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Chapter 19

Effect of Mucilage Extraction on the Functional Properties of Chia Meals

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Additional information is available at the end of the chapter

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1. Introduction

Chia (*Salvia hispanica* L.) is an annual herbaceous plant that belongs to the *Lamiaceae* family, which is native to southern Mexico and northern Guatemala. The *Salvia hispanica* fruit consists of four nutlets, similar to an indehiscent achene, which contain a single seed. These nutlets are commonly called "seeds" [1]. Chia seed, together with corn, beans, and amaranth were important crops for pre-Columbian civilizations in America, including the Mayan and Aztec populations [2, 3]. With time its use was abandoned, but by at the end of the last century there was a resurgence of interest in chia due to its nutritional value [4]. Chia is considered an alternative crop to diversify and stabilize the economy of Northwestern Argentina [5]. The plant produces numerous small white and dark seeds that mature in autumn [6]. These seeds contain about 30% oil, and they mainly consist of unsaturated fatty acids [4, 7]. Chia seeds are a natural source of omega-3 fatty acids, antioxidants, proteins, vitamins, minerals and dietary fiber [5, 7, 8].

Chia meal (residue of the seeds after oil extraction) is a good source of proteins (19-23%) [9], dietary fiber (33.9-39.9%) [10], and compounds with antioxidant activity [7]. It also exhibits some interesting functional properties for its use in the food industry [11]. Functional properties are generally associated with the presence of proteins [12] and also of dietary fiber [13-16].

Dietary fiber (DF) consists of a heterogeneous mixture of compounds that are classified according to their physical properties and effects of their intake into: soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) [17], referring to the solubility of fibers in water. Plant secretions such as pectins and gums, components such as mucilage, and chelating

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agents such as phytates are sources of SDF; whereas cellulose, lignin, some fractions of hemicellulose, phenolic compounds such as tannins and lipid structures such as waxes, suberins and cutins constitute IDF [18].

The functional properties of food components can be defined as any physicochemical property that affects and/or modifies some of its characteristics and that contributes to the quality of the final product. Knowledge about of functional properties such as color, particle size, water holding, absorption and adsorption capacity, as well as those linked to the affinity for lipid components is very useful for the food industry, because during the processing some modifications can occur that must be taken into account according to the usage of the final product and its marketing conditions [19]. For example, water-holding capacity (WHC) is related to the freshness and softness effect present in bakery products, and the oil-holding capacity (OHC) is related to the un-fatty effect in fried food when it is low and to the juiciness and texture in meat products when it is high [18, 20, 21].

In addition to the characteristics mentioned above, it is important to consider the physiological effects of the DF intake. Given the capacity of SDF to form gels, it increases the viscosity of the bolus in the gastrointestinal tract, slowing the intestinal transit, making digestion and the absorption of nutrients more efficient, providing more of a feeling of satiety. Soluble fiber are fermentable fibers that can be microbiologically decomposed in the colon, producing gases such as carbon dioxide, hydrogen and methane, and short-chain fatty acids (acetic, propionic and butyric) which are absorbed and used as energy sources. Some of the most important beneficial effects of SDF is that it regulates blood sugar and lower cholesterol levels. On the other hand, IDF is responsible for adding bulk to the stool, speeding the passage of stool through the intestine by promoting peristalsis, alleviating constipation and other gastrointestinal disorders [22, 23]. Both types of fiber may also reduce the risk of obesity, hypertension, appendicitis, and other disorders [24]. The beneficial effects noted above show the important role that DF play in human intake, and that is why a daily intake of 25-30 g is recommended, with a good SDF/IDF balance (a minimum of 30% SDF and 70% IDF, optimum 50/50 ratio) in order to benefit from both fractions of fiber [25, 26].

