EXTRACTION AND CHARACTERIZATION OF CHIA (*Salvia hispanica* L.) MUCILAGE FOR APPLICATION IN FOOD PRODUCTS

VIVIANE DA COSTA BORGES[1], MYRIAM DE LAS MERCEDES SALAS-MELLADO[2]

ABSTRACT

The present study aimed to carry out the extraction, characterization and comparison of oven-dried and freeze-dried chia mucilage, for use in food products. The chia seed mucilage was extracted and dried by two different methods: freeze-drying and conventional drying in an oven. The composition, microstructure and functional properties of the two mucilage were evaluated for application as food ingredients. The analyses held in the dried mucilage were % of yield, the determination of the functional properties: water holding capacity, oil retention capacity, emulsifying capacity and solubility, besides color and microscopy. The dried and freeze-dried chia mucilage showed similar proximal composition and distinct functional properties that confirm their use as ingredients in food products. The scanning electron microscopy analysis showed that the oven-dried mucilage has a structure similar to a film while the lyophilized mucilage has a fibrous and spongy structure.

Keywords: FOODS, FUNCTIONAL PROPERTIES, INGREDIENT.

EXTRAÇÃO E CARACTERIZAÇÃO DA MUCILAGEM DE CHIA (*Salvia hispanica* L.) PARA APLICAÇÃO EM PRODUTOS ALIMENTÍCIOS

RESUMO

O presente estudo teve como objetivo realizar a extração, caracterização e comparação de mucilagem de chia seca em estufa e liofilizada, para uso em produtos alimentícios. A mucilagem das sementes de chia foi extraída e seca por dois métodos diferentes: liofilização e secagem convencional em estufa. A composição, microestrutura e propriedades funcionais das duas mucilagens foram avaliadas para aplicação como ingrediente alimentar. As análises realizadas na mucilagem seca foram % de rendimento, determinação das propriedades funcionais: capacidade de retenção de água, capacidade de retenção de óleo, capacidade de emulsificação e solubilidade, além de cor e microscopia. A mucilagem de chia seca e liofilizada mostrou composição proximal semelhante e propriedades funcionais distintas que confirmam seu uso como ingredientes em produtos alimentícios. A análise por microscopia eletrônica de varredura mostrou que a mucilagem seca em estufa possui uma estrutura semelhante a um filme, enquanto a mucilagem liofilizada possui uma estrutura fibrosa e esponjosa.

PALAVRAS-CHAVE: ALIMENTOS, INGREDIENTE. PROPRIEDADES FUNCIONAIS,

[1]Discente – Universidade Federal do Rio Grande (FURG)/EQA/PPG-ECA – e-mail: vivicborges88@gmail.com
[2]Docente – Universidade Federal do Rio Grande (FURG)/EQA/PPG-ECA – e-mail: mysame@yahoo.com

1. INTRODUCTION

Salvia hispanica L., also known as chia, is a herbaceous plant of the Labiatae family, Spermatophyta division and Plantae kingdom [2].Chia seeds are used as a nutritional supplement, as well as in the manufacture of cereal bars, breakfast cereals and biscuits in the United States, Latin America and Australia [11].

In 1996, chia seeds were recognized by FAO as a potential source of polysaccharides, mainly resulting from their mucilage. This mucilage is constituted by a polysaccharide whose molecular weight was determined by gel filtration and ranges from 0.8-2.0 x 10^{-6} daltons. The chia mucilage, considered a natural gel of chia seeds is a new ingredient, composed mainly of xylose, glucose and glucuronic acid forming a branched polysaccharide [13, 16].

Chia seed has in its composition a percentage of 5-6% (m/m) of mucilage, which is a secretion rich in polysaccharides, responsible for the retention of water by the seed contributing to its increase in volume when placed in an aqueous medium [16]. The gel formed has qualities that allows its application in various products in the food industry [2], such as thickener, gel former and quelator [6].

The mucilage appears as an existing transparent film which is a transparent mucilaginous gel, reaches its maximum thickness after 2 h of hydration, forming two layers, an inner consisting of branched structures and an outer one. A strong link between the seed and mucilage is also observed, assuming that the mucilage is located in the outer layer that forms the seed coat, in the so-called mucilage cells [16].

Due to the functional properties of chia mucilage, it has the potential to be used as a thickener in food formulations, and can be used as an emulsifying agent, depending on its capacity and emulsion stability. It can also be applied to frozen food products due to their freeze-thaw stability [9].

