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Chemical Characterization of CHIA (*Salvia hispanica L.)* for Use in Food Products

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Abstract Chia is a seed native to the region that extends from the North of Mexico to Guatemala, and it has been target of study for food enrichment. Many of its newly developed functional foods contain bioactive compounds including dietary fiber, antioxidants and other substances. The objective of this study was to evaluate chia seed (*Salvia hispanica L*) from her chemical components and prove their claim for functional properties. Chia seeds contain high levels of lipids (34.4%) and are rich in Omega-3, Omega-6 and Omega-9, which constituted 62, 17.4 and 10.5% of the total lipids, respectively. Chia seed also contain fibers (23.7%) and proteins (19.6%). Their extracted phenolic compounds (32.35 $\mu g_{GAE}.mL_{extract}^{-1}$) showed antioxidant activity. From the results obtained in the analysis, one should explore the use of this seed in food products, aiming at adding nutritional value and producing foods which contribute to the well-being and health of humans.

Keywords: *antioxidants, functional food, Omega-3, Salvia hispanica L.*

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1. Introduction

The search for novel foods is a relevant practice worldwide. *Salvia hispanica L*., also known as chia, is an herbaceous plant cultivated semi-annually, and it belongs to the family *Labiatae*, division *Spermatophyta* and kingdom *Plantae* [1]. Chia is native to the region that stretches from North Mexico to Guatemala. Its seeds were widely used by Aztec tribes for food, medicine and paints [2]. Chia seed contains a significant amount of lipids (approximately 40% of the total weight), with almost 60% of the lipids comprising Omega-3 fatty acids. Dietary fiber constitutes more than 30% of the total weight of the seed, and approximately 19% of the seed contains proteins of high biological value [3]. Unsaturated Omega-3 fatty acids are nutritionally important for good health and are beneficial for individuals suffering from heart disease, diabetes and immune response disorders [4].

Functional foods have received heavy attention in recent years as components of healthy lifestyle changes. The term "functional" is used to refer to a food that is regularly consumed to provide physiological benefits or to reduce the risk of chronic disease in addition to its basic nutritional functions. Many new foods contain bioactive functional compounds including fiber, prebiotics, probiotics, oligosaccharides, phytochemicals, antioxidants, and other substances that confer functional properties or beneficial effects on human health [5]. Functional foods offer health benefits in addition to the nutritional value inherent in their chemical compositions, and they may have potential roles in reducing the risk of chronic degenerative diseases [6]. Important functional foods that need to be consumed on a daily basis are fruits, vegetables, legumes and grains; these materials generally provide the body a high intake of vitamins, minerals, phytochemicals (antioxidants and anticarcinogenic molecules) and fibers, which are essential for the proper functioning of the organism and for the maintenance of health [5].

Natural antioxidants protect the human body against free radicals, inhibit many chronic diseases, and prevent lipid oxidation in food. Phenolic compounds are important components of many edible plants, including soybean, canola, flaxseed and olive, which are used as food or food ingredient sources [7]. Synthetic antioxidants are widely used for its performance; however, they present different toxicological problems [8]. The legislation on food safety has gradually become more rigorous, requiring the use of toxicity tests for synthetic antioxidants. Additionally, consumers tend to use natural products as antioxidants, as these appear safe and do not require pre-testing [9]. Chia seeds are a promising source of antioxidants due to the presence of polyphenols, chlorogenic and caffeic acids, myricetin, quercetin and kaempferol [3,10], which protect consumers against adverse conditions such as cardiovascular diseases and certain cancers [11,12]. Thus, the objective of this work was to evaluate chia seed (*Salvia hispanica L.*) for its chemical components and to extract its phenolic compounds for the evaluation of their antioxidant activity.

2. Materials and Methods

The seeds of chia (*Salvia hispanica L.)*, were provided by Chá e Cia – Medicinal Herbs for Tea, located in Jacareí, São Paulo. Twigs were removed from seeds with tweezers. Chia seeds were ground with a double knife crusher (Arno, model PL pic-liq), sieved through mesh with a granulometry of 16 mesh, packed in plastic bugs and stored at 4°C until the performance of the tests.

