Research Article

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Optimization of mucilage extraction from chia seeds (*Salvia hispanica* L.) using response surface methodology

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Abstract

BACKGROUND: Chia mucilage has potential application as a functional ingredient; advances on maximizing its extraction yield could represent a significant technological and economic impact for the food industry. Thus, first, the effect of mechanical agitation time (1–3 h) on the exudation of chia mucilage was analyzed. Then, response surface methodology was used to determine the optimal combination of the independent variables temperature (15–85 °C) and seed: water ratio (1: 12–1: 40.8 w/v) for the 2 h exudation that give maximum chia mucilage yield. Experiments were designed according to central composite rotatable design.

RESULTS: A second-order polynomial model predicted the variation in extraction mucilage yield with the variables temperature and seed: water ratio. The optimal operating conditions were found to be temperature 85 °C and a seed: water ratio of 1: 31 (w/v), reaching an experimental extraction yield of 116 $\pm$ 0.21 g kg^{-1} (dry basis). The mucilage obtained exhibited good functional properties, mainly in terms of water-holding capacity, emulsifying activity, and emulsion stability.

CONCLUSION: The results obtained show that temperature, seed: water ratio, and exudation time are important variables of the process that affect the extraction yield and the quality of the chia mucilage, determined according to its physicochemical and functional properties.

Keywords: chia seed mucilage; extraction yield; response surface methodology; optimization

INTRODUCTION

Chia mucilage is an anionic heteropolysaccharide consisting mainly of the sugars xylose and glucose in a 2: 1 ratio, with significant amounts of uronic acids (glucuronic and galacturonic acids).[1] This polysaccharide is exuded from the chia seeds when they are placed in an aqueous solution; and upon full hydration, filaments became apparent and conformed to a transparent 'capsule' attached to the seed.[2,3] One of the most common methods used to extract the mucilage from the seeds is aqueous extraction.[4,5] The extraction conditions have significant effects not only on yield, but also on the purity and relative viscosity of the extracted crude polysaccharides.[6]

Different methods have been developed for the extraction of chia mucilage, involving various technological alternatives. In all cases, the first stage consists of the exudation of the mucilage in an aqueous medium; however, methods differ in how the mucilage is separated from the seed. Some methods separate the mucilaginous liquid from the seed (centrifugation or vacuum filtration) and then dehydrate the extracted product,[7–10] whereas other methods remove the water prior to the separation of the dry mucilage by applying different drying techniques, such as using a drying oven with forced-air circulation[2,11] or freeze-drying.[1,9]

In the extraction processes, where there are multiple operating variables interacting, it is likely that those variables affect the responding factors. Therefore, it is necessary to apply an optimization method to determine the optimal experimental conditions. Response surface methodology (RSM) is a set of mathematical and statistical techniques that is widely used in the food industry to define the combined effects that different variables have on a desired response, thus optimizing the operating conditions, with the advantage of reducing the number of experimental trials.[12] An

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experimental strategy frequently used in the development of food processes combines a central composite rotatable design (CCRD) and RSM to thoroughly examine several parameters with a minimum experimental time, determining the most relevant factors and their ranges of influence, as well as the interactions between the factors.[13,14] Control of the operating conditions of different processes is of great interest for the food industry because it contributes to the optimization of a technological process and provides information based on current consumer trends with respect to the effects associated with nutrition and health.

Muñoz *et al.*[2] studied the extraction of mucilage from Chilean chia seeds using a Box–Behnken experimental design, achieving the optimization of the process at 80 °C with a seed: water ratio of 1: 40 (70 g kg^{-1} (dry basis, d.b.) extraction yield). Owing to the growing interest in this subproduct of chia seeds given its various applications in the production of functional foods, such as emulsions,[15–17] ice-creams,[11] bakery products,[18–20] and biodegradable films,[21–23] advances in terms of maximizing its extraction yield can result in a significant technological and economic impact for the food industry. The aim of this study was to optimize the extraction process of chia mucilage, first by analyzing the effect of mechanical agitation time during extraction of the mucilage, and then by determining the optimal operating combination of the variables temperature and seed: water ratio in the extraction process.

MATERIALS AND METHODS

Material

The commercial chia seeds used in this study were provided by DUSEN S.R.L., Argentina (March, 2015). They were placed in hermetic plastic containers and stored at 5 ± 1 °C until further use.

