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Vegan brownie enriched with phenolic compounds obtained from a chia (*Salvia hipanica* L.) coproduct: Nutritional, technological, and functional characteristics and sensory acceptance

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A R T I C L E I N F O

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A B S T R A C T

This study aimed to develop a nutritious vegan brownie with fiber and bioactive compounds, sensorially accepted by consumers using phenolic compounds extracted from defatted chia flour (a coproduct of the production of chia oil) and chia mucilage. The brownies were developed by evaluating the replacement of 2, 4, and 6% of the flour with the freeze-dried extract obtained from defatted chia flour. The extract obtained from the coproduct presented ferulic acid (1.91 ± 0.006 mg g^{-1}) and rosmarinic acid (3.79 ± 0.03 mg g^{-1}). The brownies prepared with 2, 4, and 6% extract showed 70%, 89%, and 92% of ferulic acid and 60%, 75%, and 91% rosmarinic acid added to the formulation, respectively. Proximate composition analyses demonstrated that replacing egg with chia seed mucilage decreased fat and protein and increased fiber in vegan brownies. However, the addition of phenolic extract obtained from defatted chia flour increased the fat content of vegan brownies. A residual lipid fraction present in the chia flour was probably extracted during the extraction of phenolic compounds. The technological analyzes (firmness, specific volume, symmetry, and uniformity) showed that the physical characteristics of the brownies were not affected by the different formulations used. However, the color of the products was affected by the addition of the mucilage, which appears grayish, and by the extract containing the phenolic compounds, which presents a yellowish color. The 4% replacement brownie was chosen for sensory analysis with the vegan control brownie. The results of the sensorial analysis indicated an excellent acceptance (76%) of the brownie with 4% of the extract, not differing from the vegan control. In this way, we demonstrated the possibility of producing a sensorially acceptable vegan brownie using a phenolic extract obtained from a coproduct of the food industry.

1. Introduction

Chia seeds (*Salvia hispanica* L.) present a rich nutritional composition of dietary fibers (33.04 g.100 g^{-1}), proteins (20.58 g.100 g^{-1}), and polyunsaturated fatty acids (32.33 g.100 g^{-1}) (Fernández-López et al., 2018), especially those from the omega-3 group (63.79%), presenting a low moisture (5.94 g.100 g^{-1}) (Ciftci et al., 2012). Furthermore, these seeds are sources of bioactive compounds with high antioxidant activity (Killeit, 2019), such as rosmarinic acid, caffeic acid, gallic acid, ferulic acid, and p-coumaric acid (Fernández-López et al., 2018). Chia consumption has been associated with health benefits due to its composition, such as improving the blood lipid profile (Silva et al., 2016) and hypotensive effect (Segura-Campos et al., 2013) and hypoglycemic effects (Ho et al., 2013; Silva et al., 2016). Chia mucilage also has interesting properties for preparing films, dressings, or creams for topical use. Its composition, rich in polyunsaturated fatty acids, presents excellent anti-inflammatory compounds that can help heal wounds (Oliveira Filho et al., 2021).

In addition to the nutritional properties of the seeds, the mucilage extracted from them with water has interesting properties for use in food. It can be used as a food additive to control viscosity, stability, texture, and consistency, mainly due to its good stability to thermal

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treatments up to 244 °C (Falco et al., 2017). These characteristics are important to replace animal-derived ingredients in plant-based foods aimed at a vegetarian and vegan audience that is growing (Lira et al., 2023). Another product derived from chia seeds is defatted flour, an industrial organic coproduct from dry oil extraction. Defatted flour still has antioxidant properties and essential fatty acids with high potential for use in the food industry Guindani et al. (2016). The flour presents lower lipid (7.40 g.100 g^{-1}) and higher protein (27.02 g.100 g^{-1}), dietary fiber (48.02 g.100 g^{-1}), and moisture (6.84 g.100 g^{-1}) contents than the seeds (Fernández-López et al., 2018).

Research employing chia tripled in recent years due to its benefits as a food ingredient, mainly in bakery products (Zettel and Hitzmann, 2018). Therefore, this study aimed to prepare a vegan brownie with phenolic compound extracted from defatted chia flour and chia mucilage as sources of ingredients to prepare a more nutritious product. Defatted chia flour, the coproduct of the manufacture of chia oil, was used to obtain an extract rich in phenolic compounds. This extract was freeze-dried and used to partially replace wheat flour in the brownie recipe in three percentages (2, 4, and 6%). Moreover, the egg, a traditional brownie ingredient, was replaced by chia seed mucilage (w/w). Therefore, this study verified using chia ingredients in a vegan brownie formulation through physical-chemical analyses and sensory acceptance.

2. Material and methods

2.1. Raw material and reagents

Chia seeds and defatted chia flour were donated by Produzza Foods (Foz do Iguaçu, PR, Brazil). The defatted chia flour was obtained from cold-pressing oil extraction and then filtered, according to the supplier. The samples were stored in sealed packages protected from light, heat, and moisture. Analytical acetonitrile (CH_3CN, 99.9%) and absolute ethyl alcohol (C_2H_6O, 99.5%) were used for chromatographic analysis. The analytical standards caffeic acid, chlorogenic acid, rosmarinic acid, p-coumaric acid, ferulic acid, and gallic acid were purchased from Sigma-Aldrich (Cotia, SP, Brazil). The ingredients (50% cocoa chocolate, wheat flour, egg, 100% cocoa powder, salt, sugar, baking powder, butter, soy oil, vanilla essence, and white vinegar) for the development of brownie formulations were purchased in a local supermarket (Limeira, SP, Brazil).

2.2. Mucilage extraction

The mucilage extraction from chia seeds was performed according to a method proposed by Muñoz et al. (2012). Whole chia seeds and distilled water were stirred at a ratio of 1:40 (w/v) at 80 °C for 2 h. The mucilage was separated from the seeds using an 80-mesh sieve and was dried at 50 °C for 12 h. The mucilage extraction yield was calculated according to Equation (1).

$$Mucilage\ yield(\%) = 100 \times \frac{Dried\ mucilage\ (g)}{Whole\ chia\ seeds\ (g)} \tag{1}$$

2.3. Extraction of phenolic compounds from chia coproduct

The phenolic compounds were extracted from defatted chia flour using a hydroethanolic solvent in an ultrasound bath system (Elma, P60H, Stuttgart, Germany). The method consisted of mixing 5 g of defatted chia flour with 50 mL of ethanol: water solution (70:30 v/v), according to conditions previously established by laboratory tests. In sequence, the mixture was placed in a beaker and inside a heated ultrasonic bath filled with water. The extraction was performed at 37 kHz, 100 W, and 50 °C, for 30 min, according to the methodology described by Punzi et al. (2014) with modifications. The extract was concentrated in a rotary evaporator (IKA, RV 10 digital- Staufen, Germany) at 40 °C and 75 rpm for 30 min, then frozen in an ultra-freezer (Thermo Scientific, 88400D – Waltham, EUA) at −80 °C for 24 h and subsequently freeze-dried (Liobras, L101- São Carlos, Brazil) (Ibarz and Barbosa-Cánovas, 2002). The extraction was performed only once, and the extraction yield was determined according to Equation (2).

$$Extraction\ yield(\%) = 100 \times \frac{Freeze - dried\ extract\ \ (g)}{Defatted\ chia\ flour(g)} \tag{2}$$

After the lyophilization process, the dry extract of the defatted chia flour was stored at −20 °C in the freezer until used to prepare the brownie. Extract analyses are described in section 2.6.

