Research Article

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# Microstructure, chemical composition and mucilage exudation of chia (*Salvia hispanica* L.) nutlets from Argentina

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## Abstract

**BACKGROUND: The micromorphology and anatomy of nutlets, myxocarpy (mucilage exudation) and mucilage structure of Argentinean chia were described using scanning electron microscopy (SEM). The proximal composition of nutlets and mucilage was also studied.**

**RESULTS: Chia nutlets are made up of a true seed and a pericarp enclosing the seed; they are small, glabrous, elliptic and apically rounded. The pericarp has cuticle, exocarp, mesocarp and bone cells vertically arranged and endocarp. The myxocarpy was carefully recorded by SEM. After 5 min in contact with water, the cuticle of nutlets is broken and the exocarp cell content gradually surrounds the rest of the nutlet. The proximal composition of chia nutlets was studied; fat is the major component ($327 \pm 8.0$ g kg$^{-1}$) followed by protein ($293 \pm 4.0$ g kg$^{-1}$) and fiber ($276 \pm 1.0$ g kg$^{-1}$). Extractions of chia nutlets with water at room temperature yielded $38 \pm 1.0$ g kg$^{-1}$ (dry basis) of mucilage. The fresh mucilage structure was similar to a network of open pores. The freeze-dried crude mucilage contained more ash, residual fat and protein than commercial guar and locust bean gum. The solubility of 10.0 g L$^{-1}$ w/v solution of chia freeze-dried crude mucilage in water increased with temperature, being maximal at 60 °C (870 g kg$^{-1}$).**

**CONCLUSION: The results obtained show a fast exudation of chia mucilage when nutlets are in contact with water. The freeze-dried crude mucilage hydrates easily in water, even at low temperatures. Chia nutlets have mucilaginous substances, with interesting functional properties from a technological and physiological point of view.**


**Keywords:** *Salvia hispanica* L; mucilage; morphology; SEM; nutlet

## INTRODUCTION

*Salvia* L. is the largest genus of the family Lamiaceae, and is represented by about 1000 species. Based on the most recent classification, this genus is part of the subfamily Nepetoideae, tribe Mentheae, subtribe Salviinae.[1] Chia (*Salvia hispanica* L.) is an annual herbaceous plant belonging to this taxonomic hierarchy; it was widely used in pre-Columbian Mesoamerica as a major commodity and it was valued as a food, medicine and oil. Today, there is renewed interest in chia as an excellent source of omega-3 fatty acids, protein, antioxidants and dietary fiber for a healthy diet.[2–6] In 2009, it was approved as a novel food by the European Parliament and the European Council.[7] There is no evidence of adverse effects or allergenicity caused by whole or ground chia seeds;[8,9] thus chia seeds and derived products are promising sources of food.

The fruit of chia, as in other plants of the Lamiaceae family, is a schizocarp consisting of indehiscent locules which separate to form four fruitlets, referred to mericarps or nutlets.[10–12] Commercially, each of these fruitlets is called as a 'seed', but actually the (true) seed is contained within each fruitlet.[13,14] In this text we will refer to the fruitlet of chia as a 'nutlet'. Each of these nutlets has a stratified pericarp: cuticle, epicarp, mesocarp, a layer of bone cells and endocarp; the latter is in contact with the true seed.[14–16] The color of the *S. hispanica* nutlet ranges from black and black-spotted to white, although most chia commercialized today is black-spotted, followed by a low percentage of white nutlets.[17] These nutlets are ellipsoid in shape and the average principal dimensions are 2.11 mm length, 1.32 mm width and 0.81 mm thickness,[18] increasing linearly with moisture content in the range 46–177 g kg$^{-1}$ dry basis.[19]

When nutlets are soaked in water, a clear mucilaginous gel is exuded, which remains tightly bonded to the nutlet.[20,21] Mucilage plays a significant role in anchoring the nutlet to the soil. Even

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though the presence of mucilage may provide a considerable selective advantage under certain conditions, the production of mucilage may be costly for the plants, and it may be quickly lost where it has no function.[6,22] Recent information indicates that mucilage in Labiatae is only present in the subfamily Nepetoideae, in 75% of the genera and species.[16] The biological/ecological function of mucilage is still not clear and the correlation between the presence of mucilage and certain environmental conditions has not yet been established.