Chia mucilage (SDF), a complex carbohydrate of high molecular weight, is an important component of the seed due to its physiological role. The mucilage is secreted when the seed comes into contact with water, generating high-viscosity solutions [27, 28]. Many studies have examined the functional properties of different types of gums (*Linux usitatissimum, Opuntia Picus indica, Alyssum homolocarpum, Psyllium plantago)* [29-32]. However, little information has been reported on the functionality of chia seed mucilage as a stabilizing or thickening agent of food products.

The objective of the present work was to perform a comparative evaluation of the functional properties of chia meals (*Salvia hispanica* L.) obtained from seeds with and without mucilage.

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2. Materials and methods

2.1. Seeds

Chia seeds were obtained from commercial sources in Salta, Argentina (25º S and 65.5º W). They were cleaned manually by removing the foreign matter such as stones, dirt and broken seeds. They were packed in hermetic plastic vessels and stored at 5ºC until further use.

2.2. Meal without mucilage (Msm)

Meal without mucilage refers to the residue obtained after the oil extraction process using a Soxhlet apparatus following the IUPAC Standard Method [33] (*n*-hexane under reflux, 8 h, 90 °C,) of seeds that had previously had the mucilage extracted.

2.2.1. Mucilage extraction

The mucilage was extracted of chia seeds previously soaked in water (1:4) for 4 hours at room temperature. This mixture was distributed into plastic trays and covered with aluminum foil and frozen at -80°C, lyophilized, and the mucilage was removed by a sieving process (20 sieve mesh ASTM, 840 μm) (3 sections of 15 min each).

2.3. Meal with mucilage (Ms)

The data corresponding to the meal with mucilage that was considered in the analysis of the results corresponds to that reported by Capitani *et al*. [11]. The meal was obtained after oil solvent extraction (*n*-hexane) in a Soxhlet apparatus (Buenos Aires, Argentina) by thermal cycles at 80ºC for 8 h, following the IUPAC Standard Method [33], of chia seeds previously ground in a laboratory grinder (Moulinex, horizontal blade grinder, Buenos Aires, Argentina).

Meals (Msm and Ms) were homogenized and stored in plastic vessels at 5ºC until further use.

2.4. Scanning Electron Microscopy (SEM)

The whole seeds and seeds after mucilage removal were adhered to a cover slip, coated with a thin gold film (600 Å) in a sputter coater (Pelco 91000) and observed in a scanning electron microscope (LEO model EVO 40) at 5 kV. Longitudinal sections were sliced with a razor blade, after being plunged into liquid nitrogen to ensure the maintenance of their internal structure, and analyzed by microscopy using the same procedure and magnification ranges between x136 and x5000.

2.5. Characterization of meals

2.5.1. Proximate composition

Moisture, crude fiber and ash content were determined according to AOCS recommended practices Ba 2ª-38, Ba 6-84 and Ba 5ª-49, respectively [34]. Oil and nitrogen content (N) were

determined following IUPAC Standard Method [33] and AOAC Method [35], respectively. Protein content was calculated as nitrogen x 6.25. Carbohydrate content was estimated by calculating the nitrogen-free extract (NFE) by difference using Eq. (1).

$$\text{NFE:} 100 - (\text{oil} + \text{protein} + \text{crude fiber} + \text{ash}) \quad (1)$$

2.5.2. Total, soluble and insoluble dietary fiber

Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were determined according the enzymatic gravimetric method [36].

2.5.3. Neutral Detergent fiber (NDF), Acid Detergent fiber (ADF), lignin, cellulose and hemicellulose

The vegetable cell was separated into two parts (Van Soest method): cell content (highly digestible) and cell wall (partially digestible). The cell wall was analyzed and its components (cellulose, hemicellulose and lignin) were determined. The technique makes use of acidic and neutral detergent [35, 37].

2.5.4. Antioxidant activity

The extraction of phenolic compounds was carried out according to the method of Re *et al*. [38]. Ten mL ethanol were added to 1 g sample, then it was homogenized in Vortex for 2 min, decanted and filtered (0.45 μm nylon paper). The supernatant was transferred into a flask and evaporated using a rotavapor apparatus (BUCHI R124, Germany) to concentrate the sample. It was then redissolved in 1000 μL ethanol.