Chia mucilage extraction

Mucilage was obtained by the procedure proposed by Capitani *et al.*[3] with some modifications: whole chia seeds were soaked in water (1: 40 w/v) at 60 °C with constant stirring in order to induce the mucilage release. Then the crude mixture containing water, gum, and seeds was frozen at −20 °C for 96 h, followed by freeze-drying (−50 °C, 26 Pa, 120 h) (FD-1A-50, Boyikang Laboratory Instruments Inc., China). The dried mucilage was separated from the seeds by rubbing in a ZONYTEST stirrer (Buenos Aires, Argentina) over a 20 ASTM mesh screen (840 μm) for three periods of 15 min each.

Effect of exudation time on the extraction efficiency of chia mucilage

First, the effect of the exudation time (1, 2, and 3 h) on the extraction yield of chia mucilage was analyzed.

Table 1. CCRD for the independent variables (actual and coded levels)

	Coded level		Actual level	
Trial	X_1	X_2	Temperature (°C)	Seed: water ratio (w/v)
1	−1	−1	25	1: 12
2	−1	1	25	1: 36
3	1	−1	75	1: 12
4	1	1	75	1: 36
5	−1.4	0	15	1: 24
6	1.4	0	85	1: 24
7	0	−1.4	50	1: 7.2
8	0	1.4	50	1: 40.8
9	0	0	50	1: 24
10	0	0	50	1: 24
11	0	0	50	1: 24

X_1, temperature, X_2, seed: water ratio.

Experimental design and statistical analysis

For the exudation time that gave the highest extraction yield, the best combination of extraction temperature and seed: water ratio was evaluated in order to maximize the extraction yield of chia mucilage. RSM combined with a CCRD was used for that purpose. The design consisted of 11 experiments, adding three central points and four axial points to the full 2^2 factorial design. All the experiments were conducted in duplicate. Both coded and actual values of the independent variables studied are listed in Table 1. The independent variables and their ranges were selected based on preliminary studies. STATGRAPHICS Centurion XV software (version 15.2.06, StatPoint, Inc.) was used to perform the statistical analysis of the results, develop multiple regression models from the experimental data, and predict process conditions that may improve the extraction performance. Experimental data were fitted to a generalized second-order model using the following equation:

$$Y_i = b_0 + b_1X_1 + b_2X_2 - b_{11}X_1^2 + b_{12}X_1X_2 - b_{22}X_2^2 \qquad (1)$$

where Y_i, the dependent variable, is the predicted response (extraction yield), X_1 and X_2 represent the coded levels of the independent variables (temperature and seed: water ratio respectively). The coefficients of the polynomial are represented by b_0 (constant term), b_1 and b_2 (linear effects), b_{11} and b_{22} (quadratic effects), and b_{12} (interaction effect). Modeling was started with a quadratic model including linear, squared, and interaction terms. Analysis of variance was conducted to determine significant effects of the process variables on the response, and to fit the second-order polynomial models to the experimental data. Only coefficients making a significant contribution at $P < 0.05$ were selected for building the models.

For statistical calculation, the variables were coded according to

$$X_i = \frac{x_i - \bar{x}}{d_i/2} \qquad i = 1,\ 2 \qquad (2)$$

where X_i is the value dimension of the independent variable, x_i is the value of the independent variable in real units, $\bar{x}$is the mean of the highest and lowest value of x_i for the 2^2 design, and d_i is the difference between the maximum and minimum values of x_i.

Experimental validation of the optimized conditions

The extraction yield response was optimized by being maximized according to the model. In order to validate the regression model generated, three extraction tests were carried out under the optimal operating conditions, because they were different from those of the experiments used to develop the model.

Determination of residual mucilage

The seeds obtained after the mucilage extraction under optimized conditions were subjected to a second extraction under the same operating conditions.