Work evaluated the use of mucilage as replacer of emulsifier and stabilizer in ice cream [4], and other work [12] verified the reduction of 56.6 of fat in bread and 51.6 in cakes, when chia mucilage was used as fat replacer in the formulation of the products.

The present study aimed to carry out the extraction, characterization and comparison of two types of drying of chia mucilage and its potential of use in food products.

2. MATERIALS AND METHODS

2.1.Obtainment of chia flour

Chia seeds used were provided by Chá & Cia Company, Jacarei, São Paulo, Brazil. To obtain chia flour, chia seeds were ground in a double-knife blender (ARNO model PL, Brazil) and sieved to a particle size of 14 mesh.

2.2.Extraction, drying and determination of %yield of chia mucilage

The extraction of the mucilage was performed according to] MUÑOZ et al. [15], with some adaptations, where chia seeds were hydrated in a 1:40 (seed:water) ratio and stirred in a shaker (Cientec, model CT-712RNT, Brazil) at a speed of 125 rpm for 2h. Two stages were executed to separate the seed mucilage, vacuum filtration using a vacuum pump (Quimis, model Q355, Brazil), and centrifugation in a centrifuge (Hanil, model Supra 22, Korea) at 11,600 x g for 20 min, where the supernatant obtained was the chia mucilage. To obtain dry mucilage, the aqueous suspension was spread in shapes and placed in an oven (Quimis, model Q-314 D242, Brazil) with a temperature of 50°C for 24 h, and to obtain the lyophilized mucilage the suspension was frozen in Ultrafreezer (Indrel, modelo IULT 90-D, Brazil) at -80°C for 48 h and dried in a lyophilizer (Liotop, model L108, Brazil). To determine the seed/water ratio during the hydration step, mucilage yield analysis was carried out. 1:20, 1:30 and 1:40 (seed/water) ratio concentrations were tested and the yield was calculated by measuring the mucilage weight obtained over the chia seeds weight .Yield was obtained in percentage (%).

2.3. Water and Oil Holding Capacity

The chia flour and the dried and lyophilized mucilage were analyzed. The water holding capacity (WHC) and the oil retention capacity (ORC) were determined following the methods described by CHAU et al. [7] with modifications. For the determination of WHC, 0.1 g of sample was added to 10 ml of distilled water, then stirred for 1 min in a vortex mixer (Warmnest, model VX-38, Brazil) and centrifuged at 2,200 × *g* for 30 min in a centrifuge (Marconi, model MPW-350, Brazil). The supernatant was discarded and the remaining wet sample in the centrifuged tube was weighed. The results were expressed as the mass (g) of water retained per mass (g) of the sample. The ORC was determined using the same method as WHC with water replaced by soya oil.

2.4. Emulsifying Capacity

The emulsifying capacity (EC) was measured according to the method of COOREY et al. [8] with modifications. A suspension of 1% (w/w) of flour and/or mucilage was prepared in distilled water in a volumetric flask and transferred to a 500 mL flask. The suspension was then homogenized with 100 ml of soya oil during 10 min with the aid of an Ultraturrax (IKA, model T25, Brazil). The samples were centrifuged (Hanil, 22K Supra, Korea) at 3,870 x g for 15 min. The volume of the emulsion layer was measured in a test tube. The result of the EC was expressed as a percentage and calculated using Equation 1.

$$\text{EC (\%)} = 100 \times \text{the total emulsified layer (ml) /total suspension volume} \quad (1)$$

2.5. Solubility

The solubility of chia flour and dried and lyophilized chia mucilage were determined according to the method described by BETANCUR-ANCONA et al. [3]. First, a 40 mL suspension of 10 $g.L^{-1}$ w/v sample was prepared in a 50 mL centrifuge tube. The solution was stirred for 30 min in a shaker (Cientec, model CT-712RNT, Brazil) at room temperature. Later, the suspension was centrifuged at 3,415 x g for 15min in a centrifuge (Hanil, model 22K Supra, Korea). An aliquot of 10mL of the supernatant was dried in an oven at 120°C for 4 h. The solubility was calculated according to Equation 2

$$\text{Solubility } (g.kg^{-1}) = \text{dry weight at 120°C} \times 400/\text{ sample weight} \quad (2)$$

2.6. Color

Color analysis of chia flour and dried and lyophilized mucilage were held in a Minolta® CR400 colorimeter. This was determined following the color system in the space L*a*b* or CIE-L*a*b* defined by CIE (International Commission on Illumination) in 1976, evaluating the L* (lightness), a* and b* (chromaticity coordinates) values.