Chia nutlets contain 50–60 g $kg^{-1}$ mucilage, which is part of the soluble dietary fiber.[23,24] The addition of dietary fiber in foods can affect their texture, playing a role as texturing and stabilizing agents. In this sense, soluble dietary fiber contributes to the stabilization of food structure (dispersions, emulsions, etc.) through gel formation or thickening of the continuous phase.[25] Lin *et al.*[20] proposed a tentative structure of the basic unit of the polysaccharide, i.e. a tetrasaccharide with 4-*O*-methyl-$\alpha$-D-glucoronopyranosyl residues occurring as branches of $\beta$-D-xylopyranosyl on the main chain. This mucilage can be easily extracted and hydrated to achieve water retention of 27 times its weight in water and it has a great potential as a functional ingredient to be used as a thickener in foods.[21]

Scanning electron microscopy (SEM) is a technique particularly suited for structural and topographical examinations of seeds and fruits. In recent years, the importance of SEM in the study of nutlets has been demonstrated for various genera of Lamiaceae.[26] However, few studies have been carried out on *S. hispanica*. Therefore, the objectives of this work were to provide the characterization of the microstructure and description of the pericarp structure of Argentinean chia nutlets; to analyze their proximal composition; to study the mucilage exudation process when nutlets are in contact with aqueous media; to examine the proximal composition, and functional and microstructural characteristics of the mucilage. This information is useful in order to contribute to the botanical characterization and industrial application of chia nutlets.

## MATERIALS AND METHODS

### Material

Chia used in this study was a commercial source obtained from Salta, Argentina (25° S, 65.5° W). The nutlets were cleaned manually and foreign matter, such as stones, dirt and broken nutlets, was removed. Afterwards, they were packed in hermetic plastic vessels and stored at $5 \pm 1$ °C until use.

### Proximal composition of chia nutlets

AOCS procedures were used to analyze moisture (Ba 2a-38 method), crude fiber (Ba 6-84 method) and ash (Ba 5a-49 method)[27] contents of chia nutlets and the mucilage obtained from them. Oil content was determined following the IUPAC Standard Method 1.122.[28] Total nitrogen content was determined by the Kjeldahl method according to AOAC[29] and the protein calculated as %N × 6.25. Carbohydrate content was estimated as nitrogen-free extract (NFE) by difference using Eqn 1:

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

The energy content was calculated by multiplying the values of crude protein, fat and carbohydrate contents by factors of 4, 9 and 4, respectively, and summing; results were expressed in kcal.[30] All determinations were done in triplicate.

### Determination of the cell wall contents

The ground nutlets were separated into cell contents, highly digestible; and cell walls, partially digestible (Van Soest method). The cell wall was analyzed and its components (cellulose, hemicellulose and lignin) were determined. The technique uses acidic and neutral detergent.[29,31] All determinations were done in triplicate.

### Mucilage extraction

Samples of 10 g whole nutlets were placed in a tray (9 cm × 14 cm × 5 cm) and distilled water was added in 1:10 (w/v) proportion. They were covered with aluminium foil and maintained at room temperature for 4 h. Then, samples were frozen at −50 °C, followed by freeze-drying (20 °C, 30 μmHg, 98 h). The dried mucilage was separated from the nutlet by rubbing over a 20 ASTM mesh screen (840 μm) during three periods of 15 min each. This is the freeze-dried crude mucilage sample.

### Proximal composition of the freeze-dried crude mucilage

Moisture, protein, fat, ash and carbohydrate contents of chia freeze-dried crude mucilage were determined according to the methods used for the proximal composition of nutlets. All determinations were done in triplicate.

### Mucilage solubility

The solubility of freeze-dried crude mucilage was determined using the method reported in Betancur-Ancona *et al.*[32] Briefly, 40 ml of 10.0 g $L^{-1}$ w/v mucilage suspension was prepared in a 50 mL centrifuge tube previously tared. A magnetic agitator was placed in the tube, and it was kept at constant temperature (25, 30, 50, 60 and 80 °C) in a water bath for 30 min. The suspension was then centrifuged at 3415 × *g* for 15 min and the supernatant decanted. An aliquot of 10 mL of the supernatant was dried in an air convection oven (FAC, Argentina) at 120 °C for 4 h, in a crucible (constant weight was reached). The percentage of solubility was calculated as follows:

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

### Nutlet, myxocarpy and mucilage microstructure characterization by SEM

Whole and longitudinally sliced nutlets, mucilage, release of mucilage (myxocarpy) and different concentrations of mucilage suspensions were analyzed by SEM.

For mucilage suspensions, the samples (2.5, 5.0, 7.5 and 10.0 g $L^{-1}$ w/v) were previously dehydrated. The different suspensions were initially fixed for 1 h in 25 mL $L^{-1}$ (v/v) glutaraldehyde in 0.067 mol $L^{-1}$ phosphate buffer and each dehydrated for 15 min in a graded ethanol series (30%, 50%, 75% and 100%), and then with acetone 100%. Solvents were replaced by liquid $CO_2$ in a critical point drier (Polaron, Loughborough, UK) and the critical point was achieved.

