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ORIGINAL PAPER

Chia (*Salvia hispanica* L.) cookies: physicochemical/microbiological attributes, nutrimental value and sensory analysis

Natali Alcântara Brandão[1] · Mariana Borges de Lima Dutra[1] · Ana Laís Andrade Gaspardi[1] · Maira Rubi Segura Campos[2]

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Abstract

The objective of this study was to evaluate physicochemical/microbiological attributes, nutrimental value and sensory acceptance of cookies formulated with chia (*Salvia hipanica* L.). Six different formulations were made replacing wheat flour by: 20% chia flour (F20); 20% chia seeds (S20); 10% chia flour + 10% chia seeds (FS20); 30% chia flour (F30); 30% chia seeds (30S); and 15% chia flour + 15% chia seeds (FS30). The cookies were characterized at five storage times (0, 10, 20, 30, and 40 days) for color, volume, maximum breaking strength, water activity, moisture content and, microbiological analyses. The nutrimental value was determined analyzing the proximate composition (moisture content, ash, lipids, proteins, crude fiber, total carbohydrates) and, total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF). Sensory evaluation was performed using the ideal scale for crispness, open-ended method and check-all-that-apply (CATA), acceptance test, and purchase intention test. After 40 days of storage, an increase in the specific volume, water activity, and moisture content of the cookies was observed, despite the lower microbial counts (< 10 CFU/g) for all periods evaluated. The cookies exhibited high protein contents (14.46%, FS30) and high total dietary fiber (73.20%, F20), due to the high content of these components in chia seeds. The samples FS30 and S20 presented greater acceptance for the descriptors good texture, good color, good odor, sweet taste, good taste, and pleasant taste. The addition of chia seeds and chia flour in cookies improved the nutritional value and the sensory acceptance of the products.

Keywords Chia · Cookies · Sensory evaluation · Physicochemical · Microbiological · Nutrimental

Introduction

Interest in chia has been increased due to its numerous health benefits, including the prevention of cardiovascular diseases, some types of cancer and prevention of weight gain, with a significant consumer demand in current society, particularly in Mexico, Southwest of the United States, and South America [1]. The search for a healthy diet through the substitution of less nutritious ingredients for others of higher nutritional value, without compromising the taste of food has been a common practice [2]. Chia (*Salvia hispanica* L.) is an annual herbaceous plant belonging to the Lamiaceae family, from southern Mexico and northern Guatemala [3]. Chia seeds contain about 40% lipids (almost 60% as omega-3) [2], besides proteins of high biological value (about 19% of the total weight) [4, 5]. In addition, they contain natural minerals, vitamins, and antioxidants such as tocopherols (238–427 mg kg^{-1}) and polyphenols [2]. In 1996, chia seeds were recognized by the FAO (United Nations Food and Agriculture Organization) as a potential source of polysaccharides, essentially resulting from their mucilage. One of the characteristics of this mucilage is the particularity of aqueous solution creating a low-concentration gel film around the seeds [6]. Ixtaina et al. [3] have reported that chia seeds contain high fiber contents (34.6%), which is about 9% higher than those found in other cereals and can increase satiety and decrease energy consumption [4]. Dietary fiber is considered an ingredient food due to its important roles in the body, such as intervening in the metabolism of lipids

✉ Maira Rubi Segura Campos
maira.segura@correo.uady.mx

1 Federal Institute of Education, Science and Technology South of Minas Gerais, Inconfidentes, Brazil

2 Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Periférico Norte Km. 33.5, Tablaje Catastral 13615, Col. Chuburná de Hidalgo Inn, 97203 Mérida, Yucatán, Mexico

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and carbohydrates and physiology of the gastrointestinal tract, besides ensuring a slower absorption of nutrients and promoting satiety [7].

Chia has great potential in the food industry, since its consumption is an interesting alternative to improve the nutritional value of foods. Chia seeds have been used as supplements in the production of bread, energy bars, cookies and other functional foods [6].

In addition to the search for healthy foods with high nutritional value, consumers have searched for foods with good sensory characteristics [8]. The optimization of some parameters, such as shape, color, appearance, aroma, flavor, texture, consistency, and the interaction between different components aimed at good quality and good acceptance is important in the development of a new product [9]. The maintenance of sensory quality of a food can favor consumer's loyalty to a specific product in an increasingly demanding market [10].

Sensory assessments using word association methods may be a simple and valid approach to gathering information about consumers' perception of a food, including check-all-that-apply (CATA) and open-ended tests. The CATA method consists of a list of words or phrases from which consumers should select all words they deem appropriate to describe a product [11]. The preference map technique uses multivariate statistical analysis to obtain, in a multidimensional space, a graphical representation of the differences in acceptance of products, identifying the individuals and their preferences [12].

Cookies stand out in the global market for having good sensory and commercial acceptance, being well accepted by children and adults. In recent years, cookies have been considered as a product of great commercial interest due to its practicality in production, marketing and consumption, as well as a long shelf life.

Thus, the objective of this study was to evaluate physicochemical/microbiological attributes, nutrimental value and sensory acceptance of cookies formulated with chia (*Salvia hipanica* L.).

Materials and methods

Cookies manufacture

A blend of refined wheat flour and whole wheat flour, sucralose, egg white, vanilla, salt, baking powder, seed flour, and chia seeds were used for the cookies manufacture. All raw materials were purchased at the local market in the city of Merida, Yucatan, Mexico. The chia seeds were first sieved to remove the soils and ground in a blender (Oster Classic Osterizer 4126, USA) for about 30 s.

Six different cookies formulations were made with different flour and/or chia seed incorporations levels, as follows: F20 (20% chia flour), S20 (20% chia seeds), FS20 (10% chia flour + 10% of chia seeds), F30 (30% chia flour), S30 (30% chia seeds), and FS30 (15% chia flour + 15% chia seeds).