2.7. Proximal composition

The proximal composition of chia flour and mucilage was determined [1] with the following analyses being performed:

a) Moisture: determined according to method No. 44-15A.

b) Ashes: determined according to method No. 08-01. A time of five hours was used at 600°C, in muffle.

c) Protein: determined by the Kjeldahl (Nx6.25) method No. 46-13.

d) Lipids: The fat content was determined by the Soxhlet method, No. 30-20.

e) Crude Fiber: determined according to method No. 32-05-01.

f) Carbohydrates: The total carbohydrate content was estimated by difference.

2.8. Scanning electron microscopy

Analysis of scanning electron microscopy (SEM) was performed to analyze the microstructure of 6 samples: chia seed, chia flour, freeze-dried mucilage, dried mucilage, chia seed after lyophilized hydration, and chia seed after oven-dried hydration. The surface of the samples was coated with gold to improve conductivity, and the samples were analyzed by electron microscope (JEOL, model JSM-6610LV) at an accelerating voltage of 10 kV.

2.9. Analysis with optical microscope and stereomicroscope

Analysis with Optical Microscope (Labimex, model PZO, USA) and Stereomicroscope (Tecnival, model SQZ-DS4, Brazil) was performed to analyze the structure of 6 samples: chia seed, chia flour, freeze-dried mucilage, dry mucilage, chia seed after lyophilized hydration, and chia seed after oven-dried hydration. The microscope images are magnified 40x and stereo images of the magnified of 2.5x. Photos were taken with a phone (Samsung, model galaxy SIII, South Korea) coupled to the machine to record the samples of the structures.

2.10. Data processing

All analyses were performed in triplicate. The analysis results were statistically analyzed using analysis of variance (ANOVA) and compared using the Tukey test ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

The chia seed has 5-6% of mucilage that was considered difficult to remove. In Table 1, one can see that the seed: water concentration that reached highest yield was the ratio 1:40, being the one chosen to carry out the extraction of dried and lyophilized mucilage. MUÑOZ et al.[16] reported that was obtained higher yield value 7% in chia mucilage extraction using the concentration 1:40.

TABLE 1. Extraction yield of chia mucilage.

Concentration	DM Yield (%)	LM Yield (%)
01:40	4.96 ± 0.15^{ab}	5.23 ± 0.15^{a}
01:30	4.57 ± 0.10^{b}	4.94 ± 0.17^{ab}
01:20	3.72 ± 0.11^{c}	3.89 ± 0.18^{c}

DM = dry mucilage. LM = lyophilized mucilage. Means followed by the same letter do not differ significantly ($p < 0.05$) from each other in the row and column.

Chia mucilage is a new ingredient and presents interesting characteristics for the development of food products. The proximal composition and the functional properties of flour and chia mucilage are shown in Table 2.

TABLE 2. Proximal composition and properties of chia flour and mucilage.

Components/Properties		Chia flour	Lyophilized mucilage	Dry mucilage
Moisture (%)		7.06 ± 0.28[b]	11.48 ± 0.51[a]	12.28 ± 0.16[a]
Proteins (%)		22.58 ± 0.26[a]	7.08 ± 0.14[b]	6.94 ± 0.43[b]
Lipids (%)		36.03 ± 0.13[a]	2.46 ± 0.47[b]	2.53 ± 0.61[b]
Ashes (%)		5.95 ± 0.08[c]	7.07 ± 0.08[b]	7.64 ± 0.03[a]
Crude fiber (%)		26.28 ± 0.31[b]	34.81 ± 1.06[a]	35.19 ± 0.71[a]
Carbohydrates (%)		9.16	48.58	47.70
WHC(g/g)		27.86 ± 1.18[b]	159.12 ± 14.61[a]	177.12 ± 4.45[a]
ORC(g/g)		19.01 ± 1.11[b]	60.15 ± 5.28[a]	23.45 ± 0.81[b]
EC (%)		23.33 ± 1.44[c]	44.16 ± 1.44[a]	33.33 ± 1.44[b]
Solubility (g/kg)		116.63 ± 7.42[c]	147.48 ± 2.84[b]	164.83 ± 3.79[a]
	L*	40.16 ± 0.85[c]	92.98 ± 1.54[a]	86.28 ± 0.14[b]
Color	a*	3.96 ± 0.37[a]	-0.95 ± 0.19[c]	0.61 ± 0.04[b]
	b*	14.32 ± 0.69[a]	2.94 ± 0.64[c]	4.66 ± 0.03[b]

WHC = water holding capacity. ORC = oil retention capacity. EC = emulsifying capacity. Means followed by the same letter in the row does not differ significantly ($p < 0.05$) from each other.