A spectrophotometric method was used to determine the antioxidant activity using a Hitachi U-1900 UVeVIS spectrophotometer (Japan). The antioxidant activity was quantified by a dying assay of the radical cation $ABTS^+$ measuring $ABTS^+$ reduction as the percentage of absorption inhibition at 734 nm, just 6 min later. The radical cation ABTS and potassium persulfate were obtained from Sigma Aldrich. Chlorogenic acid was used as standard antioxidant. Results were expressed as μmol/L Trolox g/sample, considering that chlorogenic acid diminishes twice the amount of absorption than Trolox [39].

2.5.5. Functional properties

2.5.5.1. Water-Holding (WHC) and Oil-Holding Capacity (OHC)

Water and oil holding capacities were determined according to the method of Chau *et al*. [40]. Briefly, 1 g (dry base (d.b.)) sample was weighed and then stirred into 10 mL distilled water or corn oil (density 0.92 g/mL, Arcor). These suspensions were centrifuged at 2200 x g for 30 min (Rolco Centrifuge Refrigerate, Model CR-5850, 22 cm radius, Buenos Aires, Argentina) and the supernatant volumes were measured. Water-holding capacity was expressed as gram water held per gram sample, and oil-holding capacity as gram oil held per gram sample.

2.5.5.2. Water Absorption Capacity (WA_bC)

This property was determined according to the AACC method 88-04 [41]. Approximate water absorption capacity was first determined by weighing out 2 g (d.b.) sample, adding water until saturation (approx. 35 mL) and centrifuging at 2000 x g for 10 min in a Rolco Model CR-5850, 22-cm radius centrifuge (Buenos Aires, Argentina). Approximate water absorption capacity was calculated by dividing the increase in sample weight (g), by initial weight, quantifying the water needed to complete the original sample weight (2 g d.b.) to 15 g. Water absorption capacity (WAbC) was then determined by placing samples in four tubes, adding different quantities of water (1.5 and 0.5 mL water above original weight, and 1.5 and 0.5 mL water below; one in each tube), agitating vigorously, and centrifuging the samples at 2000 x g for 10 min in a Rolco Model CR 5850. The supernatant was discarded and the residue weighed. Average water absorbed was calculated, and WA_bC was determined and expressed as gram water absorbed per gram sample.

2.5.5.3. Organic Molecule Absorption Capacity (OMAC)

This capacity was determined according to the method of Zambrano *et al*. [19]. A three gram (d b.) sample was placed in excess quantity corn oil (approx. 25 mL) for 24 h at room temperature, and then centrifuged at 2000 x g for 15 min in a Rolco Model CR-5850. OMAC was expressed as the absorbed hydrophobic component and calculated in terms of sample weight gain (g oil/sample g).

2.5.5.4. Emulsifying Activity (EA) and Emulsion Stability (ES)

These properties were evaluated according to the method [36] of Chau *et al*. [40]. Briefly, 100 mL 2 g/100 mL suspension was homogenized using an Ultra-Turrax T25 disperser (Janke & Kunkel, IKA-Labortechnik, Germany) at 7800 rpm for 2 min. Then, 100 mL corn oil (density 0.92 g/mL, Arcor) were added and homogenized at 15,000 rpm for 2 min. Emulsions were centrifuged in a 15 mL graduated centrifuge tube at 455 x g for 10 min, and then emulsion volume was measured. The EA was expressed as the remaining volume of the centrifuged emulsion corresponding to 100 mL of initial emulsion. The emulsion stability was determined by heating the emulsions to 80ºC for 30 min, cooling them to room temperature and then centrifuging the samples at 455 g for 10 min. ES was expressed as the remaining volume of the centrifuged emulsion corresponding to 100 mL of initial emulsion. On the other hand, all emulsions were evaluated by optical characterization using a Vertical Scan Analyzer (QuickSCAN, Beckman Coulter, Fullerton, USA). The QuickSCAN head scans the entire length of the sample (approximately 65 mm), collecting backscattering (BS) data every 40μm. Thus, it is possible to obtain curves showing the percentage of backscattering light flux, relative to external standards, as a function of the sample height in mm [42]. Coalescence kinetics were determined by measuring the mean values of BS as a function of time in the 25-30 mm zone (Backscattering % 25-30 mm).