For the cookies manufacture, first all the ingredients were weighed. Flours were sieved to avoid lumps. The liquid ingredients were blended for 2 min in a blender (Kitchen Aid Professional 600 Series, USA), and then the solid ingredients were added to the mixture and homogenized for 2 min. Eight grams of sample were shaped into a cylinder with 5 cm in diameter and 2 mm thick, and placed in stainless steel baking sheets covered with buttered parchment paper. The samples were baked at 150 °C for 15 min, and then cooled to 25 °C, and stored in plastic bags made from low-density polyethylene.

Physicochemical and microbiological characterization throughout 40 days of storage

The cookies were evaluated from time 0 up to 40 days, (5 evaluation periods): 0, 10, 20, 30, and 40 days. Color measurements, specific volume, maximum breaking strength, water activity, moisture content, and microbiological analyses (filamentous fungi, yeasts, and aerobic mesophiles) were determined, all in triplicate, according to the following methodologies. Color measurements were performed according to Rhim et al. [13] in a colorimeter (Color Guide Gardner, Germany) using a white plate for calibration. The readings were performed in triplicate, according to the Hunter scale of CIE color system (L^* a^* b^*), where L^* represents brightness, ranging from black to white (0 to 100), a^* and b^* represent Chroma, with a^* ranging from green to red (−500 to 500) and b^* from blue to yellow (−200 to 200). The specific volume of the samples was measured by the seed displacement test [14]. Maximum breaking strength tests were performed according to the methods proposed by AACC 74-09. The samples were subjected to uniaxial compression between two parallel plates in a Universal Instron 4411 machine-USA, using a test tube with a diameter of 12.75 mm, a load cell of 500 N, with an extension band of 4.5 mm, and speed of 25 mm/min. The water activity was measured using Aqua Lab (dew point water activity meter 4 TEV) [15]. Moisture content was determined according to the method 925.01 of AOAC [16] by drying the samples in an oven at 105 °C until constant weight. Filamentous fungi, yeasts, and total bacteria (aerobic mesophiles) counts were carried out according to the Mexican regulation [15]. Filamentous fungi and yeasts counts were determined after incubation at 25 °C for 7 days using Potato Dextrose Agar (PDA, Merk-USA) as the culture medium. Plate Count Agar (PCA, Merk-USA) was used for counting the aerobic mesophiles, with incubation at 37 °C for 48 h.

Proximate composition

The proximate composition of the cookies was assessed by the determination of moisture content, ash, lipids (ethereal extract), proteins (total nitrogen), crude fiber, and carbohydrates according to the following methodologies: The moisture content was determined in an oven (Fisher Scientific 851-f, USA) at 105 °C until constant weight according to the method 925.01 of AOAC [16]. Ash was determined according to the methodology 923.03 of AOAC [16], with incineration in a muffle (Thermo Scientific, Thermolyne F48020-33-80, USA) at 550 °C for 4 h. Lipids were determined by extraction with hexane as described in the method 920.39 of AOAC [16], in Foss Soxtec 8000 HT System TECATOR (Sweden) extraction unit. Proteins were determined by the Kjeldahl method as described in the methodology 954.01 of AOAC [16] in BICH-KjelFlex k-360 model (AutoKjekdahl Unit K-360, Buchi-Switzerland), and calculated using 6.25 as the conversion factor. Crude fiber was determined according to the methodology 962.09 of AOAC [16], by acid hydrolysis followed by basic hydrolysis in Ankom 2000 fiber analyzer (A200-USA). The Carbohydrates were calculated using the following equation:

Carbohydrates (%) = 100 – (moisture (%) + ash (%) + protein (%) + lipids (%) + fibers (%)) [16].

Total Dietary Fiber (TDF), Insoluble Dietary fiber (IDF), and Soluble Dietary Fiber (SDF)

Total dietary fiber was determined according to the enzymatic method proposed by Prosky et al. [17]. Firstly, 1 g of sample was placed in Erlenmeyer flask and 50 mL of 0.05 N phosphate buffer at pH 6 was added. The erlenmeyers were then placed in a water bath at 100 °C, and 0.1 mL of thermostable α-amylase (Sigma A-3306, USA) was added and stirred at 60 rpm for 15 min using a Cole-Parmer Stir Pak (Heavy Duty Mixer System, USA). After cooling, the pH was adjusted to 7.5 using NaOH 1.0 M. The erlenmeyers were again placed in a water bath at 60 °C, and 0.1 mL of protease (Sigma P-3910, USA) was added and stirred at 60 rpm for 30 min (Cole-Parmer Stir Pak-Heavy Duty Mixer System, USA). After cooling, the pH was adjusted to 4.0 with HCl 1.0 M. The erlenmeyers were placed in a water bath at 60 °C, and 0.3 mL of amyloglucosidase (Sigma A-9913, USA) was added and stirred for 30 min. Finally, 95 g/kg ethanol, preheated at 60 °C, was added to the flasks at a ratio of 1: 4 (v/v). The contents of the erlenmeyers were vacuum filtered in crucibles containing Celite (Sigma C-8656, USA). The remaining residue was subjected to washing cycles for three times with 20 mL of 780 g/kg ethanol, twice with 10 mL of 950 g/kg ethanol, and twice with 10 mL acetone. The contents of the crucible were dried at 105 °C. The protein content (N x 6.25) was determined in the residue of two crucibles, and the remaining crucibles were incinerated at 550 °C for 4 h.

$$\text{TDF}(\%) = [\text{mass of residue (g)} - \text{protein (g)} - \text{ash (g)} - \text{blank}]/\text{mass of sample (g)} \times 100.$$

Insoluble dietary fiber (IDF) was determined according to the methodology described by Prosky et al. [17], similar to the determination of TDF, except for the addition of 95% ethanol at a ratio of 1: 4.

Soluble dietary fiber (SDF) was calculated according to Prosky et al. [17], by the difference between TDF and IDF. SDF = TDF – IDF.