Proximal composition, caloric value and water holding capacity (WHC). The moisture content (method n° 935.29), ashes (method n° 923.03), lipids (method nº 920.85), proteins (micro-Kjeldahl method, n° 920.87) and dietary fiber (total digestion of the material in the 1.25% w/v H_2SO_4 for 30 min, followed by 1.25% w/v NaOH for 30 min) of chia seeds were determined according to the Association of Official Analytical Chemists [13] criteria. The carbohydrate content was obtained by difference between 100 and the sum of ashes, lipids, proteins and dietary fiber.

The caloric value of the samples was calculated from the Atwater coefficients [14], taking the caloric coefficients corresponding to proteins, carbohydrates and lipids, as shown in Equation (1).

$$\begin{aligned}&\text{Caloric value } \left(\text{kcal.100 g}^{-1}\right)\\&=\left(\text{g of protein}*4\right)+\left(\text{g of lipids}*9\right)\\&+\left(\text{g of carbohydrates}*4\right)\end{aligned} \quad (1)$$

The water holding capacity (WHC) was determined as described in Regenstein et al [15].

Fatty acids profile. Oil was extracted from chia seed by acid hydrolysis [16]. The transformation into methyl esters and fatty acid composition were determined according to the AOAC [16] criteria using a gas chromatograph (Thermo, model Focus GC, detector FID).

The chromatographic conditions used were as follows: initial column temperature, 100ºC for 4 min, final column temperature, 240ºC with speed of 3ºC.min^{-1}, injector temperature, 225ºC, and detector temperature, 285ºC. The drag gas used was helium, and the SP2560 capillary column was 100 m x 0.25 mm.

Extraction and quantification of phenolic compounds. The extraction and quantification of phenolic compounds was performed as described by Badiale-Furlong et al. [17]. A total of 5 g of the sample was incubated with 40 mL of methanol under horizontal agitation (5 x *g*) for 2h hat 25ºC, allowed to rest for 15 min, and shaken again (1h) after adding 10 mL of methanol. The extract was filtered and washed three times with hexane. The extract was clarified with 0.1M barium hydroxide and 5% zinc sulfate for 20 min, filtered and carried to a final volume of 50 mL with methanol.

Quantification of phenolic compounds was carried out spectrophotometrically using Folin-Ciocalteu reagent. Aliquots of 0.5 mL of the phenolic extracts were added to test tubes along with 0.5 mL of distilled water and 4.5 mL of alkaline solution (4% Na_2CO_3, 2% $CuSO_4$ and 4% potassium sodium tartrate in a 100:1:1 ratio). The tubes were incubated for 15 min in a 40 °C water bath. Afterwards, 0.5 mL of the Folin-Ciocalteu assay reagent (diluted 1:2 in distilled water) was added, and the tubes were incubated at room temperature for 10 minutes. The absorbance at 750 nm was measured in a spectrophotometer (IONLAB, model IL-592). For quantification, a calibration curve was prepared using gallic acid (GAE) in concentrations of 0 to 20 µg.mL^{-1}.

Phenolic compounds profile. Chromatographic patterns for the determination of phenolic compounds (cinnamic acid, chlorogenic acid, caffeic acid and quercetin) were obtained from Sigma Chemicals Co. (St. Louis, MO) with 99% purity. We used a Milli-Q system (Millipore, Bedford, MA, USA) with a 0.22 µm pore filter to purify water for the mobile phase.

For the separation, identification and chromatographic quantification of phenolic compounds, chia extract was used to be analyzed in a liquid chromatograph (HPLC) via a binary pump and UV-VIS detector with a 50-µl loop injector and a column C18, 250 x 4.6 mm 100 Å, 5 µm. For the patterns of chlorogenic, caffeic and cinnamic acids, an ultra efficiency liquid chromatograph (UPLC) set-up was composed of a binary bomb detector, PDA, gun with a 3-µL loop and C18 column – 2, 1x50 mm, 1.7 µm (Acquiy UPLC BEH – Waters).