All dried samples (whole nutlets before and after mucilage removal, longitudinally sliced nutlets and dried mucilage suspensions) were adhered to a cover slip, coated with a thin gold film (600 Å) in a sputter coater (Pelco 9100) and observed in a scanning electron microscope (LEO EVO VP, Cambridge, UK) under high vacuum with 5 kV acceleration voltage. Longitudinal sections of nutlets were sliced with a razor blade, after being plunged into liquid nitrogen to ensure keeping their internal structure, and analyzed

  

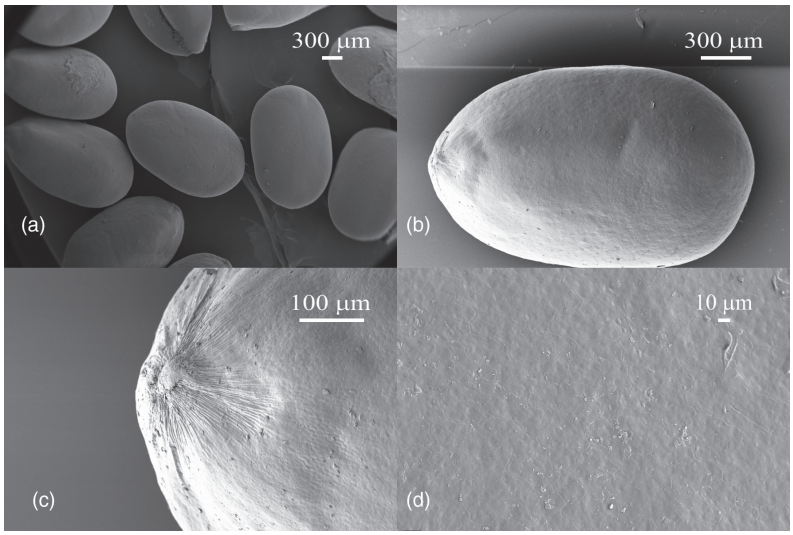


**Figure 1.** SEM images of chia nutlets: (a) nutlet set; (b) lateral view; (c) abscission scars; (d) pericarp surface.

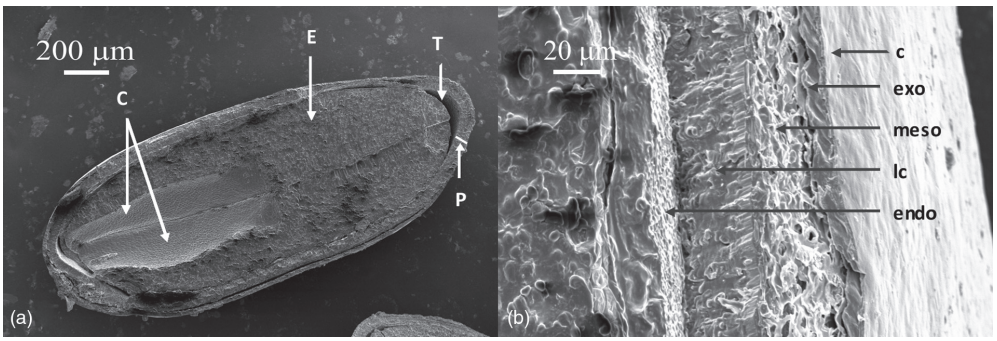


**Figure 2.** SEM images of chia nutlets: (a) longitudinal section showing the internal structure; (b) pericarp transverse section; P, pericarp; T, testa; E, endosperm; C, cotyledons; c, cuticle; exo, exocarp; meso, mesocarp; lc, layer of bone cells; endo, endocarp.

by microscopy using the same procedure. The release of mucilage (myxocarpy) was studied by soaking in water and observing chia nutlets by SEM at different soaking times (5, 10, 30 and 60 min).

## RESULTS AND DISCUSSION

### Morphological and anatomical characterization of chia nutlets by SEM

The nutlet size of chia calculated from SEM was $2.01 \pm 0.10$ mm length, $1.24 \pm 0.08$ mm width and $0.83 \pm 0.03$ mm thickness. These dimensions are in the same range as those reported by Ixtaina *et al.*[18] and Muñoz *et al.*[21] The nutlets appear as glabrous (without trichomes), elliptic (Fig. 1a, b) and apically rounded. The abscission scar was almost circular and its diameter was approximately 400 μm (Fig. 1c). The nutlet sculpturing surface is smooth and of Type I (foveate: surface with small pits), according to the classification given by Özkan *et al.*[33] for other *Salvia* species (Fig. 1d). Size, shape and ornamentation characteristics of the nutlet appear to have significant taxonomic value for distinguishing different species of *Salvia*.[34]

Each chia nutlet is made up of a true seed enclosed within the pericarp. The true seed consists in a seed coat (testa), the endosperm and the embryo, which is made up mostly of cotyledons (Fig. 2a). Basically, the pericarp is similar to other Nepetoideae by having cuticle, exocarp, mesocarp, bone cells vertically arranged and endocarp (inner epidermis) (Fig. 2b). The exocarp and mesocarp cells are parenchymatous. In the exocarp (nutlet epidermis), there are often cells which produce mucilage when they are wetted. By studying many species of *Salvia*, Hedge[35] found that they have a similar basic structure but show differences in thickness of the pericarp, proportions of the individual layers and color. In this study, the thickness of pericarp nutlets ranged from 66.7 to 68.6 μm, the layer of bone cells (32.0–37.0 μm) being the thickest part of the pericarp. The innermost layer of mesocarp diverges from other layers in containing prismatic crystals.