2.6. Statistical analysis

The results obtained were analyzed using ANOVA and Tukey's test ($p \leq 0.05$), using Infostat software [43].

3. Results and discussion

The nutlet of Salvia hispanica consists of the seed and a pericarp surrounding the seed. The true seed, in turn, consists of a coat (testa), the endosperm and the embryo, consisting mainly of two cotyledons [1]. Basically, the pericarp of the chia seed is similar to that other Nepetoideaes because it shows cuticle, exocarp, mesocarp, layers of sclereids and endocarp. The cells of the mesocarp and exocarp are parenchimatic. Figure 1 shows a scanning electron microscopy of a *Salvia hispanica* nutlet. In the exocarp there often are cells that produce mucilage when the nutlets get wet (Figure 1D).

Figure 2 shows SEM microscopy of *Salvia hispanica* L. seeds after mucilage extraction. In these images it can be observed that the mixocarpy phenomenon occurs in the outer layers (cuticle and exocarp). After removing the mucilage, the nutlet surface is characterized by small hill-like eminences, spaced, that cover the entire surface, corresponding to the mesocarp cells. Chia seeds presented a similar structure to that of two mucilaginous species (*Carrichtera annua* and *Anastatica hierochuntica)*, which could be associated with the presence of concentric aggregates of glucuronic acid [44]. The retention of the mucilage close to the seed can be due to the association of the mucilage with the columella (a secondary wall cell produced after mucilage secretion) and portions of the cell wall [45].

The proximate composition of chia meals with and without mucilage is presented in Table 1. Both meals were characterized by a high protein content, higher than that reported for sunflower meals of different origin (20.6-23.1%) [46] and canola meals (36.1-40.0%) [47, 48], and within the range of the values reported for linseed meals (38.9-43.3%) [48, 49].

On the other hand, both types of meals presented a high crude fiber content, with values higher than those reported for sesame, soybean, linseed and canola meals, 5.8%, 3.5%, 5.27% and 11.54%, respectively [50, 48].

In Table 2 it is possible to observe that both types of meals presented a high content of TDF, consisting mainly of IDF. Even though the value of SDF was relatively low, this could be attributed to the fact that, during the determination of this fiber, some components were not quantified because they cannot precipitate during the treatment with ethanol, and thus SDF was underestimated [26]. It is worth noting that the meal obtained from seeds that previously had their mucilage extracted (Msm) exhibited a statistically higher content of IDF ($p<0.05$) than that for Ms, at the expense of a significant decrease in its SDF content. These results are consistent with the data obtained from the analysis of NDF, consisting of cellulose, hemicellulose and lignin (structural polysaccharides that contribute to the IDF fraction), which was statistically higher ($p<0.05$) in Msm (Table 3). Regarding Ms, it presented a better IDF/SDF balance, with a 89/11 ratio.