Sensory evaluation

Presentation of samples

Samples were presented at room temperature in disposable containers coded with 3-digit random numbers containing about 6 g of sample, 5 cm in diameter, in sequential monadic form [18]. The tests were performed in individual booths with white fluorescent lighting.

Ideal scaling test

To evaluate the crunchiness of the cookies and determine the ideal incorporation level of chia flour and/or seeds, an ideal scaling test was performed using a 9-point structured hedonic scale anchored at the extremes "extremely less crunchy than the ideal" and "extremely crunchier than the ideal" [19]. For that, 60 consumers indicated how close the sample was to an ideal product.

Open-ended test

Sixty volunteers used four words or expressions to describe the characteristics of each cookie [11], and the 15 most frequent descriptors were selected for use in the CATA method.

Check all that apply (CATA) test

After the frequency analysis of the responses of the open-ended method, the terms that stood out were used as descriptors in the CATA method. The samples were served to 120 consumers, who marked on an evaluation card all descriptors that, according to their perception, were related to each sample [20].

Acceptance test

One-hundred and twenty consumers evaluated the appearance, aroma, flavor, texture, and overall impression of the samples. For the test, a 9-point structured hedonic scale was used, anchored in the extremes by “I disliked very much” and “I liked very much” [18].

Purchase intention

The intention to purchase the product was evaluated using a 5-point structured scale ranging from “certainly would not buy” to “certainly would buy” [19].

External preference map

An external preference map was constructed from the results of overall impression obtained in the acceptance test and the descriptors obtained in the open-ended test, evaluated by the CATA method.

Statistical analysis

All data were analyzed by ANOVA and Tukey test with a confidence level of 95% ($p \leq 0.05$). The results of the physicochemical, proximate composition, and total dietary fiber were analyzed using the software STATGRAPHICS® (Centurion XV 2006, USA). The results of the open-ended test, CATA, external preference map, acceptance test and ideal scaling were analyzed using the Sensomaker® software developed by Pinheiro et al. [21] (Lavras, Brazil, 2013). A frequency histogram was constructed for the purchase intention test using the software Microsoft Office® Excel 2010 (Microsoft, USA).

Results and discussion

Physicochemical and microbiological characterization of the samples throughout 40 days of storage

The parameters color, specific volume, maximum breaking strength, water activity (AW), moisture content, and the microbiological characterization were evaluated for 40 days at 0, 10, 20, 30, and 40 days of storage.

Color

The results of the color parameters L*, a*, and b* of the cookies are shown in Table 1.

In general, the sample F30 presented lower L* values (luminosity) when compared with all samples at days 10, 20, 30, and 40, while the samples S20 and S30 exhibited the highest L* values for all periods studied. The difference in brightness between the samples may be due to the particle size of chia flour and chia seeds, since chia flour has a lower particle size, thus making a more homogenous

Table 1 Color parameters L*, a* and b* of chia cookies at different times

Parameter	Samples	Time (days)				
		0	10	20	30	40
L*	F20	47.30 ± 0.19^{cA}	45.83 ± 0.50^{dB}	44.41 ± 0.23^{dC}	44.63 ± 0.81^{cC}	46.86 ± 0.60^{cA}
	S20	$51.96 \pm 1.14^{aC,B}$	$53.35 \pm 1.15^{bB,A}$	$54.07 \pm 0.41^{b,A}$	$53.31 \pm 0.24^{aB,A}$	51.18 ± 0.90^{bC}
	FS20	43.60 ± 0.38^{dD}	$49.52 \pm 0.24^{cC,B}$	51.05 ± 1.32^{cA}	48.70 ± 0.50^{bC}	$50.53 \pm 1.11^{bB,A}$
	F30	43.62 ± 0.35^{dB}	45.44 ± 0.36^{dA}	42.32 ± 0.26^{eC}	$42.81 \pm 0.65^{dC,B}$	43.73 ± 0.74^{dB}
	S30	50.04 ± 1.57^{bC}	57.08 ± 0.68^{aA}	56.62 ± 0.50^{aA}	52.68 ± 1.48^{aB}	56.34 ± 0.51^{aA}
	FS30	46.59 ± 1.43^{cC}	$48.60 \pm 1.84^{cC,B}$	$50.34 \pm 0.46^{cB,A}$	47.94 ± 0.10^{bC}	51.61 ± 1.40^{bA}
a*	F20	$7.67 \pm 0.35^{cC,B,A}$	6.64 ± 0.29^{cC}	$7.75 \pm 0.64^{bB,A}$	$8.39 \pm 0.86^{a,bA}$	$6.89 \pm 0.63^{cC,B}$
	S20	$6.50 \pm 0.30^{dC,B}$	8.26 ± 0.55^{bA}	6.06 ± 0.34^{cC}	$7.12 \pm 0.75^{d,cB}$	8.47 ± 0.36^{aA}
	FS20	10.81 ± 0.54^{aA}	9.72 ± 0.47^{aB}	9.55 ± 0.44^{aB}	$7.84 \pm 0.33^{c,bC}$	$7.50 \pm 0.23^{c,bC}$
	F30	6.29 ± 0.29^{dD}	$7.17 \pm 0.08^{c,bC}$	5.94 ± 0.40^{cD}	9.04 ± 0.51^{aA}	$8.08 \pm 0.61^{b,aB}$
	S30	8.96 ± 0.17^{bA}	$7.20 \pm 1.29^{c,bB}$	$6.29 \pm 0.20^{cC,B}$	$6.46 \pm 0.53^{dC,B}$	5.84 ± 0.19^{dC}
	FS30	7.83 ± 1.07^{cB}	9.52 ± 0.43^{aA}	6.42 ± 0.42^{cC}	$7.07 \pm 0.28^{d,cC,B}$	$7.12 \pm 0.58^{cC,B}$
b*	F20	19.61 ± 0.04^{cA}	16.68 ± 0.24^{cC}	16.87 ± 0.21^{cC}	$17.73 \pm 1.42^{bC,B}$	18.18 ± 0.37^{cB}
	S20	21.66 ± 0.41^{aA}	21.56 ± 0.32^{aA}	19.75 ± 0.57^{bB}	22.20 ± 0.42^{aA}	20.38 ± 0.29^{bB}
	FS20	20.70 ± 0.11^{cB}	21.21 ± 0.11^{aA}	21.54 ± 0.31^{aA}	21.56 ± 0.47^{aA}	21.32 ± 0.14^{aA}
	F30	$17.84 \pm 0.04^{dB,A}$	16.63 ± 0.27^{cC}	$17.04 \pm 0.86^{cC,B}$	$17.41 \pm 0.38^{bC,B}$	18.06 ± 0.53^{cA}
	S30	$21.02 \pm 0.77^{b,aA}$	20.97 ± 0.48^{aA}	20.98 ± 0.24^{aA}	21.22 ± 0.26^{aA}	20.52 ± 0.35^{bA}
	FS30	17.86 ± 0.79^{dB}	18.51 ± 0.44^{bB}	20.98 ± 0.24^{aA}	18.03 ± 0.76^{bB}	18.07 ± 0.68^{cB}