Proximal composition of chia mucilage

AOCS procedures were used to analyze moisture (Ba 2a-38 method), crude fiber (Ba 6e84 method), and ash contents (Ba 5a-49 method).[24] Oil content was determined following the IUPAC Standard Method 1.122.[25] Total nitrogen content was determined by the Kjeldahl method according to AOAC,[26] and the protein content was calculated as nitrogen x 6.25. Carbohydrate content was estimated as nitrogen-free extract (NFE) by difference using

$$\text{NFE} = 100 - \left(\text{oil} + \text{protein} + \text{crude fiber} + \text{ash}\right) \quad (3)$$

Functional properties of chia mucilage

Water-holding capacity (WHC), oil-holding capacity (OHC), water absorption capacity (WAbC), water adsorption capacity (WAdC), organic molecule absorption capacity (OMAC), emulsifying activity (EA), and emulsion stability (ES) were determined following the procedures described by Capitani *et al.*[27]

RESULTS AND DISCUSSION

Effect of exudation time on the extraction efficiency of chia mucilage

The variation in chia mucilage yield for the different exudation times analyzed (1, 2, and 3 h) at 60 °C and with a seed: water ratio of 1: 40 is presented in Fig. 1.

As can be observed, when exudation time was increased from 1 to 2 h, the yield increased significantly ($P < 0.05$) by 26.3%, reaching the highest extraction yield at 2 h (97 g kg^{-1} (d.b.)). At 3 h, the yield decreased significantly by 11.9% with respect to 2 h of exudation. Taking into account the anionic characteristic of the chia mucilage and possible interactions with the constituents of the seed pericarp, future investigations will be necessary to explain this latter behavior. The exudation time that gave the maximum extraction yield was consistent with the results reported by Muñoz *et al.*[2] and Campos *et al.*,[11] who obtained the maximum yield for chia mucilage at 2 h and 2.4 h of hydration of the chia seeds in water respectively, which could be considered the maximum time for the experimental exudation of mucilage.

Fitting models and optimization

The mean values of the extractions yields of chia mucilage for each one of the 11 experiments carried out according to the experimental design developed are presented in Table 2. The temperature applied during the exudation process affected the color of the chia mucilage, which was darker with increasing temperature during extraction. This behavior can be attributed to the Maillard products produced during heating, with a higher roasting temperature resulting in a greater browning index. Thus, there are two reactions that could result in the caramelization browning: sugar–sugar reactions when heated at high temperatures, and the Maillard reaction, which results from reactions between reducing sugars and proteins and their derivatives (amino acids and amides).[28] On the other hand, the change of color may be due to the passage of some impurities, such as natural pigments, tannins, coat, tegument, and germ, favored by high temperature.[29–31]

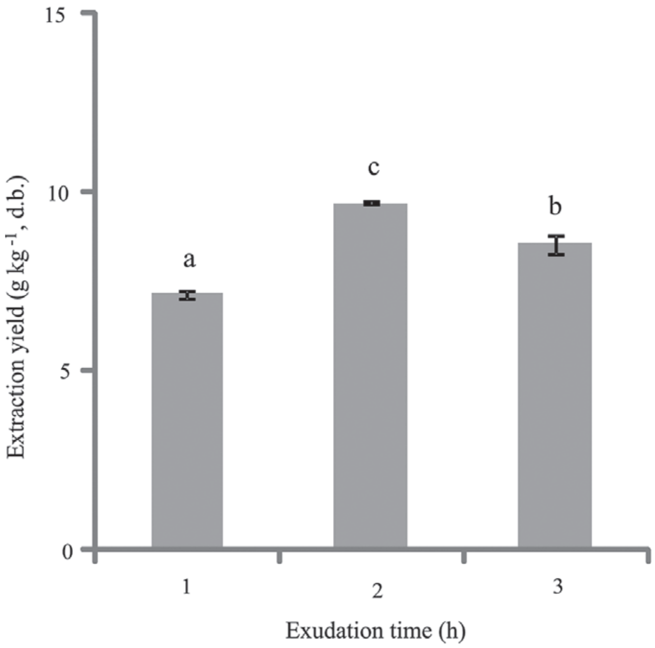

Figure 1. Effect of exudation time on the extraction yield of chia mucilage. Different letters indicate significant differences between each exudation time ($P < 0.05$). Each value is an average of two determinations ($n = 2$).