In general, chia flour has high levels of proteins (22.58) and lipids (36.03). Its protein content is higher than many cereals, including wheat (14%), maize (14%), rice (8.5%), oat (15.3%) and barley (9.2 %) [10]. The composition of chia flour was similar to that reported by SALGADO-CRUZ et al. [17].

The proximal composition of dried and lyophilized mucilage is very similar, and both differ from chia flour composition. The freeze-dried and oven-dried mucilages showed larger proteins, lipids and ash content than values reported by COOREY, TJOE and JAYASENA [9], probably due to the mucilage extraction method that does not separate mucilage seed in its entirety. Was obtained a chia seed mucilage with high protein and ash content, 11.2% and 8.4% respectively, the method used by these authors to separate mucilage from seeds was friction in the strainer, followed by a lyophilization drying [5].

In other work, SEGURA-CAMPOS et al. [18] obtained a mucilage of the chia seed containing 28.96% crude fiber, and CAPITANI et al. [5] verified that the chia seed had a crude fiber content of 27.6% and the mucilage extracted had a content of 13.5%.

We can conclude that the crude fiber content of the chia flour presented in Table 2 is in line with what was expected, and that it was possible to obtain mucilages with higher crude fiber content when compared to previous work, probably due to differences in the method of extraction of the mucilage.

It can be verified that the chia mucilage has very different functional properties when compared with chia flour, showing higher WHC, ORC, EC and solubility values. These mucilage characteristics may be due to its high fiber content. The main physiological effect of the fiber comes from its ability to absorb and hold water. This capability results from the presence of polysaccharides.

Between the two mucilage obtained, it was verified statistical difference between the values of the functional properties, except in the WHC. The oven-dried mucilage showed higher solubility, and lower ORC and EC values when compared to lyophilized mucilage. Both mucilage presented higher WHC values ranging from159.12 to 177.12 compared with that found by SEGURA-CAMPOS et al. [18] who obtain a value of 103.2 of the mucilage obtained from chia flour and defatted chia flour.

The ORC of lyophilized mucilage was very similar to that found by COOREY, TJOE and JAYASENA [9], who studied the chia seed mucilage and chia flour mucilage, which showed an ORC of 58.61 g/g and 19.18 g/g respectively, with lyophilization being the drying process used.

The highest solubility among the studied samples was 164.83 g/kg for oven-dried mucilage, though this value is still low compared to that found by CAPITANI et a. [5] of 660 g/kg for chia seed mucilage of Argentine origin.

All this functional properties confirm that chia mucilage can be used as a new ingredient for the development of food products.

The color of chia flour is dark brownish with a Lightness L* value of 40.16 and chromaticity coordinates of a* = 3.96 and b* = 14.32, however mucilage were brighter with high luminance values of 92.98 and 86.28,showing that the lyophilized mucilage being brighter than the oven-dried mucilage, because the first did not suffer any thermal process therefore no developing a dark color.

Figure 1 shows the images obtained by scanning electron microscopy. In Figure 1a, the image of the intact chia seed can be seen as an elliptical shape and smooth surface, with no apparent roughening. Chia flour (Figure 1b) has high granularity, due to its high content of lipids, which did not allow a further reduction in particle size, forming clumps that hinder separation.