The mobile phase elution was performed with a gradient of acetic acid (0.5%, v/v) and water, acetic acid and butanol (350:1:10 v/v/v), an aqueous solution of 0.1% phosphoric acid and 0.1% methanol and methanol, which varied as described in Table 1 and Table 2, with 0.45 and 1.0 mL min^{-1} flow, resulting in a total time of 10 and 50 min race. The injected volume was 3 and 5 µL.

Table 1. Elution gradient program of solvents for the separation of phenolic compounds (cinnamic, caffeic and chlorogenic acids) in chia seeds

Time Interval (min)	Acetic acid (%)	Water/Acetic acid/Butanol (350:1:10) (%)
0.0 – 0.4	10	90
2.4 – 3.4	5	95
3.5 – 4.5	1	99
4.8 – 4.9	5	95
8.0 – 10.0	10	90

Table 2. Elution gradient program of solvents for the separation of phenolic compounds (quercetin) in chia seeds

Time Interval (min)	Phosphoric acid/Methanol (%)	Methanol (%)
0.0	78	22
33.0	0	100
40.0 – 50.0	78	22

To identify the compounds in the mixture (cinnamic, caffeic and chlorogenic acids), the retention times and peak spectra of the samples were compared with the patterns. Each pattern was analyzed individually to determine its retention times and prescriptive UV curves, the patterns were later combined for quantification.

Analytical curves and the linear UV-visible detector responses for the phenolic compounds were evaluated against an analytic curve constructed by injecting five standard solutions in concentrations of 0.8-20 mg.Kg-1 chlorogenic acid, cinnamic acid, caffeic acid and quercetin. The limits of detection for each compound were 1.5-3.0 mg.kg^{-1} for chlorogenic acid and caffeic acid, 5.0-15.0 mg.kg^{-1} for cinnamic acid and 0.04-0.1 mg.kg^{-1} for quercetin. The content of phenolic glycoside dust was obtained as the difference because hydrolysis is required to quantify and verify the bioavailability of these compounds [18].

2.1. Evaluation of Antioxidant Activity of Phenolic Compounds

Free radical sequestration capacity of the 2.2-diphenyl-1-picrylhydrazyl (DPPH). The consumption of free radicals from DPPH by the phenolic extracts and control reaction was monitored as described by Herrero et al. [19] with modifications to determine the decrease in absorbance units (AU) in the solutions containing phenolic extracts. The measurements were performed on a spectrophotometer (IONLAB, model IL-592) at a wavelength of 515 nm, using a gallic acid equivalent control. A total of 1 mL of the phenol extract was added to tubes containing 3.0 mL of a methanol solution of DPPH ($5.2x10^{-5}$ mol.L^{-1}). For blank reactions, 1 mL of methanol replaced the phenolic extracts. Reaction tubes were incubated at room temperature in the dark, and the color change from yellow to violet was measured after 0, 30, 60, 90, 120, 150, 180 and 210 min. The DPPH solution was prepared daily, stored in amber bottles covered with aluminum foil, and kept in the dark at 4°C until its use.

The ability to sequester the free radical was expressed as a percent inhibition of oxidation of the radical and calculated according to Equation (2).

$$\%\ Inhibition = \frac{AU_{\text{blank}} - AU_{sample}}{AU_{\text{blank}}} x100\% \qquad (2)$$

Where AU_{blank} correspond to the absorbance units of blank and AU_{sample} correspond to the sample absorbance units.

Inhibition of enzymatically catalyzed oxidation. Inhibition of enzymatically catalyzed oxidation was performed according to Oliveira et al. [20]. Peroxidase was extracted from potato (*Solanum tuberosum*). The enzymatic extract was obtained from 20 g of homogenized potato pulp that was stirred in blender for 3 min with 100 mL of phosphate buffer pH 6.5 (20 mM). The homogenate was centrifuged at 3220 x *g* at 4°C for 10 min and filtered. The crude extract (supernatant) was maintained at approximately 4ºC.