2.4. Brownie formulation and preparation

Table 1 presents the five different brownie formulations studied. Control formulation, using a traditional recipe and ingredients (BTC), vegan control formulation (BVC), and vegan formulations with 2, 4, and 6% of freeze-dried phenolic extract replacing wheat flour (BPE-2, BPE-4, BPE-6, respectively).

The preparation of the brownies was based on Milberg, Zicarelli (2014) methodology. The chocolate was melted and added to the soybean oil, while the mucilage, salt, and sugar were beaten in a mixer, and vinegar and vanilla were added. The mucilage was previously hydrated using 3 g of mucilage and 100 g of water, mixed in a culinary mixer, and left to rest for 30 min. Mucilage replaced eggs in the brownie recipe: 30 g of mucilage was employed in the place of each egg, according to Gallodos et al. (2020). The chocolate melted with oil was integrated into the mixture. Then, the white wheat flour, lyophilized extract, cocoa powder, salt, sugar, and baking powder previously sifted were carefully incorporated into the preparation using a rubber spatula from the sides to the center. Finally, the batter was baked at 170 °C for approximately 20 min. The brownies BPE 2, 4, and 6 were formulated by replacing wheat flour with freeze-dried extract rich in phenolic compounds obtained from defatted chia flour in the proportions of 2%, 4%, and 6%, according to section 2.3.

2.5. Brownies technological properties

The firmness characteristics of the brownies were determined in a texture analyzer (Stable Micro Systems, TA.XT Plus - Goldaming, England), according to the AACC (2010) (method number 74-9.01). For that, two 2 cm-thick slices of each sample were compressed at the center of the texturometer platform using a 36 mm diameter cylindrical probe (SMS P/75) at a distance of 2.5 mm, 40% compression, and 5 g trigger force. The speed conditions were 1.0 mm/s for the pretest and test and 10.0 mm/s for the post-test.

Table 1
Mass of ingredients used for the brownie's formulation.

Ingredients	BTC	BVC	BPE-2	BPE-4	BPE-6
White wheat flour (g)	100	100	98	96	94
Lyophilized extract (g)	–	–	2	4	6
Egg (g)	60	–	–	–	–
Mucilage (g)	–	30	30	30	30
Cocoa powder (g)	9	9	9	9	9
Chocolate (g)	35	35	35	35	35
Salt (g)	0.47	0.47	0.47	0.47	0.47
Sugar (g)	140	140	140	140	140
Baking powder (g)	0.95	0.95	0.95	0.95	0.95
Butter (g)	40	–	–	–	–
Soy oil (g)	–	24	24	24	24
Vanilla essence (g)	3	3	3	3	3
Water (g)	45	45	45	45	45
White vinegar (g)	–	2	2	2	2

BTC: brownie traditional control; BVC: brownie vegan control; BPE-2, BPE-4, BPE-6: brownie vegan formulations with 2, 4 and 6% substitution of wheat flour for freeze-dried phenolic extract, respectively.

The specific volume of brownie samples was determined by the relation between the apparent volume-to-weight ratio according to AACC (2010) (method number 10-05.01) after baking.

The symmetry and uniformity indices of the brownie samples were calculated according to the AACC (2010) (method 10–91). This method is based on measuring the height of the brownie at three specific points (Fig. 1). Symmetry was determined according to Equation (3).

$$Symetry = 2 \times C - (B + D) \tag{3}$$

The brownies' uniformity was determined according to Equation (4).

$$Uniformity = B - D \tag{4}$$

The water activity value of the samples was determined in a water activity analyzer (Aqua Lab, Dew Point 4 TE, USA) at room temperature.

2.5.1. Brownies color analysis

The color of the brownies was determined using a colorimeter (Konica Minolta model CR-400 – Chiyoda, Japan) according to the definitions established by the CIE (Commission internationale de l'éclairage) in 1976 (Robertson, 1977). The color parameters were expressed in terms of lightness L (L = 0 for black and L = 100 for white) and chromaticity parameters a* (green [−] to red [+]) and b* (blue [−] to yellow [+]). The value of hue angle or h was calculated using Equation (5).

$$h = tan^{-1}\frac{b*}{a*} \tag{5}$$

2.6. Nutritional properties and energetic value of brownies

The proximate composition of the brownies samples was performed according to the AOAC (2005) through the analysis of moisture (method number 935.29), lipids (Soxhlet method, number 920.85), protein (micro-Kjeldahl method N x 6.25, number 920.87), total dietary fiber (method number 985.29), and ash (method number 923.03). The total carbohydrate content (nitrogen-free extract – NFE) was obtained by difference, according to Equation (6).

$$NFE(\%) = 100 - [(moisture(\%) + ash(\%) + proteins(\%) + fibers(\%)] \tag{6}$$

The energetic value of the brownie samples was determined using the Atwater method based on the caloric coefficients corresponding to protein, carbohydrate, and fat, according to Equation (7) (Watt and Merril, 1963).

$$Energetic\ value\left(\frac{100kCal}{g}\right) = [protein(g) \times 4] + [fat(g) \times 9] + [carbohydrate\ (g) \times 4] \tag{7}$$

2.7. Brownies functional properties

2.7.1. Extraction of phenolic compounds from de brownie batter

The extraction of phenolic compounds from the raw batter and baked brownies was performed for quantification. For this, 2 g of each raw batter or baked brownie was mixed with 50 mL of ethanol: water solution (70:30 v/v), according to previously established conditions. Then, the samples were placed in an ultrasound bath at 37 kHz frequency and 100 W of power for 30 min at 50 °C according to the method developed by Punzi et al. (2014) with modifications. The samples were centrifuged (Centurion Scientific, K241R – West Sussex – United Kingdom) at 27 G for 30 min. Then, the supernatant was filtered using a syringe filter (0.20 μm, nylon, Analítica - São Paulo, Brazil) and stored at −20 °C for further analysis. The ferulic acid and rosmarinic acid recovery were determined based on their respective content in raw batter and baked brownies, according to Equation (8).

$$Phenolic\ recovery(\%) = 100 \times \frac{Final\ phenol\ content}{Inicial\ phenol\ content} \tag{8}$$

Where *final phenol content* is the content of ferulic or rosmarinic acid in the batter and baked brownie and *inicial phenol content* is the content of ferulic or rosmarinic acid added to the raw brownie batter.

2.7.2. Identification and quantification of phenolic compounds

An ultra-performance liquid chromatography method on a 2695D separation module (Waters, Acquity H-Class - Milford, EUA) equipped with a 2998 diode array detector (UPLC-PDA) was developed to identify and quantify the compounds extracted from chia coproduct and their retention in batter and baked brownies. The compounds were separated on a Kinetex C18 column (100 × 4.6 mm id, 2.6 μm, Phenomenex, Torrance, USA) at 47 °C using a flow rate of 1 mL/min. The mobile phase consisted of 0.1% acetic acid (v/v) in water (solvent A) and 0.1% acetic acid (v/v) in acetonitrile (solvent B). The following gradient was used: 0–3 min, 97% A; 5–6 min, 94% A; 6–10 min, 90% A; 10.5–14 min, 87% A; 16–17 min, 85% A; 18 min, 80% A; 19–20 min, 75% A; 21–22 min, 70% A; 23–26 min, and 97% A. Detections were monitored with two individual channels at 270 nm and 350 nm, with the best measurement at 350 nm. Compounds identification was performed by comparing their retention time and UV–vis spectra to standards of caffeic acid, chlorogenic acid, rosmarinic acid, p-coumaric acid, ferulic acid, and gallic acid. The compounds identified in the sample (rosmarinic acid, ferulic acid) were quantified using Empower 2 software (Waters, Milford, USA) using standard curves (0.39–100 ppm).