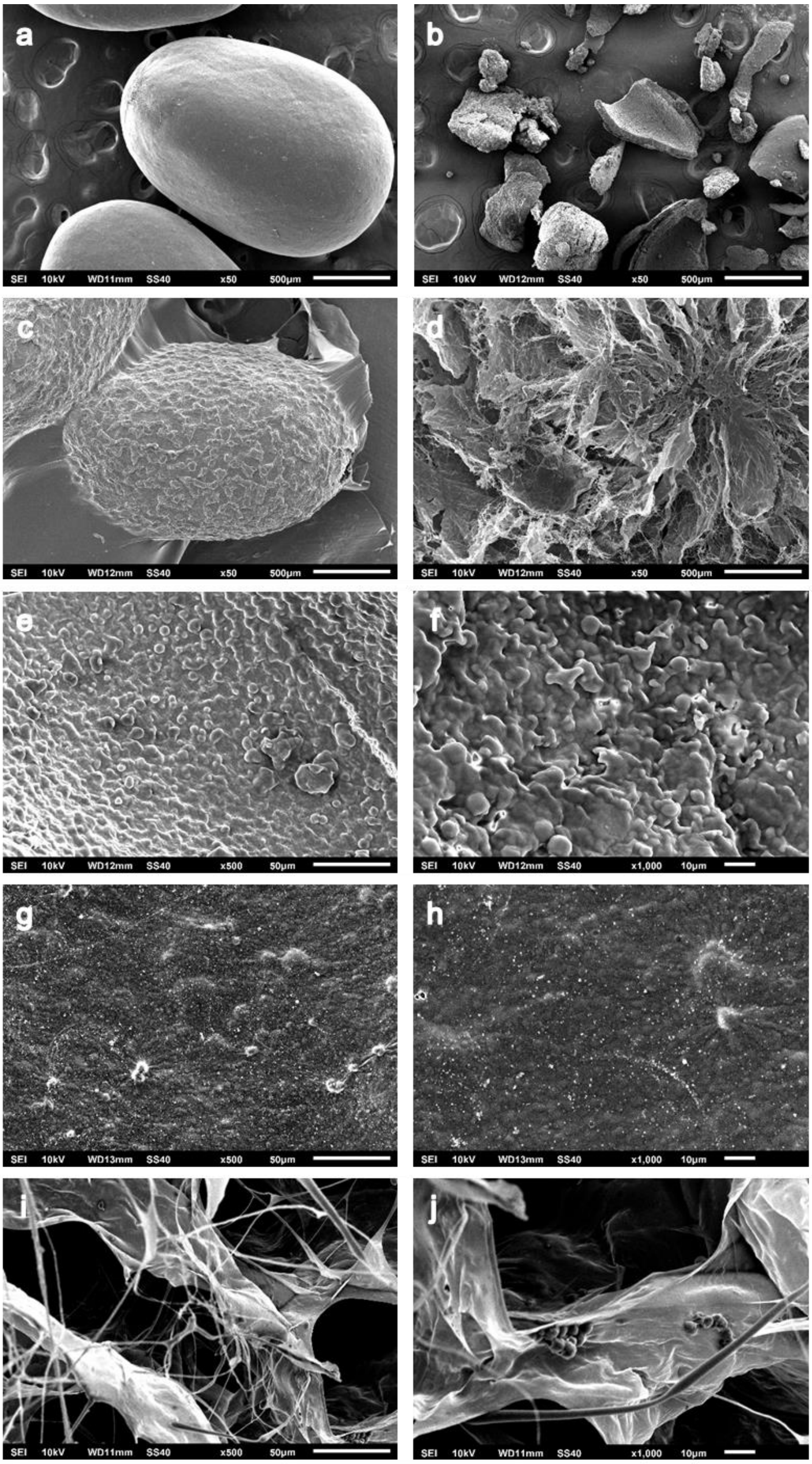

FIGURA 1. SEM images (a) chia seed (x50), (b) chia flour (x50), (c) hydrated and oven-dried chia seed (x50), (d) hydrated and lyophilized chia seed (x50), (e) chia flour (x500), (f) chia flour (x1000), (g) oven-dried mucilage (x500), (h) oven-dried mucilage (x1000), (i) lyophilized mucilage (x500), (j) lyophilized mucilage (x1000).

It can be seen in Figure 1c that after the seed was hydrated and dried in an oven, there was a formation of a roughened layer on its surface, and a thin film around it, clearly due to production of mucilage. However, when the hydrated seed was lyophilized (Fig.1d), the chia seed structure is not observed, as it was completely surrounded by the freeze-dried mucilage.

The chia flour structure (Figure 1e-f) has a high roughness on its surface, while the dry mucilage (Figure 1g-h) has a slightly roughened structure and white dots, which may be evidence of the presence of lipids. The lyophilized mucilage (Figure 1i-j) however has a very distinct structure from the others in several aspects, because its structure is porous, fibrous and dense, showing traces of fat globules as well. Was reported that the association between the various mucilage components forms a network structure of open pores, which provide interesting rheological properties and gel formation. The extraction, purification, drying and/or new modification processes may significantly affect the composition and molecular structure of natural plants consisting of biopolymers [5].