Means followed by the same letter lowercase, in the same column, or means followed by the same letter uppercase, in the same row, do not differ from each other at $p \leq 0.05$ by Tukey’s test dough, which resulted in cookies with lower luminosity, that is, darker than those made only with chia seeds.

The sample S30 presented lower a* values (with a greater tendency to green color) at 30 and 40 days of evaluation, differing significantly from all the others in these times. The sample FS20 presented higher a* values (with greater tendency to red color) at times 0, 10 and 20.

The samples F20 and F30 made with the addition of chia flour showed lower b* values for all periods, that is, they presented a darker and less yellow color due to the dark color of flour, which is better distributed in the dough, and masks the yellow coloration of the samples. The samples S20, FS20, and S30 were the yellowest samples, with the highest b* values, with no significant differences among them.

During storage, small changes in L*, a*, and b* values were observed for all samples, which means that the color of the cookies remained constant during 40 days of storage. These results are interesting from an industrial point of view, once they represent color stability of the food products.

Specific volume

The results of the specific volume (mL/g) of the samples are shown in Table 2.

An increase in the specific volume was observed for the samples S20, F30, S30, and FS30 at day 40, when compared to the first day of evaluation (time 0), while the samples F20 and FS20 showed a reduction of the specific volume over time. No significant differences were observed for the samples F30 and FS30 at day 10. Only the sample F20 differed significantly from the others at 20 days of evaluation. At day 30, no significant differences were observed for the specific volume of the samples F20 and FS20. At day 40, the samples F20, S20, and S30 did not differ among them ($p \leq 0.05$), and the samples FS20 and F30 presented similar behavior. The difference in the specific volume of the samples was due to the different addition levels of chia flour and/or seeds, once

Table 2 Specific volume (mL/g), maximum breaking strength (N), water activity (Aw) and moisture content (%) of chia cookies at different times

Parameter	Samples	Time (days)				
		0	10	20	30	40
Specific volume (mL/g)	F20	3.14 ± 0.65^{aA}	1.84 ± 0.19^{dB}	1.91 ± 0.07^{bB}	$2.13 \pm 0.19^{c,bB}$	$3.06 \pm 0.59^{b,aA}$
	S20	$2.16 \pm 0.1^{c,bB}$	$2.66 \pm 0.24^{aB,A}$	2.3 ± 0.08^{aB}	$1.97 \pm 0.53^{d,cB}$	$3.34 \pm 0.68^{b,aA}$
	FS20	$2.77 \pm 0.15^{b,aA}$	$2.42 \pm 0.1^{b,aA}$	2.45 ± 0.20^{aA}	$2.13 \pm 0.11^{c,bA}$	2.66 ± 0.83^{bA}
	F30	$2.18 \pm 0.09^{c,bB}$	$2.25 \pm 0.11^{c,bB}$	2.35 ± 0.13^{aB}	1.64 ± 0.32^{dC}	2.74 ± 0.21^{bA}
	S30	1.23 ± 0.63^{dD}	$2.05 \pm 0.08^{d,cC}$	$2.44 \pm 0.09^{aC,B}$	$2.93 \pm 0.46^{aB,A}$	$3.3 \pm 0.24^{b,aA}$
	FS30	$1.72 \pm 0.17^{d,cD}$	$2.22 \pm 0.23^{c,bC}$	$2.5 \pm 0.20^{aC,B}$	$2.6 \pm 0.25^{b,aB}$	3.83 ± 0.11^{aA}
Maximum breaking strength (N)	F20	497.22 ± 1.20^{bB}	498.46 ± 1.30^{cB}	497.58 ± 2.08^{aB}	505.03 ± 2.53^{aA}	491.18 ± 1.11^{cC}
	S20	504.14 ± 0.56^{aA}	502.93 ± 1.23^{aA}	493.04 ± 1.87^{bB}	492.48 ± 1.32^{dB}	503.56 ± 2.02^{aA}
	FS20	493.59 ± 0.30^{cC}	501.07 ± 2.02^{baA}	497.48 ± 1.41^{aB}	499.18 ± 0.39^{cBA}	497.35 ± 1.72^{bB}
	F30	492.74 ± 3.04^{cB}	501.79 ± 1.24^{baA}	494.67 ± 2.64^{baB}	502.25 ± 1.39^{bA}	500.32 ± 1.84^{bA}
	S30	498.33 ± 0.00^{bA}	494.77 ± 0.56^{dB}	493.98 ± 0.57^{bB}	497.51 ± 1.73^{cA}	498.78 ± 1.86^{bA}
	FS30	492.55 ± 1.30^{cB}	499.44 ± 1.52^{cbA}	492.32 ± 1.96^{bB}	493.40 ± 0.69^{dB}	493.76 ± 1.36^{cB}
Water activity (Aw) *	F20	0.50 ± 0.00^{aD}	0.55 ± 0.01^{aB}	0.53 ± 0.00^{aC}	$0.56 \pm 0.00^{aB,A}$	0.56 ± 0.00^{bA}
	S20	$0.42 \pm 0.01^{c,bD}$	$0.49 \pm 0.02^{c,bB}$	0.45 ± 0.00^{bC}	0.46 ± 0.00^{cC}	0.53 ± 0.01^{cA}
	FS20	0.37 ± 0.02^{dD}	0.45 ± 0.01^{cB}	0.43 ± 0.02^{cC}	0.42 ± 0.00^{dC}	0.54 ± 0.01^{cA}
	F30	0.43 ± 0.01^{bB}	0.36 ± 0.05^{dC}	0.45 ± 0.00^{bB}	0.47 ± 0.00^{bB}	0.57 ± 0.02^{aA}
	S30	$0.34 \pm 0.02^{eC,B}$	$0.52 \pm 0.03^{b,aA}$	0.33 ± 0.00^{eC}	0.36 ± 0.00^{eB}	0.49 ± 0.00^{eA}
	FS30	$0.40 \pm 0.01^{cD,C}$	0.43 ± 0.04^{cB}	0.38 ± 0.00^{dD}	$0.42 \pm 0.00^{fC,B}$	0.51 ± 0.00^{dA}
Moisture content (%)	F20	9.52 ± 0.00^{aA}	7.32 ± 0.03^{aD}	7.53 ± 0.06^{aC}	6.85 ± 0.03^{aE}	8.17 ± 0.01^{bB}
	S20	7.61 ± 0.06^{bA}	2.92 ± 0.09^{eC}	5.82 ± 0.02^{bB}	5.42 ± 0.34^{bB}	$7.87 \pm 0.52^{b,cA}$
	FS20	7.38 ± 0.16^{bB}	4.12 ± 0.01^{dD}	4.60 ± 0.12^{cC}	4.00 ± 0.05^{cD}	$7.91 \pm 0.10^{b,cA}$
	F30	8.91 ± 0.79^{aA}	5.17 ± 0.01^{cC}	5.59 ± 0.01^{bC}	3.95 ± 0.01^{cD}	$7.89 \pm 0.09^{b,cB}$
	S30	5.40 ± 0.67^{cB}	5.73 ± 0.02^{eB}	3.37 ± 0.11^{eC}	3.08 ± 0.07^{dC}	6.77 ± 0.37^{cA}
	FS30	7.37 ± 0.32^{bB}	4.12 ± 0.01^{dD}	4.24 ± 0.19^{dC}	4.29 ± 0.01^{cC}	10.24 ± 1.03^{aA}