### Proximal composition of chia nutlets

The proximal composition of chia nutlets is presented in Table 1; fat is the major component of the nutlets. Also, the high levels of protein and fiber make chia nutlets a good source of these nutrients. In particular, the protein content is higher than in other traditional crops, e.g. wheat, corn, rice and oats.[23]

### Determination of the cell wall contents

Table 2 shows the NDF and ADF values, calculated using the Van Soest method.[31] The NDF value represents the cell wall content, while ADF represents cellulose and lignin; NDF content is negatively correlated with digestibility. The high values of NDF in relation to those reported for sunflower[36,37] indicate that chia nutlets have a lower percentage of cell content (i.e. highly digestible). On the other hand, the higher levels of ADF found in chia nutlets are associated with a higher percentage of cellulose instead of lignin.

  

**Table 1.** Proximal composition of chia nutlets

| Component | Content (g kg$^{-1}$) |
|---|---|
| Moisture | 70 ± 4.0 |
| Crude protein | 293 ± 4.0 |
| Crude fat | 327 ± 8.0 |
| Ash | 48 ± 1.0 |
| Crude fiber | 276 ± 1.0 |
| Carbohydrate (by difference) | 56 ± 2.0 |
| Food energy (kcal) | 4320 ± 3.0 |

Data are means ± SD of triplicate determinations. Values are expressed on a dry weight basis.

**Table 2.** Determination of the cell wall contents (g kg$^{-1}$) of chia nutlets

| Component | Chia | Sunflower[36,37] |
|---|---|---|
| NDF | 602 ±18.0 | 340–480 |
| ADF | 426 ±12.0 | 220–310 |
| Lignin | 60 ± 2.0 | 80–110 |

Data are means ± SD of triplicate determinations. Values are expressed on a dry weight basis. ADF, acid detergent fiber; NDF, neutral detergent fiber.

### Mucilage extraction

When nutlets are in contact with water, the exocarp swells, the cuticle is broken by depleting their elasticity and the cell components are spilled out as mucilage surrounding the entire fruit surface, but being attached to it with remarkable tenacity. In nutlets of Lamiaceae, the mucilage consists of epidermal cells that swell or disintegrate into thin spiral fibrillae when they come into contact with water.[15,16] According to Ryding,[15] who studied the presence or absence of myxocarpy in most genera of Lamiaceae, the phenomenon has only been recorded in the subfamily Nepetoideae, and within the subfamily about 75% of the species are mucilaginous. Ryding[22] uses the term 'myxodiaspory' for the condition of having mucilaginous diaspores irrespective of whether these are seeds or fruits. This author[22] reported that species with small nutlets are more often myxodiasporic than species with large nutlets, and also that annuals are more often myxodiasporic than perennials. Sales *et al.*[38] reported in *S. plebeia* R. Br. that the reaction of nutlets is very variable, depending perhaps upon the maturity and age of the samples. Figure 3 shows the myxocarpy phenomenon when chia nutlets become wetted. After 5 min, it was observed that the cuticle had been broken (Fig. 3a) and the exocarp cell content gradually surrounded the rest of the nutlet (Fig. 3a–d). As shown in the SEM images, the rate of production of the mucilage after initial soaking is very fast (a few minutes). These results are in agreement with Muñoz *et al.*,[21] who reported that the water absorption of chia nutlets was very fast in the first few minutes and later it became slower, achieving equilibrium conditions at about 2 h of hydration.

### Proximal composition of chia freeze-dried crude mucilage

Extraction of chia nutlets with water at room temperature yielded 38 ± 1.0 g kg$^{-1}$ db of mucilage. Muñoz *et al.*[21] studied different conditions of mucilage extraction, achieving an optimum yield value of 69.7 g kg$^{-1}$ after 2 h of hydration at 80 °C and using a nutlet:water ratio of 1:40. Table 3 shows the proximal composition of chia freeze-dried crude mucilage. In comparison with commercial guar and locust bean gums, extracts of chia

**Table 3.** Proximal composition (g kg$^{-1}$) of freeze-dried crude chia mucilage and commercial guar and locust bean gums

| Component | Chia mucilage | Guar gum[38] | Locust bean gum[38] |
|---|---|---|---|
| Moisture | 115 ± 3.0 | 88 | 89 |
| Crude protein | 112 ± 3.0 | 45 | 74 |
| Residual fat | 31 ± 2.0 | 7 | 12 |
| Ash | 84 ± 1.0 | 7 | 9 |
| Crude fiber | 135 ± 6.0 | — | — |
| Carbohydrate (by difference) | 637 ± 5.0 | 942 | 905 |

Data are means ± SD of triplicate determinations. Values are expressed on a dry weight basis.