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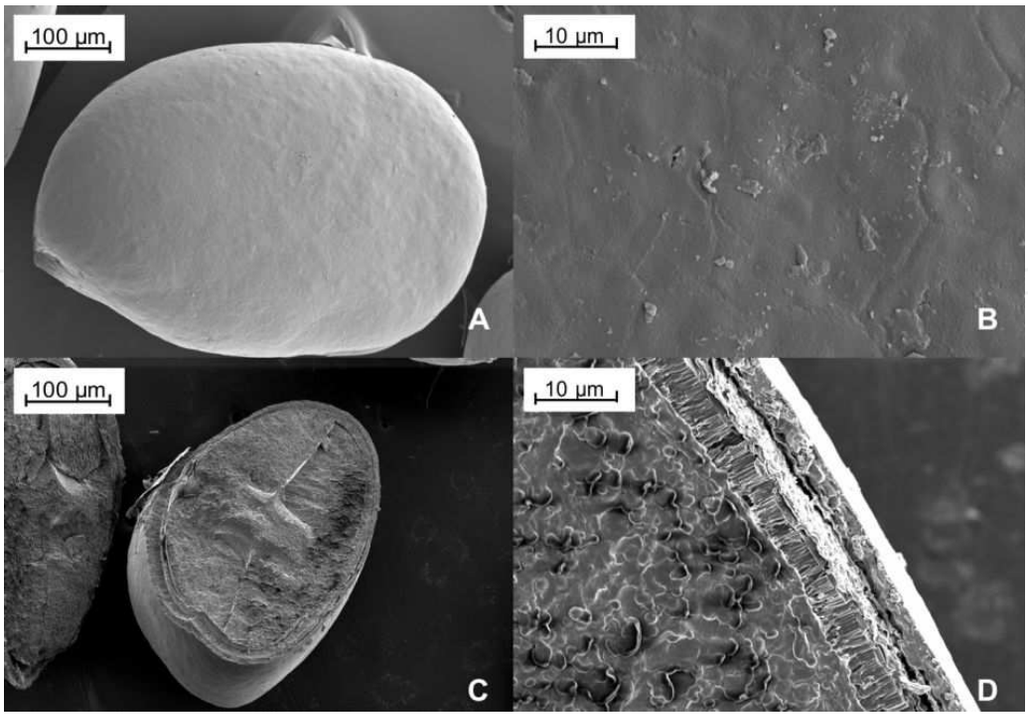


Figure 1. SEM microscopy of *Salvia hispanica* nutlets. (A) Whole nutlet, lateral view (x150), (B) pericarp surface (x5000), (C) broken nutlet, longitudinal section (x149), (D) broken nutlet (x1500).