Table 2. Mean values of the extraction yields of the optimized process for extraction of chia mucilage (actual values)

Trial	Temperature (°C)	Seed: water ratio (w/v)	Yield (g kg^{-1} (d.b.))
1	25	1: 12	54.7 ± 0.36
2	25	1: 36	53.1 ± 0.03
3	75	1: 12	105.7 ± 0.64
4	75	1: 36	113.4 ± 0.21
5	15	1: 24	53.9 ± 0.47
6	85	1: 24	121.7 ± 0.65
7	50	1: 7.2	80.3 ± 0.71
8	50	1: 40.8	91.1 ± 0.29
9	50	1: 24	90.3 ± 0.13
10	50	1: 24	92.1 ± 0.56
11	50	1: 24	88.0 ± 0.28

The experimental data were fitted to a second-order polynomial model for predicting the variation in extraction yield Y (% (d.b.)) with the variables temperature and seed: water ratio. The polynomial model for the extraction yield of chia mucilage (adjusted R^2: 0.9532; lack of fit: $P = 0.0668$) is

$$Y = 9.02 + 2.60X_1 + 0.26X_2 - 0.25X_1^2 + 0.23X_1X_2 - 0.35X_2^2 \quad (4)$$

with X_1 and X_2 being the coded values of temperature and seed: water ratio respectively.

The value of R^2 indicates that the model accounts for more than 95% of the variability in extraction yield for the selected operating variables (temperature and seed: water ratio). The lack of fit was not significant ($P > 0.05$), indicating that the proposed model could be used for predicting the extraction yield of chia mucilage within the experimental range studied.

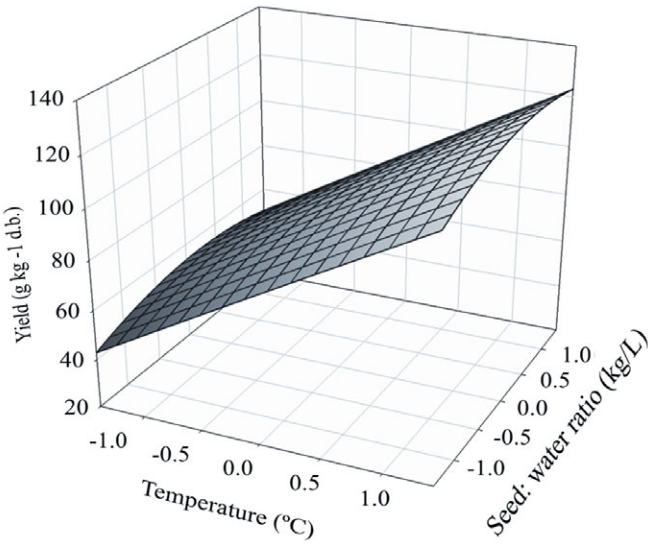

Figure 2. Response surface for the effect of temperature and seed: water ratio on yield of chia mucilage.

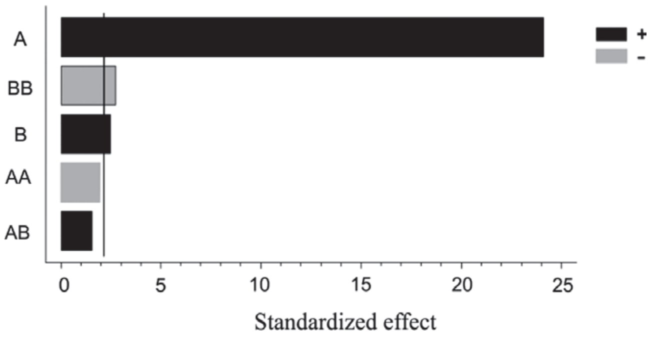

Figure 3. Pareto chart of the extraction yield of chia mucilage. The black line indicates the critical level above which the variables presented a significant effect ($P \leq 0.05$). A: temperature; B: seed: water ratio.

The response surface plot of the proposed model is presented in Fig. 2 for a better visualization, showing the effect of the independent variables temperature (coded value X_1) and seed: water ratio (coded value X_2) on the extraction yield of chia mucilage. The factors (temperature and seed: water ratio) presented statistically significant linear effects. As for the quadratic terms, only that corresponding to seed: water ratio was significant ($P < 0.05$), whereas the interaction between both independent variables was not significant ($P > 0.05$) on the extraction yield (Fig. 3). Thus, the yield of chia mucilage was higher when higher temperatures were applied during the exudation treatment, while the seed: water ratio presented a quadratic effect that was significant, reflected in a slight concave variation of yield with seed: water ratio (Eq. (4), Fig. 2). The increase in yield with temperature might be due to the decrease in viscosity of the mucilage linked to the seeds, which makes them less sticky and can be effectively released under higher temperature. The temperature dependency may also be due to the increase in solubility of the mucilage, as well as, the mass transfer rate of the water-soluble polysaccharides in the cell wall.[6] Similar behavior for the extraction yield of mucilage was achieved by Koocheki and co-workers[29,32,33] from *Eruca sativa*, *Alyssum homolocarpum*, and *Lepidium perfoliatum* and by Cui *et al.*[34] from flax seeds using response surface methodology.