The confirmation of the identification of the compounds performed by the UPLC-PDA analysis was carried out by mass chromatography (Orbi-trap Thermo Scientific, Q-Exactive-Waltham, USA). For this

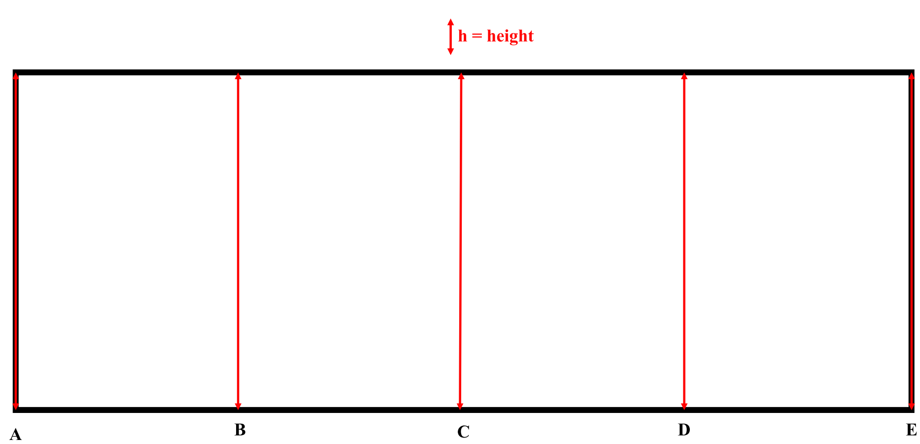

Fig. 1. Side view of a baked brownie batter. The arrows indicate the points where the height is measured for evaluating symmetry and uniformity, and the letters indicate each point.

analysis, a 1.7 μm C18 column, 2.1 × 50 mm (Waters Acquity BEH PN, 186002350 - Milford, USA), at negative polarity, flow rate: 200 μL/min ACN:H_2O with 0.1% NH4OH v/v with the following gradient: 0–5 min, 5% A; 5–10 min, 5% A; 10–11 min. 5% A; 11–13 min, 95% A was employed. Moreover, the run was carried out using a range from 100 to 1000 m/z, an injection volume of 10 μl for 13 min.

2.7.3. Total phenolic content (TPC)

The TPC of defatted chia coproduct extract and baked brownies was determined according to the method described by Singleton et al. (1999). The analysis was performed in a microplate reader (Biotek, EPOCH/2– Winooski, EUA) with software Gen 53.03 at 740 nm, using a standard curve of gallic acid (from 20 to 120 μg/mL) for quantification. The results were expressed in mg of gallic acid equivalent (GAE) per g of freeze-dried extract of defatted chia flour or baked brownie. All analyzes were performed in triplicate.

2.7.4. Antioxidant capacity by in-vitro tests

The antioxidant capacity of defatted chia extracts and baked brownies were analyzed by oxygen radical absorption capacity (ORAC) and ferric ion reducing antioxidant (FRAP) *in-vitro* methods. All analyzes were performed in triplicate.

2.7.4.1. Oxygen radical absorption capacity (ORAC). The ORAC test was carried out according to the methodology of AOAC (2013) (method 2012.23), using the microplate system and a standard curve of Trolox (from 12.5 to 400 μM) for quantification. The reading was performed by exciting the compounds at 493 nm (filter 485/20), and the emission was at 515 nm (filter 528/20). The results were expressed in μmol Trolox-equivalent antioxidant capacity (TEAC) per g of freeze-dried extract or baked brownie.

2.7.4.2. Ferric ion reducing antioxidant (FRAP). The method described by Benzie & Strain (1996) with modifications was used to determine antioxidant activity based on iron reduction. The FRAP reagent was prepared by mixing 50 mL of acetate buffer (300 mM, pH 3.6), 5 mL of TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) solution (10 mM TPTZ in HCl 40 mM), and 5 mL of ferric chloride hexahydrate (20 mM) in aqueous solution. 20 μL of different concentrations of the sample or standard curve of ferrous sulfate (from 100 to 700 μM) were added to 30 μL of distilled water and 200 μL of FRAP reagent. The mixtures were incubated at 37 °C for 8 min. Then, their absorbance values were read at 593 nm in the microplate spectrophotometer with Gen 5 3.03 software. The results were expressed in μmol Trolox-equivalent antioxidant capacity (TEAC) per g of freeze-dried extract or baked brownie.

2.8. Sensory analysis

Sensory analysis was performed through an acceptance test using a nine-point structured hedonic scale. Appearance, aroma, flavor, texture, and overall impression were the attributes evaluated according to NBR 12994 (ABNT, 2016). The scale ranged from 1 (disliked extremely) to 9 (liked extremely). The results were expressed as evaluated attribute's mean and standard deviation. The acceptability index (AI) was calculated according to Equation (9), where the Score is the mean value assigned by the panelists for each sensory attribute and is expressed as the mean (%) of these values.

$$Acceptability\ index\ (\%) = \frac{(Score \times 100)}{9} \tag{9}$$

Purchase intention was assessed using a five-point hedonic scale from 1 (definitely would not buy) to 5 (definitely would buy). The results were expressed as evaluated attribute's mean and standard deviation. The analysis was performed with 100 untrained panelists, aged over 18 years of both sexes, who did not have a food intolerance or allergy to any component of the brownie's formulations. The panelists were recruited through posters posted on the Faculty of Applied Sciences, University of Campinas (FCA-UNICAMP) walls, email lists, and dissemination through social groups of FCA-UNICAMP students. The volunteer panelists signed the informed consent form agreeing to participate in the research before carrying out the analyzes (approved by the UNICAMP Research Ethics Committee - #4.799.737).

Microbiological analysis of the brownies guaranteed the safety of the panelists who participated in the sensory analysis. The samples were tested for *Escherichia coli, presumptive Bacillus cereus* and mold and yeast count to verify the absence of pathogenic and deteriorating microorganisms. The analyzes were carried out according to the methods described by Silva et al. (2017), and the results were interpreted based on Normative Instruction n°. 60 of 2019 of ANVISA, which establishes the lists of microbiological standards for foods ready to be offered to the consumer (Brasil and da S, 2019).

Panelists analyzed the samples in the same session (day and time). The samples selected for the test were the vegan control brownie and the brownie added of phenolic extract that obtained the best performance in the analysis of total phenolics, antioxidant activity, and proximate composition (the brownie prepared with 4% of defatted chia flour extract). The samples were presented in a monadic way, providing mineral water for cleaning the palate. Samples measuring 2 × 2 cm were placed in plastic plates and coded with three random digits to avoid choice influence.

2.9. Statistical analysis

The results were analyzed using the One-Way ANOVA test to compare means and the Tukey post-hoc test to identify differences. The sensory analysis results were analyzed using the paired Student T-test to compare the means of two dependent variables. The statistical tests were performed at 95% confidence (p-value ≤0.05). All experiments were performed at least in triplicate and were analyzed using the statistical software Graph Pad Prism 9.