Enzymatic browning reactions using the phenolic compounds of chia and the control (GAE) were performed at 30 °C at pH 6.5 using 1% guaiacol as the substrate in the presence of 0.08% H_2O_2. The phenolic extracts/control (1 mL) were added as reaction inhibitors, and blank phenolic extract volume was replaced by distilled water. To these solutions, the remaining reaction components were added, including 1.5 mL of phosphate buffer pH 6.5, 1 mL of distilled water, 1 mL of hydrogen peroxide 0.08%, 0.5 mL of 1% guaiacol and 1 mL of peroxidase enzymatic extract. The tubes were shaken, and the absorbance was measured at 470 nm in an IONLAB model IL-592 spectrophotometer after 10, 15, 20, 30 and 40 min. The antioxidant activity was expressed as the percent inhibition of the color-change reaction relative to the control, as shown in Equation (2).

Treatment of the data. The results were compared by analysis of variance (ANOVA), and the average results were compared by the Tukey test with 95% statistical significance (α), $p<0.05$, using Statistica 5.0 software. All analyses, except the fatty acid and phenolic compound profiles, were carried out in triplicate.

3. Results and Discussion

Chemical composition of chia seeds (*Salvia hispanica L.*). The proximal composition analysis (Table 3) showed a higher content of dietary fiber, 22.1 g.100 g^{-1}, than previously reported by Tosco [21]. The lipid content, 33 g.100 g^{-1}, was similar to the amount reported by Ixtaina et al. [3]. The levels of protein and ash were similar to the values found by Ayerza and Coates [11] and were 23 g.100 g^{-1} and 4.6 g.100 g^{-1}, respectively. The moisture, ash and lipid contents were 6.3, 4.3 and 34.9 g.100 g^{-1}, respectively, which were similar to results published by Segura-Campos et al. [22], but the protein and fiber content 24.0 and 35.8 g.100 g^{-1}, respectively, were different.

With all these features, chia seeds can be used as emulsifiers and stabilizers due to their high fiber content, and as an ingredient for products gluten-free, and with low carbohydrate content. In addition, there is the possibility of extracting oil for production of capsules of ω -3, and protein concentrates obtained due to their protein content. So, chia seeds can be used for enrichment of products, such as cookies, cereal bars and bakery.

Table 3. Proximal composition and calorific value of chia seed

Component	% w.b.[a]	% d.b.[a]
Humidity (g.100 g^{-1})	6,2 ± 0,517	-
Ashes (g.100 g^{-1})	4,3 ± 0,035	4,6 ± 0,035
Proteins (g.100 g^{-1})	18,3 ± 1,613	19,6 ± 1,720
Dietary fiber (g.100 g^{-1})	22,2 ± 0,323	23,7 ± 0,424
Lipids (g.100 g^{-1})	32,4 ± 0,214	34,4 ± 0,353
Other carbohydrates (g.100 g^{-1})	16,5 ± 1,628	17,7 ± 1,465
Caloric value (Kcal.100 g^{-1})	431,2 ± 3,123	459,9 ± 2,394

[a]w.b.: wet basis, d.b.: dry basis.

According to Lima et al. [23], flaxseed has a caloric value of 495 Kcal.100 g^{-1}, similar to chia seeds (Table 3). Like chia seeds, flaxseeds are a renewable source rich in ω-3 and other functional components [24], and their use in formulations increases the ω-3 fatty acid content of popular foods. The high caloric value presented by chia seeds is associated with its high levels of lipids. From a nutritional standpoint, lipids have a high energy value (9 Kcal.g^{-1}) and are important precursors of fat-soluble vitamins (A, D, E and K) and essential fatty acids (linoleic, linolenic and arachidonic acids) [25]. Similar to the value found in this study, Jin et al. [26] found a caloric value for chia seed of 562 Kcal.100 g^{-1}