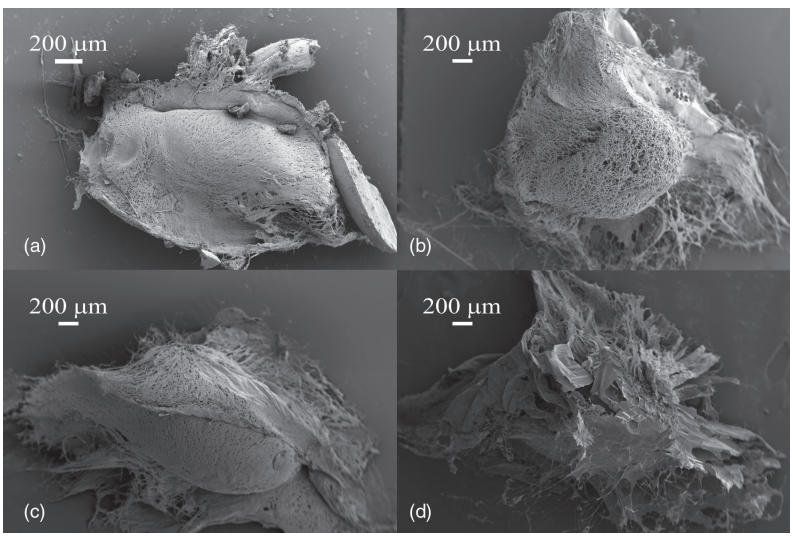


**Figure 3.** SEM images of chia nutlets soaked in water for different lengths of time: (a) 5 min; (b) 10 min; (c) 30 min; (d) 60 min.

  

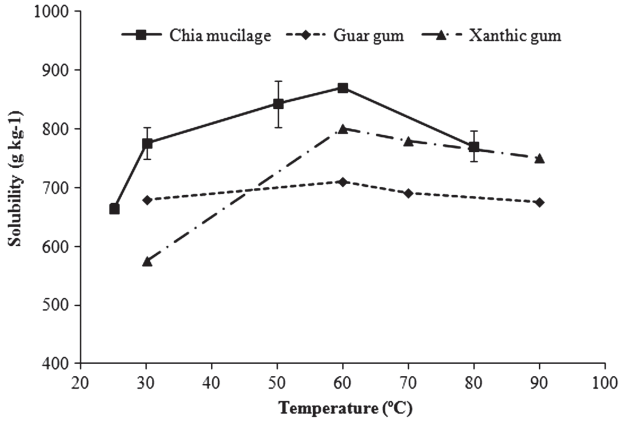


**Figure 4.** Solubility (g $kg^{-1}$) of chia mucilage as a function of temperature. Data of guar and xanthic gums[40]

mucilage contain more ash, residual fat and protein, and a lower level of carbohydrates.[39]

### Mucilage solubility

The solubility of chia mucilage in 10 g $L^{-1}$ w/v water was elevated and higher than that of guar and xanthic gums[40] in the studied temperature range (25–80 °C), varying between 660 and 870 g $kg^{-1}$. The highest solubility of chia mucilage was achieved at 60 °C, decreasing at higher temperatures (Fig. 4). These values of solubility may be due to the mucilage structure, which is slightly branched, since the water gum dispersion depends mainly on its chemical structure. Furthermore, the decreasing solubility at elevated temperatures could be attributed to the gelling effect associated with some gums at such temperatures.[40]

### Characterization of chia nutlets after mucilage removing and mucilage microstructure by SEM

Figure 5 shows SEM images of chia nutlets after mucilage extraction. In these images it can be observed that the myxocarpy 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.

Fresh and freeze-dried crude mucilage samples are shown in Fig. 6. The association between different components that formed the mucilage forms a network structure of open pores, which provide interesting rheological properties (gel formation). When mucilage was freeze-dried, it took the appearance of 'overlapping sheets'. The extraction, purification, drying and/or further modification processes can significantly affect the chemical composition and molecular structure of natural plant-based biopolymers. Mirhosseini and Amid,[41] who studied the effect of different drying techniques on flowability characteristics and chemical properties of natural carbohydrate–protein gum from durian fruit seed, reported that the freeze-dried gum showed the highest porosity, solubility and foaming capacity among differently dried seed gums. These authors reported that this behavior might be due to the least thermal degradation, which probably resulted in less compact structure than other samples. Figure 7 shows the micrographs of the chia mucilage dispersion in water. These images show clear strands, which became denser when mucilage concentration was increased. In this case, it is necessary to take into account that for high-vacuum SEM samples were dried by critical point drying and this process could lead to the presence of structures that are not normally present in the living tissues (artifacts).[38]

## CONCLUSIONS

This study contributes to the micromorphological and anatomical characterization of chia nutlets, which may be helpful in the identification of the studied species. These nutlets are elliptic, glabrous, small and with a smooth-sculptured surface. The pericarp is constituted by cuticle, exocarp, mesocarp, bone cells vertically arranged and endocarp. The thickness of pericarp ranged from 66.7 to 68.6 μm, the layer of bone cells being the thickest part.