3. Results and discussion

3.1. Mucilage extraction yield

The mucilage yield was 7.67%, a value consistent with the findings of other studies (Felisberto et al., 2015; Fernandes and Salas-Mellado, 2017). Felisberto et al. (2015) obtained a mucilage yield of 7.86% and studied its use to replace fat in pound cakes. Fernandes and Salas-Mellado (2017) obtained lower mucilage yields of 5.1% for freeze-dried mucilage and 4.69% for dried mucilage. They applied the dried mucilage as an ingredient in bread and chocolate cake formulations.

3.2. Nutritional and technological properties of brownies

The proximate composition and technological characteristics of brownies are presented in Table 2. The brownie prepared with 6% phenolic extract (BPE-6) had significantly (p-value ≤0.05) the lowest moisture content, presenting the driest batter. According to Bennion and Bamford (1997), the interactions between the chia mucilage fibers and the starch in wheat flour can delay the process of starch retrogradation and prevent moisture loss during cooking. In this sense, the higher replacement of flour by extract (6%) may have reduced the amount of starch in the brownie, affecting its water retention and producing a product with less moisture. Accordingly, the control brownies (BTC and BVC) that presented more flour than BPE-2, BPE-4, and BPE-6 also presented higher moisture.

The fat content was increased (p-value ≤0.05) in the samples of vegan brownies according to the replacement of flour with phenolic extract. Probably during the extraction of phenolic compounds, a

Table 2
Nutritional properties (g/100g), energetic value (kcal.g^{-1}) and technological properties of the formulated brownies.

Parameter	BTC	BVC	BPE-2	BPE-4	BPE-6
Moisture (%)	1.93 ± 1.93 ^{a}	13.11 ± 3.02 ^{a,b}	12.76 ± 1.65 ^{a,b,c}	11.97 ± 1.07 ^{a,b,c}	10.40 ± 1.99 ^{b,c}
Fat (%)	14.07 ± 0.26 ^{c,a}	12.57 ± 0.01 ^{d}	12.74 ± 0.06 ^{c,d}	12.95 ± 0.01 ^{c}	13.52 ± 0.01 ^{b}
Proteins (%)	6.69 ± 0.31 ^{a}	4.58 ± 0.12 ^{b,c,d}	4.70 ± 0.23 ^{b,c,d}	4.84 ± 0.17 ^{b}	4.78 ± 0.07 ^{b,c}
Total dietary fiber (%)	2.86 ± 0.01 ^{d}	3.53 ± 0.04 ^{c}	3.41 ± 0.05 ^{c}	3.67 ± 0.02 ^{b}	3.78 ± 0.06 ^{a}
Ash (%)	1.59 ± 0.39 ^{a}	0.78 ± 0.06 ^{a,b,d}	1.22 ± 0.12 ^{a,b,c,d}	1.27 ± 0.08 ^{a,c}	1.28 ± 0.61 ^{a,b}
Other carbohydrates (%)	58.86	65.44	65.16	65.36	66.18
Energetic value (kcal)	388.80	393.17	394.11	397.32	405.53
Firmness (N)	10.06 ± 0.02 ^{a,b,c}	10.03 ± 0.02 ^{a,c,d}	10.03 ± 0.01 ^{a,c,d}	10.08 ± 0.01 ^{a,b}	10.10 ± 0.03 ^{a}
Specific volume (cm^{3}.g^{-1})	2.48 ± 0.01 ^{a}	2.48 ± 0.01 ^{a,b}	2.48 ± 0.01 ^{a,b,c}	2.47 ± 0.01 ^{a,b,c}	2.47 ± 0.01 ^{b,c}
Symmetry (cm)	−0.84 ± 0.04 ^{a,b,c,d}	0 ^{a}	−0.75 ± 0.25 ^{a,b}	−0.75 ± 0.25 ^{a,b,c}	−1.75 ± 0.75 ^{d}
Uniformity (cm)	−0.31 ± 0.31 ^{a}	0 ^{a}	−0.25 ± 0.25 ^{a}	−0.75 ± 0.75 ^{a}	−0.25 ± 0.25 ^{a}
Water activity	0.78 0.03 ^{a}	0.76 ± 0.01 ^{a,b}	0.75 ± 0.02 ^{a,b,c}	0.74 ± 0.02 ^{a,b,c,d}	0.72 ± 0.02 ^{b,c,d}

Proximate composition results expressed on a dry basis. Values expressed as triplicate mean ± standard deviation. The same letter in the line indicates that there is no significant difference between the means by Tukey's test (p-value ≤0.05). BTC: traditional control formulation; BVC: vegan control formulation; BPE-2, BPE-4, and BPE-6: formulations with 2, 4 and 6% replacement of wheat flour by phenolic extract, respectively.

residual lipid fraction present in the defatted chia flour was also extracted. Moreover, BVC presented less than 10% fat content compared to BTC. Fernandes and Salas-Mellado (2017), replacing from 0 to 100% of fat with chia mucilage, observed a reduction in fat content from 9.35 to 2.10 g/100g in their samples. Furthermore, egg replacement promotes a reduction in the fat content (Aranibar et al., 2019; Coorey et al., 2014; Fernandes and Salas-Mellado, 2017), which may be advantageous for specific health situations such as high cholesterol, obesity, and diabetes (Bruins et al., 2019; Noce et al., 2021).

Replacing eggs with chia mucilage decreased the brownies' fat and protein content. On the other hand, this replacement increased the fiber content. Vegetable protein sources, such as chia, are generally low in total fat and saturated fat content and high in fiber and phytochemical compounds, bringing health benefits. In a well-structured food context, the amount of protein should not be a problem, as the vegan individual will be able to obtain the necessary amounts mainly from the combination of cereals and legumes (Slywitch, 2022). Thus, the proposed vegan brownies are an attractive option to compose a balanced diet. The ash content of the brownies was not significantly affected by their formulation. Wheat flour is a source of minerals since in Brazil it is fortified with iron according to RDC No. 150 of April 13, 2017 (Brasil and da S, 2017). Moreover, chia ingredients may also increase the ash content of products. Muffins in which saturated fat was replaced by chia mucilage had a higher ash content (Melo et al., 2022). The lower total carbohydrate content of BTC than BVC, BPE-2, BPE-4, and BPE-6 can be attributed to the replacement of eggs by mucilage, a polysaccharide. The carbohydrates in brownies include starches, sugars, gums, and organic acids, resulting from the different ingredients used. Fernandes and Salas-Mellado (2017) observed that the higher the fat replacement by mucilage in cakes, the higher the carbohydrate contents, also reducing the energetic value of the food. Although an analysis of total carbohydrates in the phenolic extract and chia seed mucilage was not carried out, the carbohydrates determined by difference may refer to different types of saccharides in these ingredients.