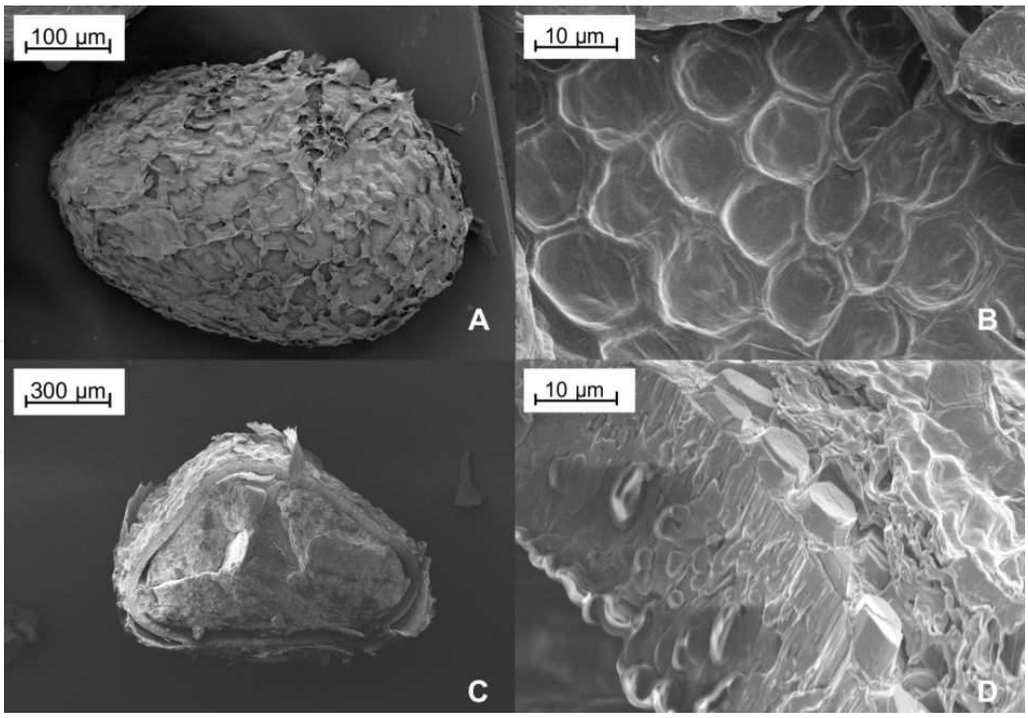


Figure 2. SEM microscopy of *Salvia hispanica* L. nutlets after mucilage extraction. (A) Whole nutlet (x136), (B) nutlet surface (x5000), (C) broken nutlet (x150), (D) broken nutlet (x3500).

Component	Msm	Ms #
Moisture	10.66 ± 0.04 [a]	10.47 ± 0.16 [a]
Protein*	42.43 ± 0.71 [a]	41.36 ± 0.28 [a]
Crude fiber	27.75 ± 0.97 [a]	27.57 ± 0.07 [a]
Ash	7.82 ± 0.13 [a]	7.24 ± 0.15 [a]
Oil	0.22 ± 0.25 [a]	0.21 ±0.08 [a]
NFE	24.17 ± 0.76 [a]	23.62 ± 0.94 [a]

[#] Capitani *et al*. [11]

Mean value (n = 3)

Values followed by different letters differ significantly ($p \leq 0.05$), according to Tukey's test.

*Factor: 6.25; NFE: nitrogen-free extract.

Table 1. Proximate composition of chia (*Salvia hispanica* L.) meals (% d.b.)

Component	Msm	Ms #
TDF	47.13 ± 0.17 [a]	46.06 ± 0.86 [a]
IDF	45.62 ± 0.37 [b]	41.13 ± 0.47 [a]
SDF	1.51 ± 0.24 [a]	4.93 ± 0.65 [b]

[#] Capitani *et al*. [11]

Mean value (n = 3)

Values followed by different letters differ significantly ($p \leq 0.05$), according to Tukey's test.

Table 2. Total (TDF), soluble (SDF) and insoluble (IDF) dietary fiber of chia (*Salvia hispanica* L.) meals (% d.b.)

Component	Msm	Ms #
NDF	63.6 ± 2.1 [b]	53.9 ± 0.3 [a]
ADF	30.8 ± 1.2 [a]	38.1 ± 1.2 [b]
Lignin	6.9 ± 0.5 [a]	4.5 ± 0.7 [a]
Cellulose	23.1 ± 0.9 [a]	34.6 ± 1.3 [b]
Hemicellulose	33.6 ± 1.0 [b]	14.8 ± 1.2 [a]

[#] Capitani *et al*. [11]

Mean value (n = 3)

Values followed by different letters differ significantly ($p \leq 0.05$), according to Tukey's test.

Table 3. Fiber composition of chia meals analyzed according to the method of Van Soest (% d.b.)

The antioxidant activity of the two chia meals compared with other types of meals is shown in Table 4. Both for Ms and Msm, the activity was high, without a significant difference between them ($p>0.05$). These values were higher than those found for wheat bran and sorghum and barley whole grain meals. But they were significantly lower than those found for chia meal obtained as a byproduct of cold-pressing oil extraction. The latter could be attributed to the fact that the meal obtained by pressing shows a higher percentage of residual oil (11.39% d.b.), which contains tocopherols, a class of compound with natural antioxidant activity [11].

Sample	Trolox equivalent antioxidant coefficient (TEAC, μmol/g)
Msm	187.4 ± 33.21 [a]
Ms[1]	226.6 ± 4.13 [a]
Chia meal from oil pressing extraction[1]	557.2 ± 28.18 [b]
Wheat bran [2]	48.5
Sorghum meal [3]	51.7
Barley meal [3]	14.9

[1] Capitani *et al*., [11]

[2] Iqbal *et al*. [57]

[3] Ragaee *et at*. [58]

Mean value (n = 3)

Values followed by different letters differ significantly ($p \leq 0.05$), according to Tukey's test.