Means followed by the same letter lowercase in the same column, or means followed by the same letter uppercase in the same row, do not differ from each other at $p \leq 0.05$ by Tukey's test the formulations with greater percentages of chia flour and/or seeds showed an increase in the specific volume after 40 days of storage.

Maximum breaking strength

The results of the maximum breaking strength of the different cookie formulations are shown in Table 2.

No significant differences were observed for breaking strength (N) of the samples S20, S30, and FS30 from the first day (day 0) to the last day of storage (day 40), with small changes in this parameter, demonstrating a sample stability throughout 40 days of storage.

Castro et al. [22] determined the texture parameters of cookies commercialized in the Chilean market using the three-point breaking test, which corresponds to a flexural test in which the maximum load is applied before rupture. The authors obtained maximum strength values up to 355 N to break the cookies.

Water activity: Aw

All samples presented higher water activity at 40 days of storage, when compared to the first day (day 0), probably due to the hygroscopicity of the cookies, which are characterized by a low-moisture product, with a tendency to absorb water during storage. The samples with addition of chia flour (F20 and F30) had the highest water activity values in most of the periods studied. Measuring Aw allows understanding the behavior of a food product. In general, water activity values close to 1 represent greater instability of the product, once most bacteria, yeasts, and fungi require minimum water activity values of 0.91, 0.88, and 0.80 respectively for growth [23]. The lower water activity values of the cookies represent stability of the product and thus consumer's safety, once there is a minimum probability of microbial growth.

Moisture contents

The moisture contents of the different cookie formulations are shown in Table 2.

Changes in moisture contents were observed for all samples, throughout the storage, and the samples FS20, S30, and FS30 presented an increase in moisture from 7.38, 5.40, and 7.37 to 7.91, 6.77, and 10.24%, respectively, from day 0 to 40 days of evaluation. Zuniga et al. [24] evaluated the moisture content of whole-cashew nut crackers during 80 days of storage and found a gradual increase in moisture ranging from 5.21 (day 0) to 9.16%.

Water absorption in food depends on several factors, including packaging, storage conditions, handling and preparation procedures and the properties of the ingredients used in the formulation. Cauvain and Young [25] have reported that the water absorption in bakery products depends mainly on two parameters: the protein content (which absorbs its same weight in water) and the fiber content of the dough (the fibers have a great union capacity with the water, being able to be responsible for the absorption of water, in up to one-third of its mass). In our study, the highest moisture content was observed in the sample FS30 after 40 days of storage (10.24%), which also presented high fiber and protein contents.

Microbiological characterization

The mesophilic aerobes counts were less than 10 CFU/g for all evaluation periods. The limits established by the Brazilian legislation in force for microbiological standards, Resolution of the Board of Directors—RDC N. 12, January 2, 2001 of the National Agency of Sanitary Surveillance is up to 5×10^2 CFU/g [26], thus these findings are within the standards recommended by the current legislation, which is a positive result once the mesophilic aerobes can cause food spoilage, especially in processed products. Lower counts (< 10 CFU/g) were also observed for mold and yeasts of the cookies for all evaluation periods. The absence of molds and yeasts indicate that the cookies were made using high quality raw materials, under adequate hygienic conditions, without failures in processing or storage, thus guaranteeing microbiological safety to the product.