The technological characteristics of bakery products, such as firmness, specific volume, symmetry, and uniformity, influence the consumer's sensory acceptance of the product. However, the different formulations tested in this study had no significant (p-value ≤0.05) effect on these technological characteristics of the brownies (Table 2). Despite this, fat content is essential for volume and maintaining bakery products' softness during storage (Felisberto et al., 2015; Fernandes and Salas-Mellado, 2017; Melo et al., 2022). In this sense, it would be interesting to observe the effects of fat content on the technological characteristics of brownies over storage time. However, several authors have shown that adding chia mucilage in formulation decreased the volume of the developed cakes, making them less aerated and denser (Borneo et al., 2010; Felisberto et al., 2015; Melo et al., 2022; Pinto et al., 2023), which is not a technological problem in brownie formulations. Positive values for symmetry index indicate growth in the center of the bakery products, a desired characteristic in cakes (Felisberto et al., 2015), but not in brownies, which usually have a straight surface. Negative values indicate a depression in the center. Thus, results equal to zero are desired for brownies. According to Felisberto et al. (2015), the addition of mucilage can affect the specific volume, symmetry, and uniformity of brownies during baking by changing the viscosity of the batter and making it less airy and denser. However, as the difference in the present study was insignificant, these results corroborate with the results of Felisberto et al. (2015) that found no significant difference in the symmetry and uniformity indices in the formulations that replaced fat with chia mucilage.

The different formulations had no significant (p-value ≤0.05) effect on the water activity of the brownies. The formulations presented water activity values between 0.7 and 0.8. The water activity in food is an intrinsic characteristic. The values vary from 0 to 1 and are linked to the food's physicochemical characteristics. This value associated with moisture information also indicates the amount of free water in the food product that can react and be available for the growth of microorganisms. In this sense, this analysis serves to predict the stability and safety of the food since high water activity can favor the growth of bacteria and filamentous fungi, favoring a rapid deterioration with alteration of the physical-chemical properties (Fontana, 2000). The studies performed by Fernandes and Salas-Mellado (2017), Melo et al. (2022), and Felisberto et al. (2015) demonstrated higher water activities (values above 0.9) in cakes produced with mucilage than with fat. However, this effect was probably not observed because brownies contain more sugar than cakes, presenting lower water activity (Menezes et al., 2009). Furthermore, in this study, the eggs that were replaced by chia mucilage.

3.3. Color parameters of brownies

The color as well as the technological characteristics of brownies influence consumer preference (Fernandes and Salas-Mellado, 2017). Table 3 presents the color parameters determined on brownie surface and crumb for each formulation. The replacement of eggs by mucilage and flour by extract decreased the brownie surface and crumb lightness parameter (L). Aranibar et al. (2019); Fernandes and Salas-Mellado (2017) and Pinto et al. (2023) obtained similar results, they observed a significant decrease in the L parameter of cakes by increasing the level of replacement of fat by chia mucilage. This darkening was attributed (Fernandes and Salas-Mellado, 2017) to the grayish color of the mucilage. The presence of the extract can also influence the product's color compared to the controls due to its yellow color from its main components, ferulic and rosmarinic acids, which are crystalline solids with yellow coloring (Oliveira-Alves et al., 2017; Sik et al., 2019). Thus, the extract addition may have allowed the higher lightness observed in the crumb of BPE-2, BPE4, and BPE-6 samples than in the BVC crumb. Moreover, the highest L value obtained for the surface of the vegan

Table 3
Color parameters (L, a*, b*, and h°) determined on the surface and crumb of the brownies.

	BTC	BVC	BPE-2	BPE-4	BPE-6
Brownie surface color parameters					
L	36.97 ± 0.87 ^{a}	31.91 ± 0.69 ^{b}	31.03 ± 0.91 ^{b,c}	31.22 ± 0.47 ^{b,c}	34.92 ± 0.85 ^{a}
a*	11.27 ± 0.90 ^{a,b}	10.91 ± 0.31 ^{b}	10.67 ± 0.63 ^{b}	9.97 ± 0.70 ^{b}	11.52 ± 0.28^{a}
b*	15.75 ± 1.69 ^{a,b}	13.6 ± 0.73 ^{a,b,c}	12.87 ± 0.96 ^{a,b,c,d}	12.47 ± 1.39 ^{c,d}	15.86 ± 0.37 ^{a}
h°	24.57 ± 0.76 ^{d}	50.48 ± 1.72 ^{b,c}	54.10 ± 0.34 a	51.6 ± 1.17 ^{a,b,c}	52.90 ± 0.97 ^{a,b}
brownie crumb color parameters					
L	35.88 ± 1.18 $^{c\ a}$	27.67 ± 1.59 ^{c,d}	31.32 ± 0.70 ^{b}	28.93 ± 1.29 ^{a,b,c}	28.85 ± 1.36 ^{b,c,d}
a*	13.89 ± 0.39 ^{a,d}	10.71 ± 0.11 ^{c}	12.11 ± 0.19 b	11.44 ± 0.79 ^{a,b,c}	11.09 ± 0.41 ^{b,c,d}
b*	20.27 ± 0.70 ^{a}	13.00 ± 0.91 ^{c,d}	16.74 ± 0.46 ^{b,c,d}	14.47 ± 1.58 ^{a,b,c,d}	14.69 ± 1.03 ^{c}
h°	24.57 ± 0.76 ^{d}	50.48 ± 1.72 ^{b,c}	54.10 ± 0.34 ^{a}	51.60 ± 1.17 ^{a,b,c}	52.90 ± 0.97 ^{a,b}

Values expressed as triplicate mean ± standard deviation. The same letter in the line indicates that there is no significant difference between the means by Tukey's test (p-value ≤0.05). BTC: traditional control formulation; BVC: vegan control formulation; BPE-2, BPE-4, and BPE-6: formulations with 2, 4 and 6% replacement of wheat flour by phenolic extract, respectively.

brownies was observed in the BPE-6 sample. The formulation did not significantly affect the a* and b* color parameters on the surface of the brownies. However, the parameters a* and b* of the brownie crumb were impacted by their different composition of ingredients. BPE-2, BPE4, and BPE-6 samples showed a more reddish (higher a* value) and yellowish (higher b* value) crumb than BVC. This difference is probably associated with the phenolic extract added to these formulations. However, the color difference is slight. In some cases, there was no significant difference, probably due to dark chocolate and cocoa, which can mask the more intense color changes caused by the extract in the brownies. Furthermore, a significant difference was observed in the angle on the chromaticity axes (h°) between the BTC sample and the BVC and BPEs samples, probably due to the different colors of the ingredients in these formulations.

3.4. Identification and quantification of phenolic compounds extracted from defatted chia flour

The compounds extracted from defatted chia flour were identified by comparing their spectra and retention times (rt) with those of caffeic acid, chlorogenic acid, rosmarinic acid, p-coumaric acid, ferulic acid, and gallic acid standards. In this sense, chromatogram peak 2 (rt: 15.08 min) and peak 3 (tr: 18.15 min) were identified as ferulic acid and rosmarinic acid, respectively. Fig. 2 presents the chromatograms and spectra of the compounds extracted from defatted chia flour and the standards of ferulic acid and rosmarinic acid.

The compounds observed in the sample did not show the same retention time as the standards, probably because the hydroalcoholic solution containing only the pure standards has a different complexity from the studied extract. However, we confirmed the profiles of the compounds through the mass spectrum. In addition to ferulic and rosmarinic acids, other peaks were observed in the chromatograms, corresponding to other compounds soluble in 70% ethanol extracted from chia flour. However, we identified just peaks 2 and 3. Ferulic ($C_{10}H_{10}O_4$) and rosmarinic ($C_{18}H_{16}O_8$) acids present a molecular mass of 194 g mol^{-1} and 360 g mol^{-1}, respectively. Thus, the mass chromatography confirmed their presence in the defatted chia flour extract since it identified a molecular masses of [M-H]- 193 m/z and [M-H]- 359m/z, corresponding to the ionized ferulic acid and rosmarinic acid molecular mass, respectively. Oliveira-Alves, et al. (2017); Rahman et al. (2017) also identified ionized ferulic and rosmarinic acids with this same molecular mass.