The results obtained show a fast exudation of chia mucilage when nutlets are in contact with water. Also, the freeze-dried crude mucilage hydrates easily in water, even at low temperatures. Chia nutlets have mucilaginous substances, with interesting functional properties from a technological and physiological point of view.

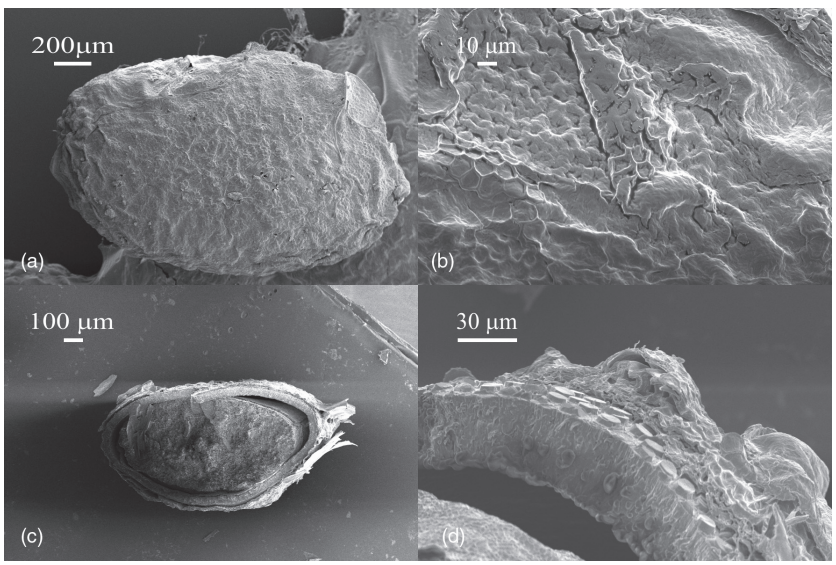


**Figure 5.** SEM images of chia nutlets after mucilage extraction: (a) whole nutlet; (b) nutlet surface; (c) broken nutlet; (d) broken nutlet.

  