Table 4. Antioxidant activity of chia (*Salvia hispanica* L.) meals compared with other meals

Regarding the functional properties, the meal with mucilage (Ms) exhibited a statistically higher absorption and water holding capacity ($p<0.05$) than that of the meal without mucilage (Msm) (Table 5). This behavior can be associated with the presence of mucilage in Ms, which acts as soluble dietary fiber, capable of holding water inside its matrix [51]. The WAbC of both meals was higher than that observed for canola and soybean meals (3.90 g/g and 3.28 g/g, respectively) and similar to that of linseed meal (6.03 g/g) [48].

Both types of chia meals presented a low absorption of organic molecules and oil-holding capacity, being significantly higher in Msm. These differences could be explained in terms of the particle size and the cellulose content of the meal [52, 53]. The determination of OHC is

important because it is related to the capacity that food components have to hold oil, affecting their flavor and mouthfeel [54]. The OMAC is associated with the interaction of the fiber with fats, bile acids, cholesterol, drugs, and toxic and carcinogenic compounds at the intestinal level. Due to their low OHC levels, both types of chia meals could be considered an important ingredient in the manufacture of fried products due to their low fatty mouthfeel contribution. It is noteworthy that the OHC values were higher than those reported for the *Jessenia polycarpa* fruit meal [55] and similar to those of the fibrous residue of *Canavalia ensiformis* and barley [53, 56] and those reported by Khattab and Arntfield [48] for linseed and canola meals (2.01 g/g and 2.09 g/g, respectively).

Property	Msm	Ms #
WHC (g/g)	5.25 ± 0.39 [a]	10.64 ± 0.60 [b]
WA_bC (g/g)	4.79 ± 0.49 [a]	6.45 ± 0.41 [b]
OHC (g/g)	2.94 ± 0.14 [b]	2.03 ± 0.08 [a]
OMAC (g/g)	2.22 ± 0.01 [b]	1.64 ± 0.02 [a]

[#] Capitani *et al*. [11]

Mean value (n = 3)

Values followed by different letters differ significantly ($p \leq 0.05$), according to Tukey's test.

Table 5. Functional properties of chia (*Salvia hispanica* L.) meals

In Figure 3 it can be noted that the differences observed in the emulsifying activity were not significant, although the stability of the emulsion prepared with Ms was statistically higher than that with Msm ($p<0.05$). This effect can be associated with the capacity of mucilage to act as a thickening agent due to its ability to increase the viscosity of the aqueous phase in an O/W emulsion, thus hindering movement of the oil droplets of the dispersed phase [59]. This property is similar in the linseed mucilage, which has a strong thickening capacity, favorably affecting the water-holding capacity and the emulsifying properties of defatted linseed flour [60].

The behavior of the meals studied with respect to emulsion stability, examined by their optical characterization with a vertical scan analyzer (QuickScan), is shown in Figure 4. Both meals presented a high initial emulsifying capacity (66.3 and 62.2 %BS. for Ms and Msm, respectively), which remained approximately constant for all the time span studied (60 min) for Ms. However, the emulsion stability of the meal without mucilage decreased markedly by the end of the 60 min (50.4 %BS).