Table 3 Proximate composition of chia cookies

Samples	Moisture %	Ash %	Protein %	Crude fiber %	Lipids %	Carbohydrates %
F20	9.52 ± 0.00^{a}	$3.11 \pm 0.03^{b,c}$	12.44 ± 0.06^{c}	13.72 ± 0.41^{b}	$13.04 \pm 0.21^{c,d}$	$48.17 \pm 0.178^{b,a}$
S20	7.61 ± 0.06^{b}	3.11 ± 0.02^{c}	11.93 ± 0.08^{c}	12.71 ± 1.10^{b}	12.55 ± 0.12^{d}	52.09 ± 0.94^{a}
FS20	7.38 ± 0.16^{b}	2.7 ± 0.04^{d}	13.26 ± 0.16^{b}	12.16 ± 0.41^{b}	16.05 ± 0.16^{b}	$48.44 \pm 0.27^{b,a}$
F30	$8.91 \pm 0.19^{a,b}$	$3.18 \pm 0.04^{b,c}$	13.72 ± 0.26^{b}	11.06 ± 0.06^{b}	21.78 ± 0.10^{a}	41.36 ± 1.13^{c}
S30	5.4 ± 0.67^{c}	$3.37 \pm 0.11^{a,b}$	13.7 ± 0.10^{b}	17.48 ± 0.50^{a}	13.22 ± 0.09^{c}	46.82 ± 0.28^{b}
FS30	7.37 ± 0.32^{b}	3.48 ± 0.10^{a}	14.46 ± 0.04^{a}	17.37 ± 1.45^{a}	16.45 ± 0.16^{b}	40.87 ± 2.07^{c}

Means followed by the same letter in the same column do not differ from each other at $p \leq 0.05$ by the Tukey' test

Proximate composition

The results of moisture content, ash, protein, crude fiber, lipids, and carbohydrates are shown in Table 3.

The samples made only with chia flour, F20 and F30, had higher moisture contents, without significant differences between them. The sample made with 30% chia seeds exhibited the lowest moisture content when compared with all the others, whereas the formulations containing blends of chia flour and chia seeds showed intermediate moisture values. The moisture values found in this study are higher to those found in traditional cookies made with wheat flour alone, which presented moisture levels of 4.6% [27] and 4.77% [28]. The higher moisture contents observed in the samples made with chia flour alone are due to the high water-binding capacity of the molecules, which was not observed in the samples made with chia seeds, once the contact surface was smaller and the samples absorbed lower moisture.

Regarding the ash levels, the sample FS30 presented the highest content (3.48%), with significant differences from the others except S30, while the lowest ash content was observed for the sample FS20 (2.7%). These results are higher than those found by Blanco-Metzler et al., [27] and Assis et al., [28] who found ash contents of 1.4 and 1.46%, respectively, in traditional cookies made with wheat flour alone. Ash represents the mineral content of cookies. Regarding mineral content, chia seeds are an excellent source of calcium, phosphorus, magnesium, potassium, zinc, and copper [29].

In relation to protein content, an increase in protein was observed with the increase in chia seeds and chia flour in the formulations. The sample FS30 presented higher protein levels (14.46%), with significant differences from the others. In contrast, the samples S20 and F20 had the lower protein levels (11.93 and 12.44%), respectively, and did not differ from each other. The protein contents observed in this study were higher than those observed in traditional cookies. Blanco-Metzler et al., [27] found 9.7% protein, while Assis et al., [28] described 6.96% proteins in traditional cookies. The high protein content observed in cookies made with chia flour and/or chia seeds are due to the protein content of chia seed, which contains about 19% of total weight, with a high biological value [5].

The samples S30 and FS30 presented the highest crude fiber levels, with values of 17.48 and 17.37%, respectively, with no significant difference between them. The other formulations had lower values of crude fiber and with no significant difference between them. The Ministry of Health of Brazil [30] states that solid foods must contain at least 3 g fiber/100 g to be considered as a fiber source and at least 6 g/100 g to be considered a high-fiber product. Thus, the present results show that the cookies made with chia flour and/or chia seeds can be considered high-fiber foods, due to the high fiber levels of chia seeds, around 34.6% [4], making the cookies a viable alternative for inclusion of a high-fiber product in the consumer market.

With respect to the lipid content, the incorporation of chia flour increased the availability of lipids in cookies, which resulted in a higher lipids levels in cookies made with chia flour alone. The highest lipid content was observed in the sample F30 (21.78%), which differed significantly from the others. The lowest lipids level was observed in the sample made with 20% chia seeds (12.55%). The lipids contents found in this study are similar to those found by Assis et al. [28] in traditional cookies (21.23%), and higher than those found in cookies made with wheat flour alone (4.8%) [27]. The high lipid levels of the cookies were due to the addition of chia flour and/or chia seeds to the formulations, once chia seeds contain about 33% lipids [3].

The lowest carbohydrates levels were observed in the samples F30 (41.36%) and FS30 (40.87%), with significant differences from the others. The carbohydrates content decreased with the addition of chia flour and/or chia seeds, probably due to the low carbohydrates of chia seeds, which, according to Ixtaina et al. [3] is about 23%, lower than the amount of fiber and lipids.

According to the proximate composition, the formulation FS30 made with 15% chia flour and 15% chia seeds had a higher nutritive value, and higher ash, proteins, and crude fiber contents, with no significant difference ($p \geq 0,05$) when compared to the sample S30 for ash and crude fiber content. Considering the nutritional quality, cookies containing higher concentrations of chia flour and/or chia seeds are attractive for consumers, who are looking for a healthy and nutritionally rich diet.

Total dietary fiber (TDF), insoluble dietary fiber (IDF), soluble dietary fiber (SDF)

The results of total dietary fiber, insoluble fiber, and soluble fiber of the cookies are shown in Table 4.