Ferulic acid is a compound present in chia and other *Salvia* species (Martínez-Cruz and Paredes-López, 2014; Oliveira-Alves et al., 2017). This compound has different biological activities, especially in combating oxidative stress (Choi et al., 2011), inflammation (Liu et al., 2017), vascular endothelial injury (Suzuki et al., 2002), fibrosis (Ali et al., 2021), apoptosis (Chen and Ma, 2019), and platelet aggregation (Hong et al., 2016). On the other hand, rosmarinic acid is one of the main phenolic compounds in chia (Oliveira-Alves et al., 2017), presenting functionalities for health and industry, as a food additive, due to its high antioxidant potential. (Marchev et al., 2021). In this sense, the defatted chia flour extract is an attractive source of valuable bioactive compounds for food applications.

The total yield of the extract was 16%, determined according to the Equation (2). For every 100 g of defatted chia flour used for extraction, 16 g of freeze-dried ethanol extract was obtained. Of these 16 g of extract, 0.031 g was ferulic acid and 0.061 g was rosmarinic acid. Additional compounds, potentially other phenolic substances, were extracted from defatted chia flour with 70% ethanol. However, we have not identified this composition. Moreover, the freeze-drying of the extract was performed to extend its shelf life and facilitate its use as a flour substitute in brownies. However, this treatment presents a cost that can be eliminated by employing the liquid extract obtained after the rotary evaporation step to eliminate ethanol. Thus, the freeze-dried extract presented 1.91 ± 0.01 and 3.79 ± 0.035 mg g^{-1} of ferulic and rosmarinic acids, respectively. Fernández-López et al. (2018) extracted 0.037 ± 0.0004 and 0.67 ± 0.02 mg g^{-1} of ferulic and rosmarinic acids, determined from a phenolic dry extract and rehydrated with methanol from defatted chia flour. The extraction process, such as the technology (ultrasound-assisted extraction, microwave-assisted extraction, accelerated liquid extraction, supercritical fluid extraction, solid-phase extraction, and others), energy, temperature, solvent, and extraction time can affect the extraction yield. Their process consisted of successive extractions with methanol and water and then with acetone and water using a sonicator and drying of the combined extracts. In this sense, since they employed an exhaustive extraction procedure, the yield difference may be associated with the raw material (defatted chia flour).

3.5. Identification and quantification of phenolic compounds added to brownies

The freeze-dried extract from defatted chia flour was added to the vegan brownie's formulation in different proportions (2, 4, and 6%), replacing part of the wheat flour. In this sense, aiming to verify the permanence of ferulic and rosmarinic acid in the batter and the baked brownie, we identified and quantified the compounds extracted from these products. Thus, Table 4 presents ferulic and rosmarinic acids recovery from batter and baked brownies.

Ferulic and rosmarinic acids remained in the brownies after the thermal baking process but in a lower content than in the batter. Although ferulic acid was found in lower content, it remained in higher concentrations than rosmarinic acid in brownie samples. This difference is related to the stability of each compound to heat and other reactions and interactions that can occur during the brownie's preparations. Other studies evaluating the use of chia ingredients have also observed the resistance of some of its phenolic compounds to baking processes. Mas et al. (2020) evaluated the replacement of 10% wheat flour with defatted chia flour in sweet cookies. However, only eleven of the twenty-five phenolic compounds in the flour were identified after processing the biscuit. Among the eleven compounds, six were hydroxycinnamic acids, an isomer of rosmarinic acid. According to the authors, the increase or decrease in the detection of phenolic compounds can be caused by chemical and physical changes due to the production process, for example, bound polyphenols may be released from the plant cell wall

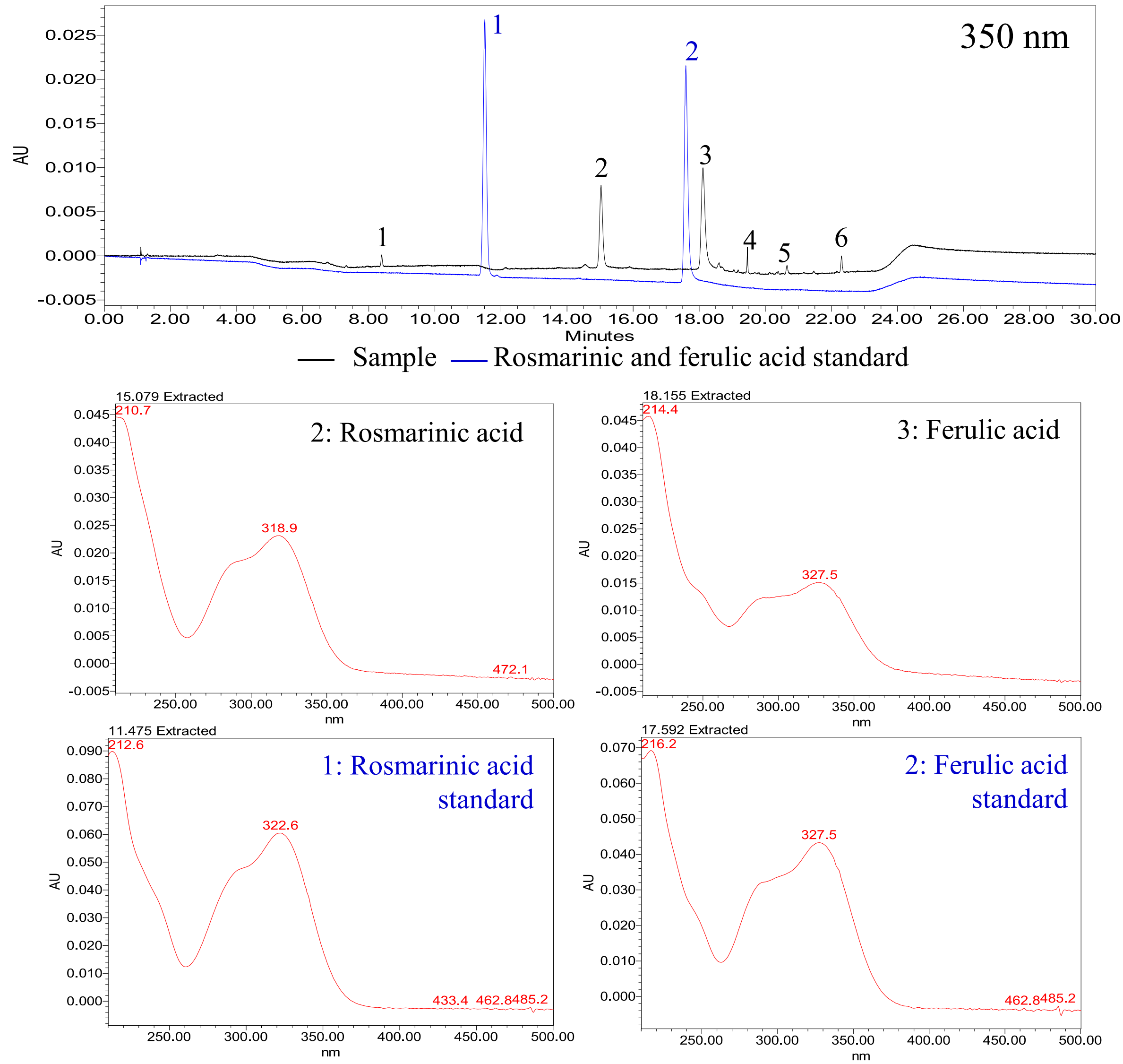

Fig. 2. Chromatograms of the defeated chia flour extract and ferulic and rosmarinic acid standards and their respective spectra.