Chia seeds contain 5-6% mucilage, which can be used as dietary fiber [10,11]. Muñoz et al. [27] studied the hydration of chia mucilage, finding that a 100 mg sample of mucilage absorbs 2.7 g of water, which is 27 times its own weight. Compared with other cereals such as oatmeal (5.5 g water/g fiber) and wheat (6.6 g water/g fiber) [28], the high water retention capacity of chia seed measured in this study (24.0 ± 0.879 g.g^{-1}) illustrates its high fiber content. Adams et al. [28] and Grigelmo-Miguel and Martín-Belloso [29] concluded that the higher soluble dietary fiber content increases the water retention capacity. Francki et al. [30] noted that soluble dietary fibers are easily fermentable by colonic bacteria, are characterized by high water retention and it have the capacity to form a gelatinous mass, which increases the viscosity of

gastrointestinal contents and slows gastric emptying, providing greater lubrication and volume of stool.

Table 4. Lipid content and fatty acid composition of chia seed

	g.100 g^{-1}
Lipids	34,39
Saturated Fats	**9,74**
Myristic acid (C14:0)	0,03
Pentadecanoic acid (C15:0)	0,03
Palmitic acid (C16:0)	6,69
Margaric acid (C17:0)	0,06
Stearic acid (C18:0)	2,67
Behênico acid (C22:0)	0,09
Tricosanoic (C23:0)	0,03
Lignocérico acid (C24:0)	0,14
Monounsaturated Fats	**10,76**
Pentadecenóico acid (C15:1)	0,03
Palmitoleic acid (C16:1)	0,09
Oleic acid (C18:1 – ω-9)	10,55
Cis-Eicosenóico acid (C20:1)	0,09
Polyunsaturated Fats	**79,47**
Linoleic acid (C18:2 – ω-6)	17,36
Linolenic acid (C18:3 – ω-3)	62,02
Cis-Eicosadienóico acid (C20:2)	0,03
Cis-Eicosatrienóico acid (C20:3)	0,03
Trans Fat	**0,03**
Elaidic acid (C18:1)	0,03
Unsaturated Fats	**90,26**

% of total lipids.

Comparing the results of chia seed oil in this study (Table 4) with Ayerza and Coates [11], we obtained similar values of palmitic acid (7%), stearic acid (3.23%), linolenic acid (60.68%) and polyunsaturated fat (PUFA) (81.15%), a lower value for linoleic acid (20.47%) and a higher value for oleic acid (7.48%). Similar to our results, Ixtaina et al. [3] classified the acids in the following order of abundance: linolenic acid (C18:3) >linoleic acid (C18:2) >oleic acid (C18:1) >palmitic acid (C16:0) >stearic acid (C18:0). However, some authors claim that the composition of oil, measured as percent fatty acids, is affected by the location of seed cultivation [11]. In addition, the proportion of ω-3/ω-6 oil from chia in this study was 3.57, which is greater than most vegetable oils including canola oil (0.45), soybean oil (0.15) and olive oil (0.13) [31]. The incorporation of ingredients with high PUFA content into the diet provides numerous health benefits [32]. The chia seed can be considered a functional food because it is a source of ω-3fatty acids, with at least 0.1 g of ω-3 in 100 g of product [33], and has high levels of total dietary fiber, up to 3 g in 100 g of product [34] and protein.

Extraction, quantification and profiling of the phenolic compounds of chia seeds (*Salvia hispanica L.*). The coefficient of determination of the analytical curve of GAE was 0.983, and the equation of the curve was y = 0,020 x (µg.mL^{-1}), where the concentration is on the x-axis and absorbance on the y-axis. As shown in Table 5, the chia phenolic extracts were within the range found by Reyes-Caudillo et al. [10] (511-881 µg.g^{-1} chia seed).