Table 4 Total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) of chia cookies

	TDF (%)	IDF (%)	SDF (%)
F20	73.20 ± 4.94^{a}	15.08 ± 0.66^{d}	58.13 ± 5.31^{a}
S20	63.72 ± 2.89^{b}	40.51 ± 0.97^{c}	23.21 ± 3.67^{c}
FS20	56.31 ± 0.472^{c}	16.81 ± 4.07^{d}	39.50 ± 4.47^{b}
F30	57.24 ± 2.31^{c}	53.37 ± 1.82^{a}	3.87 ± 2.46^{e}
S30	54.98 ± 4.28^{c}	44.14 ± 0.88^{b}	10.84 ± 3.73^{d}
FS30	63.58 ± 2.07^{b}	56.90 ± 3.46^{a}	$6.68 \pm 4.11^{e,d}$

Means followed by the same letter in the same column do not differ from each other at $p \leq 0.05$ by the Tukey's test

The sample F20 presented higher levels of total and soluble dietary fiber, with values of 73.20 and 58.13%, respectively, differing statistically from the others. No significant difference was observed between the samples FS20, F30, and S30 for the total dietary fiber. Regarding the insoluble dietary fiber, the samples FS30 and F30 presented higher levels, with values of 56.90, and 53.37% respectively, with no significant differences between them. The high contents of total dietary fibers in the cookies are due to a large amount of fiber from chia seeds.

Soluble and insoluble fibers play important functions in the human body, regulating the intestinal function, lowering blood cholesterol levels, and preventing colorectal cancer [31]. Thus, considering the health benefits of fibers and the present results, we can state that the cookies have high nutritional value, being an excellent alternative for the insertion in a healthy diet with the replacement of more caloric foods by foods with higher nutritional value.

Sensory evaluation

Ideal scaling

The ideal scaling results were: F20 (-2.30^{b}), S20 (-1.63^{ab}), FS20 (-0.80^{a}), F30 (-1.63^{ab}), S30 (-1.15^{a}), FS30 (-1.7^{ab}) where values followed by at least one same letter don´t differ by Tukey's test. The cookies with mean scores closer to zero were considered as having the ideal crispness.

The samples FS20 and S30 presented scores closer to 0, with no significant differences between them, thus they can be considered with crispness closer to the ideal. No significant differences were observed for the samples S20, F30, and FS30. The sample containing 20% chia flour presented a score very far from 0, differing statistically from the others.

Open-ended test

In the open-ended method, the consumers used four words to describe the characteristics of each sample, which led to the selection of 15 more frequent words, as follows: pleasant, bitter, good color, good odor, good texture, crunchy, slightly crunchy, slightly sweet, hard, tasteless, strong, dry, and smooth. The most cited terms were selected among all attributes, corresponding to 56% of the total terms, and the minimum frequency was 1.1% corresponding to the expression "slightly sweet". The terms obtained in this method were used as descriptors in the CATA method.

Acceptance test

The attributes aroma, appearance, flavor, texture, and overall impression of each sample were evaluated and the results are shown in Table 5.

Table 5 Acceptance scores of chia cookies formulations

Sample	Appearance	Aroma	Flavor	Texture	Overall impression
F20	5.57^{bc}	5.72^{ab}	5.47^{ab}	4.86^{c}	5.62^{a}
S20	5.72^{ab}	5.96^{a}	6.17^{a}	5.33^{bc}	5.95^{a}
FS20	6.17^{a}	5.69^{ab}	4.86^{bc}	5.34^{bc}	5.32^{a}
F30	5.13^{c}	5.18^{b}	4.42^{c}	4.71^{c}	5.38^{a}
S30	6.13^{ab}	5.92^{a}	5.24^{b}	5.53^{ab}	5.79^{a}
FS30	5.92^{ab}	5.74^{ab}	6.05^{a}	6.04^{a}	6.08^{a}

Means followed by the same letter in the same column do not differ from each other at $p \leq 0.05$ by the Tukey's test

No significant difference was observed among the samples FS20, S20, S30, and FS30 for the appearance, with greater acceptance for the sample FS20. The samples S20, S30, FS30, F20, and FS20 did not differ statistically from each other for the attribute aroma. In relation to the flavor attribute, the samples S20 and FS30 presented the highest means of acceptance not differing significantly from the F20 sample. The FS30 and S30 samples did not differ significantly from each other in relation to the texture, presenting the highest averages of acceptance in this attribute. With respect to the overall impression, no differences were observed among the samples, with the highest score observed for the sample FS30. The sample containing 30% chia flour (F30) showed lower scores for most of the attributes (appearance, aroma, flavor, and texture), while the sample FS30 had higher scores for the attributes flavor, texture, and overall impression.

Berro et al. [32] made cookies using different chia flour concentrations and evaluated the sensory acceptance of three formulations. The results indicated that the formulation with the highest percentage of chia flour (40%) showed the best acceptance scores, with values above 5.5 on the hedonic scale for all the sensory parameters studied.

External preference map

According to the preference map, the vectors directed from the zero point toward the sample indicate greater degree of liking, as shown in Fig. 1.

The external preference map, based on the results of CATA method (check all that apply) [20], allows identifying the consumer's perception in relation to food by directing vectors on the map. Samples with a greater vector targeting may be considered more preferred by consumers, while those with little vector targeting are considered to be poorly accepted by consumers. The map also shows the relationship between the acceptance and descriptors, being able to identify the characteristics that influence the acceptance of the product.