Table 4
Ferulic and rosmarinic acid content (mg.g^{-1}), recovery (%), and amount per 40 g portion of brownies (mg.40 g^{-1})[a] in baked brownies prepared with freeze-dried phenolic extract of defatted chia flour in different concentrations.

Sample	Content (mg.g^{-1})		Recovery (%)		Amount per serving (mg.40g^{-1})[a]	
	Ferulic acid	Rosmarinic acid	Ferulic acid	Rosmarinic acid	Ferulic acid	Rosmarinic acid
BPE-2	0.019 ± 0.001^{c}	0.015 ± 0.002^{c}	70% ^{c}	60% ^{c}	0.76	0.60
BPE-4	0.026 ± 0.001^{b}	0.030 ± 0.001^{b}	89% ^{b}	75% ^{b}	1.04	1.60
BPE-6	0.036 ± 0.002^{a}	0.061 ± 0.001^{a}	92% ^{a}	91% ^{a}	1.44	2.68

Values expressed as the mean of triplicate (± standard deviation). The same letter in the column indicates that there is no significant difference between the means by Tukey's test (p-value ≤0.05). BPE-2, BPE-4, BPE-6: formulations with 2, 4, and 6% substitution of wheat flour for phenolic extract, respectively.
[a] Serving size defined following IN n° 75 (BRASIL, 2020) for nutritional labeling purposes.

by kneading or heating, or they may interact with components of the new matrix, such as lipids and carbohydrates, reducing their availability and making extraction more difficult. However, they concluded that there was no evidence of a relationship between heat treatment and changes in the phenolic profile of cookies, requiring a more careful investigation.

Brigante et al. (2020) replaced 10% or 20% of wheat flour with chia seeds in cookies, observing that among the 16 compounds identified in the seeds, only five were found in cookies with 20% replacement and three in cookies with 10% replacement. They also identified a rosmarinic acid isomer in the cookie with a 20% substitution. According to the authors, physicochemical changes resulting from processing, such as interconversions between isomers, probably due to heating, can cause a reduction in the initial concentration of phenolic compounds. On the other hand, the water loss during baking increases the concentration of other compounds. Moreover, protein denaturation can release bound phenolic substances, making them accessible in the final sample and enabling their identification (Atanacković et al., 2012). However, analyzes of the control batter and baked brownie demonstrated that the other ingredients are not sources of ferulic and rosmarinic acids. The chromatograms of control samples did not present peaks related to these compounds (the chromatogram of the control brownie was presented as Supplementary Material).

The phenolic compounds present in the servings of brownies can benefit the health of their consumers as part of a healthy diet. Data on the action of ferulic acid in models that replicate human cardiovascular diseases suggested possible benefits to the health of the heart and circulatory system from doses ranging from 9.5 mg/kg to 2 g/kg in preclinical studies and from 240 to 640 μmol min/L and 1000 mg in clinical studies (Anson et al., 2011; Ardiansyah et al., 2008; Brglez Mojzer et al., 2016; Neto-Neves et al., 2021). Other studies also have confirmed rosmarinic efficacy and health benefits, reporting positive pharmacological effects in patients mainly associated with reduced oxidative stress in doses ranging from 3 g to 6 g/day (Fazli et al., 2012; Javid et al., 2018) and improvement of the lipid profile, with doses of 700 mg to 3 g/day (Asadi et al., 2018; Javid et al., 2018).

3.6. TPC and antioxidant capacity of freeze-dried phenolic extract and baked brownies

The results of total phenolic content (TPC) and antioxidant capacity by FRAP and ORAC of the defatted chia flour extract and the extracts obtained from the baked brownies are presented in Table 5. As expected, the extract presented the higher TPC and antioxidant activities than the brownies. Moreover, these results were higher than those presented in other studies. Oliveira-Alves et al. (2017a,b) quantified total phenolic content in seeds, defatted flour, and chia oil, observing the respective values of 1.16 ± 0.01 mg GAE/g, 1.11 ± 0.02 mg GAE/g, and 0.02 ± 0.01 mg GAE/g. Fernández-López et al. (2018) verified values of 4.1 ± 0.3 mg GAE/g and 5.0 ± 0.3 mg GAE/g for defatted chia flour and chia seeds TPC. Extracts obtained from defatted flour have shown antioxidant capacity by FRAP from 69 ± 3 mg GAE/g to 81 ± 3 mg GAE/g and by ORAC of 121 ± 7 mg GAE/g (Oliveira-Alves et al., 2017; Fernández-López et al., 2018). These result differences can be associated with extractive conditions, considering the solvent type, extraction procedure (ultrasound-assisted extraction, microwave-assisted extraction, accelerated liquid extraction, supercritical fluid extraction, solid-phase extraction, and others), time, and temperature. Furthermore, the characteristics of the sample itself, such as the place of cultivation, can influence the concentration of total phenolics and their antioxidant activity (Oliveira-Alves et al., 2017).

Furthermore, the brownies produced with higher amounts of phenolic extract (BPE-4 and BPE-6) presented significantly ($p \leq 0.005$) higher TPC and antioxidant activity measured by FRAP values. The TPC results were consistent with those observed by the FRAP method since the phenolic compounds present antioxidant activity. However, these results are inconsistent with the ferulic and rosmarinic acid content added to the brownies (Table 4). We expected higher TPC and antioxidant activity for the BPE-6 sample than for the BPE-4 since a higher content of the phenolic extract was used, replacing white flour in this formulation (Tables 1 and 4). On the other hand, the antioxidant capacity of control brownies (BTC and BVC) by ORAC was statistically the same as those prepared with 2 and 4% defatted chia flour extract (BPE-2 and BPE-4). In this sense, this method was the least consistent with what was expected. However, other ingredients added to brownies may contribute to TPC and antioxidant capacity results.

BVC, BPE-2, 4, and 6 present a similar composition of ingredients, differing only by the addition of different percentages of the phenolic extract (Table 1). On the other hand, the traditional control brownie (BTC) had eggs and oil; these ingredients may also have phenolic and bioactive compounds. Cocoa has polyphenols such as anthocyanins, flavanones, flavones, and phenolic acids, including traits of ferulic acid with antioxidant properties, presenting beneficial effects in cases of dyspepsia, nervous system diseases, and circulation problems (Cinar et al., 2021; Gil et al., 2021; Martin and Ramos, 2021). In addition to cocoa, the mucilage obtained from chia can also contain phenolic and antioxidant compounds. Furthermore, products from the Maillard reaction, formed during the baking process, may also act as antioxidants, according to Mesías et al. (2016).

Table 5
TPC and antioxidant activity of the phenolic extract and baked brownies.