Table 5. Content and profile of phenolic compounds ($\mu g_{GAE}.g_{amostra}^{-1}$) of chia extracts

	µg.$g_{amostra}^{-1}$
Phenolic compounds	641,71
Cinnamic acid	ND
Chlorogenic acid	4,68
Caffeic acid	30,89
Quercetin	0,17
Phenolic Glycoside*	605,97

ND (not detected); *phenolic glycoside: most likely a union glicídeo + phenolic compound.

The profile of phenolics showed a high content of caffeic acid compared with other phenols. Reyes-Caudillo et al. [10] studied the profile of phenolic compounds of chia seeds from two regions of Mexico, chlorogenic acid was predominant in crude phenolic compound extracts, ranging from 45.9-102 µg.g^{-1}_{chia}, followed by caffeic acid (3-6.8 µg.g^{-1}_{chia}). According to these authors, the phenolic compound content is affected by a number of external factors, such as weather and post-harvest conditions. Kim et al. [35] suggested that two different methods of extraction can be used to obtain more information about the concentration of phenolics in chia seeds. Because the phenolic compounds may be in the form of polymers, esters and glycosides, enzymatic hydrolysis is used to quantify and verify the bioavailability of these compounds. These assays were used in the study of Reyes-Caudillo et al. [10], who found 651 µg.g_{chia}^{-1} of phenolic glycosides in crude phenolic compound extracts, similar to the values reported here.

3.1. Antioxidant Activity of Phenolic Compounds from Chia (*Salvia hispanica L.*) Seeds

Ability to sequestrate the DPPH free radical. The antioxidant activity of the phenolic extracts was determined by the inactivation of DPPH (Figure 1). The chia phenolic extract (32.35 $\mu g_{GAE}.mL_{extract}^{-1}$) showed antioxidant activity that was statistically equal during the period from 120 to 210 min. The extract was effective in neutralizing more than 70% of the free radicals. There was a significant difference between the chia extract and the control, a synthetic antioxidant (GAE). Based on these data, it is evident that bioactive components in the extracts act as radical sequestrators and hydrogen donors [36]. Comparing our results to the study by Schmidt et al. [37], the chia phenolic compounds showed high antioxidant activity in the DPPH assay. Phenolic compounds extracted from rice bran (0.1 mg.mL^{-1}) inhibit approximately 50% of the DPPH in the reaction after 30 min. The bioactive, antioxidant components in these foods lower the incidence of cardiovascular disease and prevent the rancidity of unsaturated fatty acids [10].