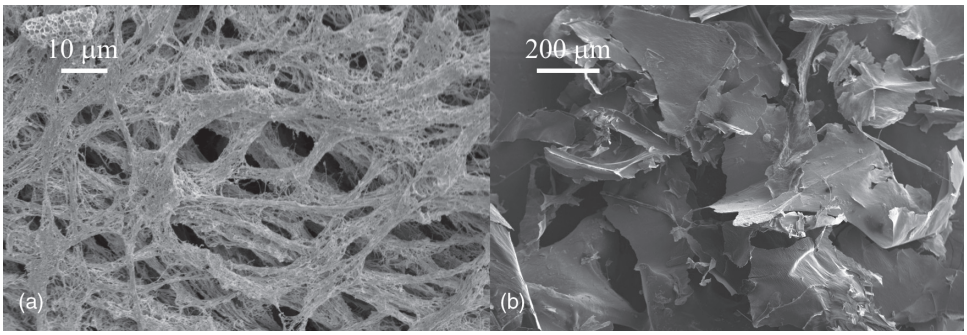


**Figure 6.** SEM images of chia mucilage: (a) fresh mucilage; (b) freeze-dried crude mucilage.

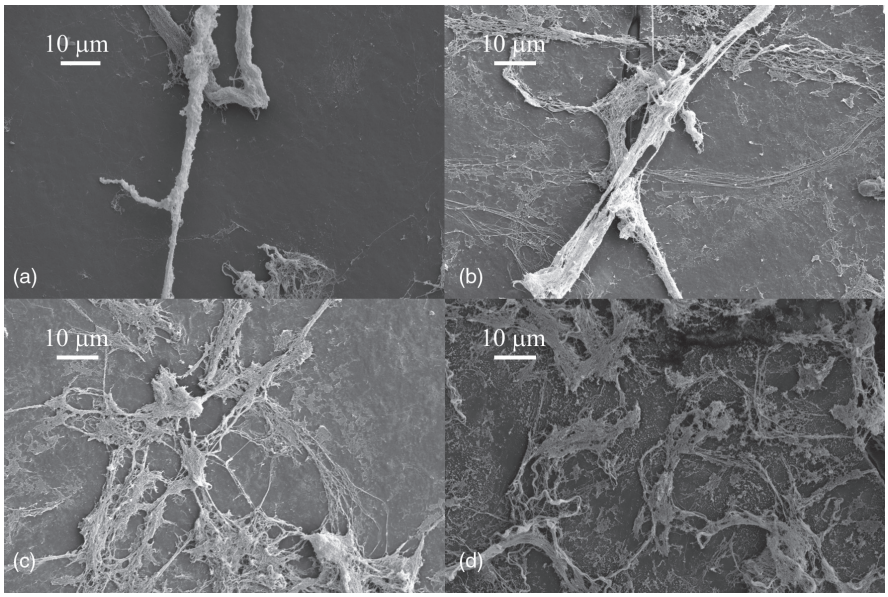


**Figure 7.** SEM images of different concentrations of chia mucilage suspensions prepared by critical point drying: (a) 2.5; (b) 5.0; (c) 7.5; (d) 10.0 g $L^{-1}$ w/v.

Thus chia mucilage could be used as a new thickening agent in the cosmetics and food industries as well as contributing to consumer health as a source of soluble dietary fiber.

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## REFERENCES

1 Harley RM, Atkins S, Budantsev AL, Cantino PD, Conn BJ, Grayer R *et al.*, Labiatae, in *The Families and Genera of Vascular Plants 7: Lamiales*, ed. by Kadereit JW. Berlin, Springer, pp. 167–275 (2004).
2 Capitani MI, Spotorno V, Nolasco SM and Tomás MC, Physicochemical and functional characterization of by-products from chia (*Salvia hispanica* L.) seeds of Argentina. *LWT – Food Sci Technol* **45**:94–102 (2012).
3 Ixtaina VY, Martinez ML, Spotorno V, Mateo CM, Maestri DM, Diehl BWK *et al.*, Characterization of chia seed oils obtained by pressing and solvent extraction. *J Food Comp Anal* **24**:166–174 (2011).
4 Ayerza R Jr, Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. *J Am Oil Chem Soc* **72**:1079–1081 (1995).
5 Taga MS, Miller EE and Pratt DE, Chia seed as a source of natural lipid antioxidants. *J Am Oil Chem Soc* **61**:928–931 (1984).
6 Ixtaina VY, Mattea F, Cardarelli DA, Mattea MA, Nolasco SM and Tomás MC, Supercritical carbon dioxide extraction and characterization of Argentinean chia seed oil. *J Am Oil Chem Soc* **88**:289–298 (2011).
7 EU Commission, Authorizing the placing on the market of chia seed (*Salvia hispanica*) as novel food ingredient under Regulation (EC) No 258/97 of Council of the European Parliament. *Offic J EU C*, 7645 (2009).
8 EFSA, Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the Commission related to the safety of chia (*Salvia hispanica* L.) seed and ground whole chia seed as a novel food ingredient intended for use in bread. *EFSA J* **278**:1–12 (2005).
9 EFSA, Scientific opinion of the panel on dietetic products nutrition and allergies on a request from the European Commission on the safety of 'chia seed (*Salvia hispanica*) and ground whole chia seed' as a food ingredient. *EFSA J* **996**:1–2 (2009).
10 Guerin GR, Nutlet morphology in *Hemigenia* R. Br. *Microcorys* R. Br. (Lamiaceae). *Plant Syst Evol* **254**:49–68 (2005).

  