Sample	TPC (mg GAE/g^{-1}) [a]	FRAP (μmol TEAC. g^{-1}) [a]	ORAC (μmol TEAC/ g^{-1}) [a]
Extract	83.00 ± 12.67	213.79 ± 28.00	139.96 ± 56.22
BTC	43.15 ± 2.04 [c]	5.22 ± 3.06 [c]	81.15 ± 13.11 [b,c]
BVC	46.77 ± 2.62 [b]	11.32 ± 2.33 [b]	69.88 ± 9.96 [c,d]
BPE-2	46.95 ± 1.52 [b]	11.32 ± 2.17 [b]	78.02 ± 13.71 [b,c,d]
BPE-4	52.77 ± 2.03 [a]	29.22 ± 3.82 [a]	84.10 ± 10.10 [b]
BPE-6	55.02 ± 2.56 [a]	30.87 ± 2.21 [a]	103.80 ± 13.32 [a]

[a] Values expressed as triplicate mean ± standard deviation. The same letter in the column indicates that there is no significant difference between the means by Tukey's test (p-value ≤0.05). BTC: traditional control formulation; BVC: vegan control formulation; BPE-2, BPE-4, and BPE-6: formulations with 2, 4 and 6% replacement of wheat flour by phenolic extract, respectively.

3.7. Sensory acceptance of BVC and BPE-4 brownies

The brownie containing 4% of defatted chia flour extract (BPE-4) was chosen for the sensory test considering that BPE-4 and BPE-6 showed little differences in the total phenolics and FRAP results. Furthermore, although the formulations did not present a significant difference in technological characteristics, BPE-4 presented a higher moisture content, which is important for the sensory test because brownies must have a moister center and lower fat contents than BPE-6, which is interesting because it reduces the calorie content. Moreover, BVC was used as a control sample for the test, targeting the participation of vegan consumers as panelists. BVC and BPE-4 samples presented safe conditions for consumption according to the results of *E. Coli*, *Bacillus cereus,* molds and yeasts analysis (Brasil and da S, 2019; Silva et al., 2017).

The panelists were constituted of 97% (n = 100) that liked brownies, and most said they consumed cakes at least once a month. Moreover, 75% declared themselves omnivorous, 16% were lacto-ovo vegetarians, 6% were strict vegetarians or vegans, and 1% did not answer this question. Table 6 describes the acceptance results obtained by the sensory tests for BVC and BPE-4.

The BVC and BPE-4 brownies received statistically (p-value ≤0.05)

Table 6
Mean values of the analyzed sensory attributes of the BVC and BPE-4.

	Sample	
Parameters	BVC	BPE-4
Appearance	8.02 ± 0.94 [a]	8.02 ± 0.90 [a]
Aroma	7.87 ± 1.07 [a]	7.98 ± 1.22 [a]
Flavor	7.74 ± 1.18 [a]	7.56 ± 1.36 [a]
Texture	7.70 ± 1.29 [a]	7.81 ± 1.26 [a]
Overall impression	7.80 ± 1.01 [a]	7.74 ± 1.19 [a]
Acceptability index (%)	76.19	76.22
Buying intention	2.01 ± 0.96 [a]	2.05 ± 1.02 [a]
Definitely would buy (%)	33.00	34.00
Probably would buy (%)	43.00	38.00
May or may not buy (%)	16.00	21.00
Probably would not buy (%)	6.00	3.00
Definitely would not buy (%)	2.00	4.00

BTC: traditional control formulation; BVC: vegan control formulation; BPE-2, BPE-4, and BPE-6: formulations with 2, 4 and 6% replacement of wheat flour by phenolic extract, respectively. Values expressed as mean ± standard deviation. The same letter in the line indicates that there is no significant difference between the means by Tukey's test (p-value ≤0.05).

the same scores for the sensory parameters (appearance, aroma, flavor, texture, and overall impression). The acceptability index was 76%, meaning the participants liked the two brownies (Roberto Spehar and de Barros Santos, 2002). Most panelists declared that they probably would buy both formulations. However, in percentage terms, a greater purchase intention was observed for the vegan control sample. The panelists noted a darker color, milder aroma, bitter and astringent residual taste, and greater intensity of the chocolate flavor in the BPE-4 sample. According to the literature, sensations of adstringency arising from the interaction of polyphenols and salivary proteins may lead to the perception of a residual taste in the mouth by panelists (Jakobek, 2015). Panelists also commented that the BVC was the formulation most similar to a traditional cake, as it was softer and had a less crispy crust. On the other hand, BPE-4 had a more brownie-like texture: a drier, crispier crust, and a moist interior. The two products were found to be heavy and low aeration, probably related to the replacement of egg by mucilage (Borneo et al., 2010), which is an advantage as it gives a similar texture to a traditional brownie.

4. Conclusion

This study demonstrated the technological and sensory aspects of using a phenolic extract of defatted chia flour in a bakery product. We present the possibility of reusing a solid industry residue to obtain an extract with ferulic acid (1.91 ± 0.0065 mg g^{-1}) and rosmarinic acid (3.79 ± 0.035 mg g^{-1}). The brownies developed by replacing 2, 4, and 6% of the wheat flour with freeze-dried extract retained 70%, 89%, and 92% of the added ferulic acid and 60%, 75%, and 91% of the added rosmarinic acid contents, respectively. Proximate composition analyses demonstrated that replacing egg with chia seed mucilage decreased fat and protein and increased fiber in vegan brownies. However, adding phenolic extract, obtained from defatted chia flour, increased the fat content of vegan brownies. Probably during the extraction of phenolic compounds, a residual lipid fraction present in the chia flour was also extracted. Moreover, the technological properties (firmness, specific volume, symmetry, and uniformity) showed that the physical characteristics of the brownies were not affected by the different formulations. The 4% replacement brownie presented good acceptability (76%) through sensory analysis. Thus, future studies could verify the bioavailability and bioaccessibility of the ferulic acid and rosmarinic acid contained in the brownies through simulated digestion analysis combined with pre- and post-process quantification to study the effects on the body. In summary, the developed brownies presented phenolic and antioxidant compounds and good nutritional profile, lower lipids, and higher fiber content than the brownie produced with eggs, suggesting that this plant-based ingredient could replace animal ingredients in food formulations.

Ethical statements

Ethical approval for the involvement of human subjects in this study was granted approved by the UNICAMP Research Ethics Committee - #4.799.737, 06/23/2021.

The participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

This research aimed to develop a phenolic extract from defatted chia flour (Salvia hispanica L.) to partially replace wheat flour and the egg with chia seed mucilage in a vegan brownie. The extract was analyzed for total phenolic concentration and profile, and antioxidant activity. The brownies were analyzed for concentration and phenolic profile, antioxidant activity, and physicochemical, technological, and sensory properties. The study provided interesting results in the development of a freeze-dried extract and its application in a bakery product, which achieved good acceptance in the sensory test and retained the compounds contained in the extract (ferulic acid and rosmarinic acid) even after the thermal process, showing advantages for the application and development of a product with bioactive compounds and potential functional properties in food. We believe that the results of the present study can contribute to the development of clean-label foods and the replacement of ingredients of animal origin with others of plant origin.

CRediT authorship contribution statement

Mariele Rodrigues Moreira: Formal analysis, Investigation, Visualization, Writing – original draft. **Vitor Lacerda Sanches:** Methodology, Supervision. **Monique Martins Strieder:** Formal analysis, Writing – review & editing. **Maurício Ariel Rostagno:** Methodology, Resources, Funding acquisition, Supervision. **Caroline Dário Capitani:** Conceptualization, Methodology, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijgfs.2023.100835.

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