Was studied the effect of different drying techniques for drying the flow characteristics and chemical properties of natural gum mixtures of carbohydrate-protein derived from fruit seeds, they reported that gum dried by lyophilization revealed a higher porosity, different from oven-dried seed gums [14]. These authors reported that this behavior might be due to reduced thermal degradation, which probably resulted in less compact structure than other samples.

Figure 2 shows the images recorded using the optical microscope and stereomicroscope.

Figure 2 shows images of intact chia seed (Figure 2a), after 2 h of seed hydration, the formation of mucilage, a mucilaginous gel which exudes from the seed, is complete. When the hydrated seed is dried in an oven (Figure 2b) the mucilage forms a foil similar to a film, and when the hydrated seed is lyophilized (Figure 2c) the mucilage forms a spongy structure covering the chia seed. The optical microscope images show that chia flour structures (Figure 2d) and dried mucilage (Figure 2e) are compact and have rough texture, while the structure of the lyophilized mucilage (Figure 2f)is different showing a fibrous structure.

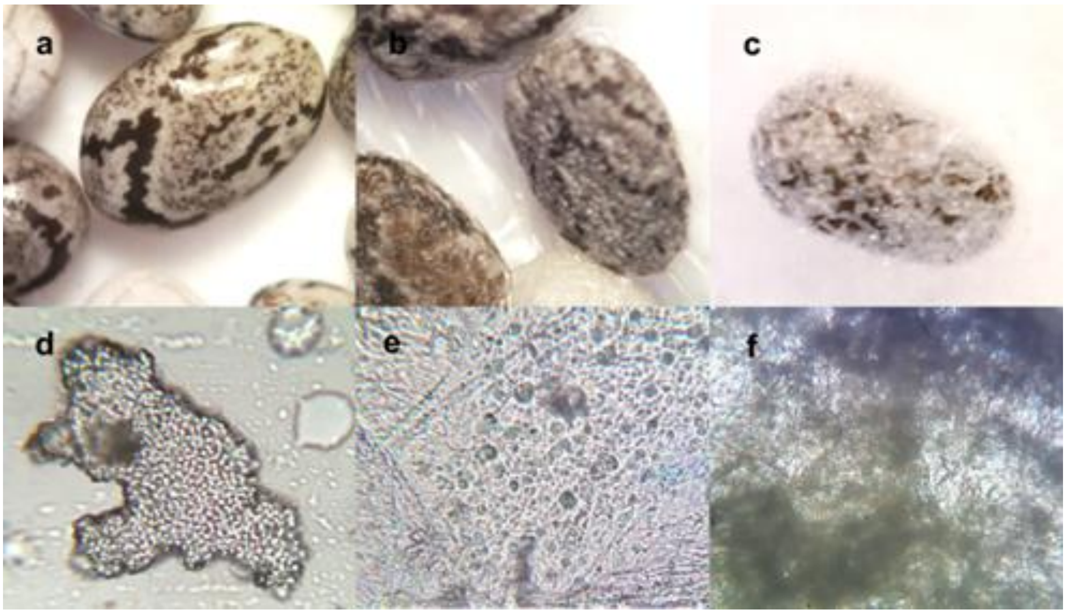

FIGURA 2. Images of the stereomicroscope (a) chia seed, (b) hydrated and oven-dried chia seed, (c) hydrated and lyophilized chia seed. Images of the optical microscope, (d) chia flour, (e) oven-dried mucilage, and (f) lyophilized mucilage.

4. CONCLUSION

Mucilage was extracted from chia seed and dried by two different methods: in an oven and by lyophilization. The proximate composition of both mucilage was similar, but due to the drying process, the mucilage had distinct functional and structural characteristics. Both types of mucilage can be applied in foods that need a high Water Holding Capacity and dried-oven mucilage presets a higher solubility compared to lyophilized mucilage. On the other hand, lyophilized mucilage may be used as emulsifying agent because of its higher Oil Retention and Emulsifying capacities. The SEM analysis of the lyophilized and dried mucilage revealed differences between their structures, oven-dried mucilage had a structure similar to a film, while the lyophilized mucilage had a spongy structure. Application studies should be performed on food products of the two mucilages obtained to verify if the difference in structure and functional characteristics will influence its performance as a thickener, gelling agent and emulsifier.

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