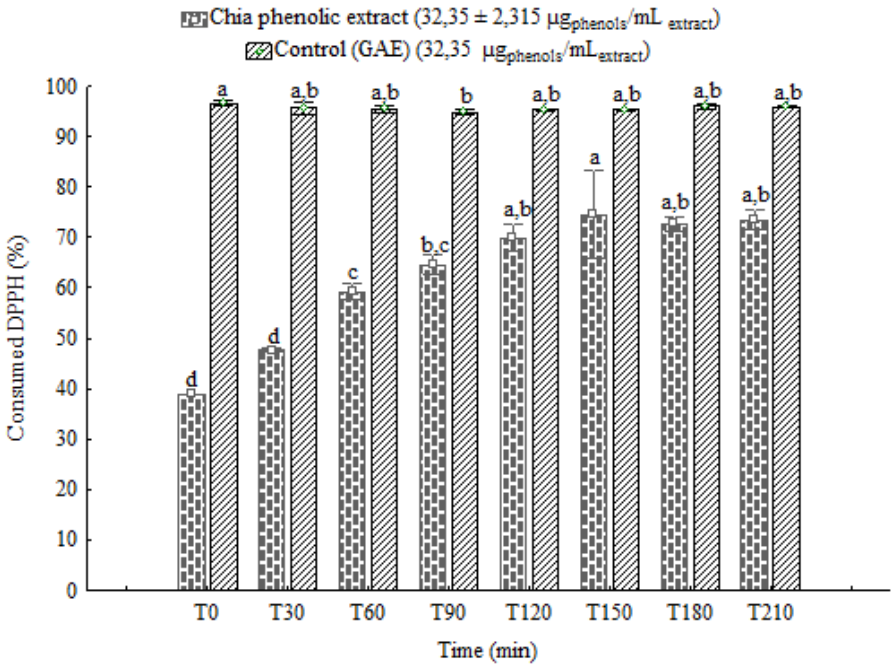

Figure 1. Percent DPPH inhibition by chia (*Salvia hispanica*, L.) extracts

Inhibition of enzymatically catalyzed oxidation. Chia phenolic extracts inhibited the oxidation of guaiacol (Figure 2), and the rate of this inhibition was constant during the assay period.

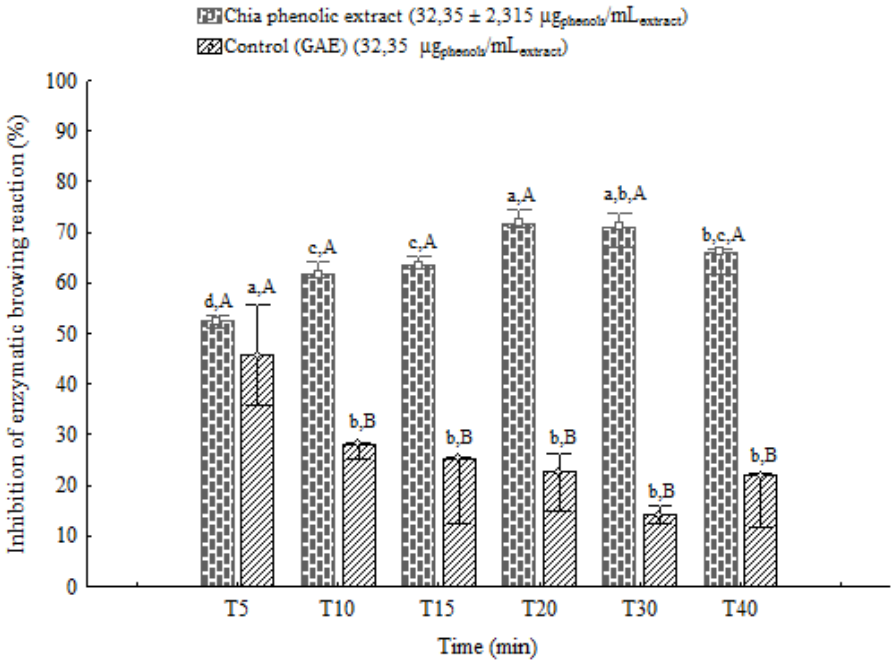

Figure 2. Percent enzymatic browning reaction inhibition by chia (*Salvia hispanica* L.) extracts

Compared with the control (GAE), there was no significant difference at 5 min of reactions, but at other times, the chia phenolic extract times showed higher antioxidant activity than the control. The chia seed antioxidant activity could have physiological effects, such as anticancer and antimutagenic activity, that remediate problems resulting from free radicals [38].

Compared with this study, Oliveira et al. [20] found higher values in wheat and rice, with 50% and 60% inhibition of peroxidase in reactions containing 2.0 and 1.3 $\mu g_{phenols}.mL^{-1}$ extracts, respectively, in 10 min. Peroxidase is an important vegetable enzyme that is involved in various reactions, including polysaccharide synthesis, indole-3-acetic acid oxidation, formation of linkages between monomers, lignification, phenol oxidation, pathogen defense, and regulation of cell elongation [39].

4. Conclusions

In conclusion, chia seeds showed high levels of lipids, proteins and fibers compared with other seeds. It is suggested that fiber, one of the components of chia, due to

its high water retention capacity, are important for the production of other products such as emulsifiers. In addition, chia seeds presented high content of phenolic compounds with antioxidant activity effectively suggesting that chia can bring health benefits when used in food products. The diversity along with the amount of nutrient composition in chia seed can help to have a healthy diet and add value in the preparation of products.

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Abbreviations Used

GAE, gallic acid.

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