Building the model with only the statistically significant variables and their interactions for a level of $P < 0.05$ gave an equation with a significant lack of fit ($P < 0.05$), so this adjusted model was not valid. Therefore, the complete model, Eq. (4), was used for optimizing the extraction process. The highest yield obtained with this model was of 124 g kg^{-1} (d.b.) for a temperature of 85 °C (X_1: 1.4) and a seed: water ratio of 1: 31 (X_2: 0.84).

In order to validate the model, the optimum operating conditions were used (temperature 85 °C and seed: water ratio 1: 31 w/v), since they did not correspond to the treatments used to develop the model. Thus, the mucilage yield observed experimentally was 116 g kg^{-1} (d.b.), giving a relative error of 6.9%. The standard deviation between the predicted and the experimental value was 0.56, being within the range observed for the experimental data used to develop the model (see Table 2). Therefore, the results obtained indicate that the second-order polynomial model is appropriate to predict the extraction yield of chia mucilage under the operating conditions of the method used.

The extraction yield obtained in this work was higher than that reported by Campos *et al.*[11] and Muñoz *et al.*,[2] who optimized the extraction of chia mucilage by applying hot-air circulation drying (49.5 g kg^{-1} (d.b.) and 69.7 g kg^{-1} (d.b.) respectively). The yield was also higher than that observed by Capitani *et al.*,[9] who obtained 38 g kg^{-1} (d.b.) yield using the same extraction method but with other operating variables. On the other hand, our extraction yield was similar to that reported in a patent that describes a method for obtaining chia mucilage that includes mechanical agitation and ultrasound as variables associated with the solubilization stage (150 g kg^{-1} (d.b.)).[35]

Residual mucilage

The seeds obtained from the optimum treatment, which were subjected to a second extraction under the predicted optimum operating conditions (temperature 85 °C and seed: water ratio 1: 31 w/v), presented residual mucilage content of 68 ± 0.42 g kg^{-1} (d.b.). This indicates that the mucilage is not extracted exhaustively in one operation alone. This behavior could be associated with previous studies reported by Capitani *et al.*[3] about the microstructural characterization of chia seeds after the extraction of mucilage (by freeze-drying), where they observed that the surface of those seeds presented small spaced mesocarp cells, which could release mucilaginous substances during a second extraction process.

Proximal composition of chia mucilage

The chia mucilage obtained under the optimum extraction conditions (temperature 85 °C and seed: water ratio 1: 31 w/v) had the following proximate composition (d.b.): moisture 79.3 ± 0.40 g kg^{-1}, protein 164.0 ± 0.01 g kg^{-1}, crude fiber 79.2 ± 0.51 g kg^{-1}, oil 90.5 ± 0.74 g kg^{-1}, ash 103.1 ± 0.12 g kg^{-1} and NFE 563.3 ± 1.14 g kg^{-1}. The protein content of the mucilage could be attributed to the natural presence of structural proteins and enzymes, and also a possible contamination with seed-germ during the extraction process.[36] In this sense, the amount of protein obtained could be related to the way the mucilage is separated from the chia seeds. The friction generated during the manual breakage of the freeze-dried mixture could produce rupture of the seeds, passing through the sieve mesh along with the mucilage, and thus affecting the level of residual protein.[15] While protein content was higher than that reported by Coorey *et al.*[7] for chia mucilage (35.4 g kg^{-1} (d.b.)) and by Timilsena *et al.*[1] for purified chia mucilage (26 g kg^{-1} (d.b.)), it was lower than the levels

Table 3. Functional properties of chia mucilage obtained according to optimum operating conditions (85 °C, 2 h, and 1: 31 w/v)

Property	Chia mucilage
WHC (g g $^{-1}$)	98.97 ± 3.69
WAbC (g g $^{-1}$)	14.93 ± 0.52
WAdC (g g $^{-1}$)	0.97 ± 0.03
OHC (g g $^{-1}$)	22.20 ± 0.47
OMAC (g g $^{-1}$)	25.67 ± 0.81
EA (mL/100 mL)	54.67 ± 1.89
ES (mL/100 mL)	57.56 ± 0.31

reported by Segura-Campos *et al.*[8] for a chia mucilage (250.7 g kg^{-1} (d.b.)) and by Capitani *et al.*[9] using the same method but different extraction variables (188.5 g kg^{-1} (d.b.)). Timilsena *et al.*[1] associated the protein content remaining in the purified chia mucilage with the possible existence of covalent bonds between proteins and lipids with the polysaccharide.

