proteína; semillas de chía, Salvia hispanica L

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