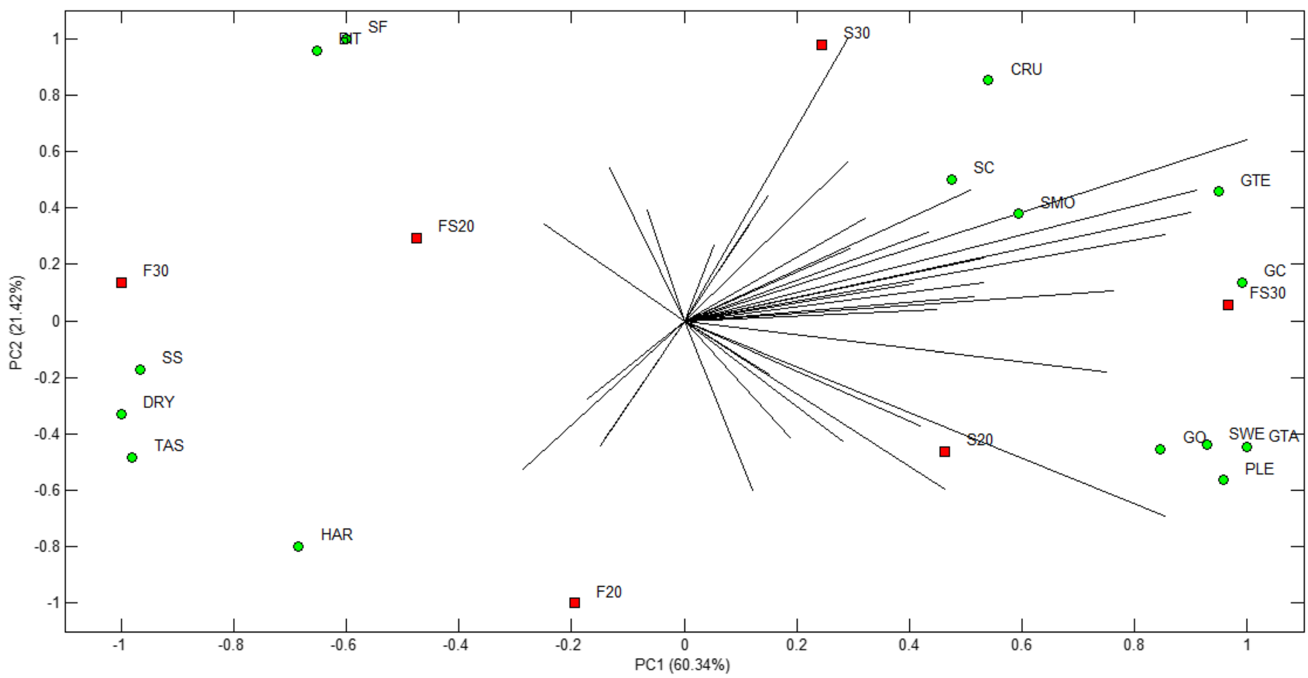

Fig. 1 External preference map of cookies, using CATA results. Pleasant: PLE; bitter: BIT; good color: GC; good taste: GTA; good texture: GTE; good odor: GO; crunchy: CRU; sweet: SWE; hard: HAR; tasteless: TAS; slightly crunchy: SC; strong flavor: SF; dry: DRY; smooth: SMO; slightly sweet: SS

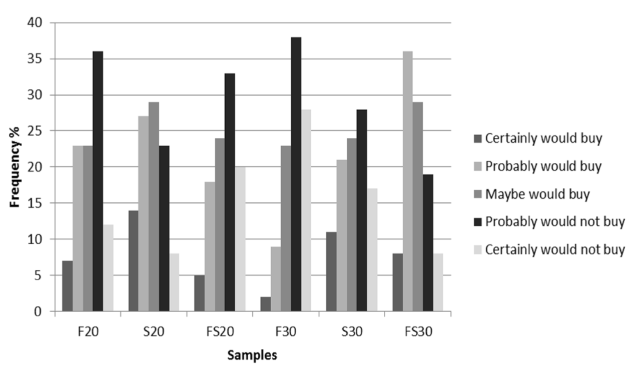

Fig. 2 Frequency distribution of the purchase intention responses of chia cookies

Thus, the samples FS30 and S20 presented greater acceptance (great targeting of vectors to these samples), and were related mainly to the descriptors good texture, good color, good odor, sweet taste, good taste, and pleasant taste. The sample S30 was related to the descriptor crunchy, which can confirm the result of the ideal scaling, once this sample presented crispness closer to the ideal. The sample F30 presented lower acceptance (with low vector targeting), being related to the descriptors slightly sweet, dry, and tasteless, confirming the results of the acceptance test, once this sample had lower acceptance scores for all attributes.

Purchase intention

The cookies made with chia flour and/or chia seeds were also evaluated for the purchase intention test (Fig. 2). The sample FS30 showed the highest positive purchase intention (44%), represented by the responses "probably would buy" (36%) and "certainly buy" (8%), which corroborates the results of the acceptance test. The samples S20 and FS30 showed the highest purchase indecision (29%) represented by the response "maybe would buy", while the sample made with addition of 30% chia flour (F30) had a higher negative purchase intention (66%), with the responses "probably would not buy" (38%) and "certainly would not buy" (28%), also confirming the lower averages found in the acceptance test.

The results of the sensory evaluation demonstrated that the cookies made with the chia flour and chia seeds blend or chia alone stood out in the sensory acceptance. The results show that the cookies with the higher chia contents were pleasant and well accepted by consumers, thus the production of cookies with a high nutritional value and a good sensory acceptance can be a good strategy from a commercial point of view.

Conclusions

The cookies made with chia flour exhibited a darker color, with low L* values (luminosity) and small changes in the color parameters L*, a*, and b* during the storage. At the end of the storage, an increase in specific volume, water activity, and moisture was observed for all cookies. On the other hand, no changes were observed for the microbial counts of the samples for all periods studied. Regarding the proximate composition, the sample FS30 stood out with the highest ash, proteins, and crude fiber, with no significant difference when compared to the sample S30 for the ash and crude fiber content. The sample F20 presented the highest total dietary fiber and soluble dietary fiber levels, while the samples FS30 and F30 presented the highest insoluble dietary fiber contents. The samples FS20 and S30 showed crispness closer to the ideal. According to the external preference map, the sample S30 was related to the descriptor crunchy. The samples FS30 and S20 presented higher acceptance scores, and were mainly related to the descriptors good texture, good color, good odor, sweet, good taste, and pleasant. The sample FS30 exhibited the highest positive purchase intention and consumers' acceptance scores, and stood out in the proximate composition.

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