11 Kaya A and Dirmenci T, Nutlet surface micromorphology of the genus *Nepeta* L. (Lamiaceae) in Turkey. *Turk J Bot* **32**:103–112 (2008).
12 Moon HK and Hong SP, Nutlet morphology and anatomy of the genus *Lycopus* (Lamiaceae, Mentheae). *J Plant Res* **119**:633–644 (2006).
13 Domínguez-Vázquez G, Berlin B, Castro Ramírez A and Estrada-Lugo EIJ, Revisión de la diversidad y patrones de distribución de Labiatae en Chiapas. *Anales Inst Biol Univ Nac Mexico* **73**:39–80 (2002).
14 Ixtaina VY, Nolasco SM and Tomás MC, Characterization of chia (*Salvia hispanica* L.) white and dark seeds and oils, in *Advances in Fats and Oils Research*, ed. by Tomás MC. Kerala. Transworld Research Network, Kerala, India, pp. 135–147 (2010).
15 Ryding O, Pericarp structure and phylogeny within Lamiaceae *subfamily Nepetoideae tribe Ocimeae*. *Nordic J Bot* **12**:273–298 (1992).
16 Ryding O, The distribution and evolution of myxocarpy in Lamiaceae, in *Advances in Labiate Science*, ed. by Harley RM and Reynolds T. Royal Botanic Gardens, Kew, pp. 85–96 (1992).
17 Ayerza R, Effects of seed color and growing locations of fatty acid content and composition of two chia (*Salvia hispanica* L.) genotypes. *J Am Oil Chem Soc* **87**:1161–1165 (2010).
18 Ixtaina VY, Nolasco SM and Tomás MC, Physical properties of chia seeds (*Salvia hispanica* L.). *Ind Crop Prod* **28**:286–293 (2008).
19 Guiotto EN, Ixtaina VY, Tomás MC and Nolasco S, Moisture-dependent physical properties of chia (*Salvia hispanica* L.) seeds. *Trans ASABE* **54**:527–533 (2011).
20 Lin KY, Daniel JR and Whistler RL, Structure of chia seed polysaccharide exudate. *Carbohyd Polym* **23**:13–18 (1994).
21 Muñoz LA, Cobos A, Diaz O and Aguilera JM, Chia seeds: microstructure, mucilage extraction and hydration. *J Food Eng* **108**:216–224 (2012).
22 Ryding O, Myxocarpy in the Nepetoideae (Lamiaceae) with notes on myxodiaspory in general. *Syst Evol* **285**:165–175 (2001).
23 Ayerza R and Coates W, Chia seeds: new source of omega-3 fatty acids, natural antioxidants and dietetic fiber. Southwest Center for Natural Products Research and Commercialization, Office of Arid Lands Studies, Tucson, AZ (2001).
24 Reyes-Caudillo E, Tecante A and Valdivia-López MA, Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chem* **107**:656–663 (2008).
25 Capitani MI, Nolasco SM and Tomás MC, Evaluación de las propiedades del mucílago de chía (*Salvia hispanica* L.) sobre la estabilidad de emulsiones O/W, in *Proceedings del VI Congreso Argentino de Ingeniería Química*, 26–29 September, Mar del Plata, Argentina. AAIQ, Buenos Aires (2010).
26 Ryding O, Pericarp structure of *Leucas* and related genera. *Nordic J Bot* **13**:637–646 (1993).
27 AOCS, *Official Methods and Recommended Practices of the AOCS* (5th edn). AOCS Press, Champaign, IL (1998).
28 International Union of Pure and Applied Chemistry (IUPAC), *Standard Methods for the Analysis of Oils, Fats and Derivates* (7th edn), ed. by Paquot C. Blackwell, Oxford (1992).
29 AOAC *Official Methods of Analysis* (15th edn). Association of Official Analytical Chemists, Washington, DC (1990).
30 Osborne DR and Voogt P *The Analysis of Nutrients in Foods*. Academic Press, London, pp. 239–245 (1978).
31 Guiragossian VY, Van Scoyoc SW and Auxtell JD, *Chemical and Biological Methods for Grain and Forage Sorghum*. Department of Agronomy International Programs in Agriculture. Purdue University, West Lafayette, IN (1977).
32 Betancur-Ancona D, López-Luna J and Chel-Guerrero L, Comparison of the chemical composition and functional properties of *Phaseolus lunatus* prime and tailing starches. *Food Chem* **82**:217–225 (2003).
33 Özkan M, Aktaş K, Özdemir C and Guerin G, Nutlet morphology and its taxonomic utility in *Salvia* (Lamiaceae: Menthae) from Turkey. *Acta Bot Croat* **68**:105–115 (2009).
34 Kahraman A and Doğhan M, Comparative study of *Salvia limbata* C.A. and *S. palaestina* Bentham (sect. Aethiopis Bentham, Labiatae) from East Anatolia, Turkey. *Acta Bot Croat* **69**:47–64 (2010).
35 Hedge IC, Observations on the mucilage of *Salvia* fruits. *Notes Roy Bot Gard Edinburgh* **30**:79–95 (1970).
36 Pinto F, Vicente A, Marsico G, Vonghia G and Montemurro O, In vivo digestibility and nutritive characteristics of diets containing almond hulls supplemented with undecorticated sunflower oilseed meal, in *Proceedings of the 13th International Sunflower Conference*, Pisa, Italy, 8–12 September 1992. International Sunflower Association, Paris (1992).
37 Trimachi G, Secchiari P, Feruzzi G, Pistola A and Casarosa L, Sunflower seed meal characterization for animal feeding, in *Proceedings of the 13th International Sunflower Conference*, Pisa, Italy, 8–12 September 1992. International Sunflower Association, Paris (1992).
38 Sales F, Hedge IC and Christie F, *Salvia plebeia* R. BR.: taxonomy, phytogeography, autogamy and myxospermy. *Pak J Bot* **42**:99–110 (2010).
39 Mazza G and Biliaderis CG, Functional properties of flax seed mucilage. *J Food Sci* **54**:1302–1305 (1989).
40 Sciarini L, Maldonado F, Ribotta P, Pérez G and León A, Chemical composition and functional properties of *Gleditsia triacanthos* gum. *Food Hydrocolloid* **23**:306–313 (2009).
41 Mirhosseini H and Amid BT, Effect of different drying techniques on flowability characteristics and chemical properties of natural carbohydrate–protein cum from durian fruit seed. *Chem Central J* **7**:1–14 (2013).


  