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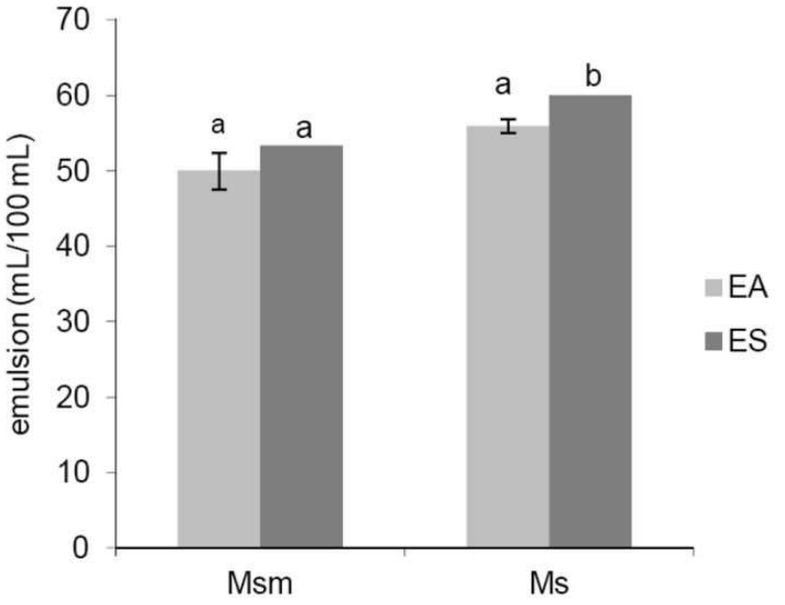


Figure 3. Activity and stability of O/W emulsions (50:50 p/p) with chia meal with and without mucilage. Values followed by different letters differ significantly (Tukey's test, p ≤0,05)

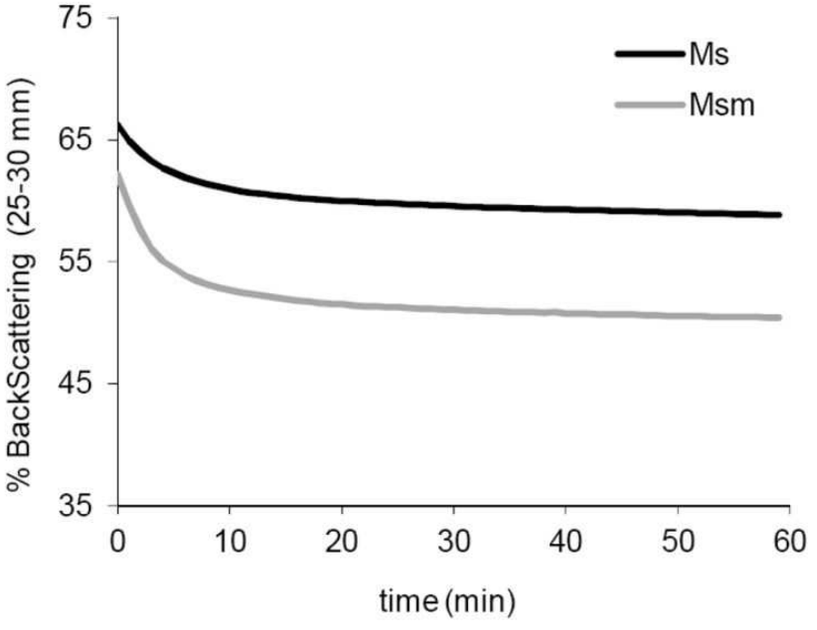


Figure 4. Destabilization kinetics of O/W emulsions (50:50 p/p) with chia meal

4. Conclusions

The results obtained show that both types of chia meals (with and without mucilage) present interesting functional properties for the food industry, which could be applied for example in the manufacture of bakery products, powdered beverages, yogurts, ice-creams, sauces and creams. They also suggest the potential use of two chia byproducts: the residual meal obtained after the oil extraction of whole chia seeds, and the use of a byproduct of the mucilage extraction. As regards the formulation of stable emulsions, the meal with mucilage is recommended for use given the role of mucilage as a thickening agent. From a physiological point of view, the presence of mucilage becomes a potentially interesting food ingredient due to its health benefits, since it has the capacity to form high-viscosity gels, slowing the intestinal transit, providing more of a feeling of satiety, and helping to prevent diseases such as obesity, colon cancer, hypercholesterolemia and diabetes.

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