Functional properties of chia mucilage

The functional properties of chia mucilage are presented in Table 3. WAbC is defined as the amount of water that a fiber source is able to absorb when placed in excess water. WAdC is the ability of a structure to adsorb water superficially when exposed to an atmosphere of constant relative humidity. WHC expresses the maximum amount of water that can be retained per gram of dry material in the presence of water and under the action of an external force (centrifugation) and that is in equilibrium with the medium. It is worth mentioning that the WHC is important in relation to the formulation and processing of high-fiber foods, that the WAbC is of interest in processes such as extrusion, where the raw material is wet before or during the process, and that the WAdC is related to the stability and deteriorative changes of a fiber source during storage.[37]

The chia mucilage exhibited a high level of WHC, similar that that reported by Segura-Campos *et al.*[8] in their characterization of chia mucilage (103.2 g g $^{-1}$), and lower than that observed by Coorey *et al.*[7] (266.55 g g $^{-1}$). According to Olivos-Lugo *et al.*,[38] a good level of WHC is required for manufacturing certain food products, for example sausages, in order to improve their structure and reduce the water loss during the cooking process. On the other hand, the WAbC was lower than that observed by Segura-Campos *et al.*[8] (44.08 g g $^{-1}$). The different value ranges for the functional properties associated with water affinity could be attributed to the different protein and fiber contents between the mucilages. It should be noted that proteins have a large number of exposed hydrophilic sites that interact with water and increase WAbC, whereas fiber has the ability to form gels and retain water.[39,40] As for the properties related to the affinity to various lipid compounds (OHC and OMAC), they were high, and that behavior could be associated with the low lipid content of mucilage (90.5 g kg^{-1} (d.b.)).

The OHC was within the range reported by Ciau-Solís[41] for chia mucilage obtained from Mexican seeds (25.79 g g $^{-1}$). However, Coorey *et al.*[7] obtained a mucilage with a higher OHC (58.61 g g ^{1}) associated with its lower lipid content (5.5 g kg^{-1} (d.b.)). A high level of OHC is adequate for preparing food emulsions.[37] In the case of EA and ES, they were high, revealing a good characteristic of chia mucilage for its application in the formulation of food emulsions. In that respect, Capitani *et al.*[15] and Guiotto *et al.*[16] suggested that incorporating 7.5 g kg^{-1} of chia mucilage to oil–water emulsions favors their stability during storage at 4 °C.

CONCLUSIONS

In this study, response surface methodology was used to model and optimize the extraction process of chia mucilage, which involves the exudation of mucilage in an aqueous medium, freeze-drying, and sieving. Temperature, seed: water ratio, and exudation time are important variables of the process that affect the extraction yield and the quality of the chia mucilage, determined according to its physicochemical and functional properties. A second-order polynomial model was used to predict the variation in extraction yield for the following operating conditions: exudation temperature and seed: water ratio, with an exudation time of 2 h. The effect of temperature on the extraction yield of chia mucilage was higher than that of seed: water ratio. Increasing the extraction temperature increased the extraction yield. The predicted optimum operating conditions to maximize extraction of chia mucilage were a temperature of 85 °C and a seed: water ratio of 1: 31 (w/v). The mucilage obtained under these conditions (extraction yield of 116 g kg^{-1} (d.b.)) exhibited good functional properties, mainly in terms of WHC (98.97 g/g) and EA and ESy (54.67 g g^{-1} and 57.56 g g^{-1} respectively), making chia mucilage a potential thickening and stabilizing agent for the food industry. It was also determined that one extraction operation alone does not extract the mucilage exhaustively; because of that, a second extraction under the predicted optimum operating conditions allowed to obtain 68 g kg^{-1} of additional mucilage. Owing to the increasing interest in this subproduct of chia seeds, given its various applications (functional foods: emulsions, bakery products, as well as biodegradable films), advances toward maximizing its extraction yield could represent a significant technological and economic impact for the food industry.

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