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Physicochemical and Lipid Characterization of Chia Seed Oil for Improving Algerian Bread Quality and Acceptability

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Abstract

In Algeria, edible oils are vital for development through income generation, rural development, and modernization of production systems. Mexican and Brazilian chia seed oils were selected as sources. Their physicochemical and nutritional characterization provides baseline information supporting research on the adaptation, cultivation, and industrial exploitation of chia under Algerian agro-climatic conditions. This study investigates the physicochemical, bioactive, and sensory properties of chia oils from Brazil and Mexico and assesses their impact on the nutritional and sensory quality of chia-enriched bread. The methodology involved analytical techniques, including FTIR, GC-MS, and ^{1}H NMR, to determine acid value, peroxide value, saponification index, fatty acid composition, and total polyphenol content. Both oils exhibited low acid values and high iodine values, indicating good quality and a high degree of unsaturation. Mexican chia oil contained more α-linolenic acid (ω-3), whereas Brazilian oil showed a higher proportion of saturated fatty acids, contributing to greater oxidative stability. Sensory evaluation showed that bread enriched with 5% Mexican chia seed oil achieved the highest acceptance (7.02/9). Principal component analysis (PCA) revealed that increasing chia oil concentration, particularly at 5 and 7.5%, was positively associated with sensory and textural attributes, including crust colour, flax-like flavour, porosity, moistness, hardness, chewiness, and bread quality. The findings demonstrate that both Mexican and Brazilian chia seed oils are functional ingredients for bread fortification. While Mexican oil showed a more favourable lipid profile, both oils improved bread quality and consumer acceptability, supporting their potential use in functional foods and food biotechnology.

Keywords: chia oil, antioxidant potential, biotechnological application, sensory analysis, bread enrichment

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Caracterización fisicoquímica y lipídica del aceite de semilla de chía para mejorar la calidad y la aceptabilidad del pan argelino

Resumen

En Argelia, los aceites comestibles son fundamentales para el desarrollo económico mediante la generación de ingresos, el desarrollo rural y la modernización de los sistemas de producción. Los aceites de chía mexicanos y brasileños fueron seleccionados como fuentes. Su caracterización fisicoquímica y nutricional proporciona información de referencia para investigaciones sobre la adaptación, el cultivo y la explotación industrial de la chía en condiciones agroclimáticas argelinas. Este estudio evalúa las propiedades fisicoquímicas, bioactivas y sensoriales de aceites de chía de Brasil y México, así como su impacto en la calidad nutricional y sensorial del pan enriquecido con chía. La metodología incluyó FTIR, GC-MS y ^{1}H RMN, para determinar el índice de acidez, el índice de peróxidos, el índice de saponificación, la composición de ácidos grasos y el contenido de polifenoles totales. Ambos aceites presentaron índices de acidez bajos e índices de yodo altos, lo que indica buena calidad y un alto grado de insaturación. El aceite mexicano presentó mayor contenido de ácido α-linolénico (ω-3), mientras que el brasileño mostró una mayor proporción de ácidos grasos saturados, lo que contribuye a una mayor estabilidad oxidativa. La evaluación sensorial mostró que el pan enriquecido con 5 % de aceite mexicano alcanzó la mayor aceptación (7,02/9). El análisis de componentes principales (PCA) indicó asociaciones positivas entre las concentraciones de 5 y 7,5 % y atributos sensoriales y texturales. Los resultados demuestran que ambos aceites son ingredientes funcionales adecuados para la fortificación del pan, con potencial para su aplicación en alimentos funcionales y biotecnología alimentaria.

Palabras clave: aceite de chía, potencial antioxidante, aplicación biotecnológica, análisis sensorial, enriquecimiento del pan

Caracterização físico-química do óleo de semente de chia, uso para melhorar o pão argelino

Resumo

Na Argélia, os óleos comestíveis são fundamentais para o desenvolvimento económico, a geração de renda, o desenvolvimento rural e a modernização dos sistemas de produção. Os óleos de sementes de chia mexicanos e brasileiros foram selecionados como fontes representativas. A sua caracterização físico-química e nutricional fornece informações de referência para pesquisas sobre a adaptação, o cultivo e a exploração industrial da chia em condições agroclimáticas argelinas. Este estudo avaliou as propriedades físico-químicas, bioativas e sensoriais dos óleos de chia brasileiros e mexicanos, bem como o seu impacto na qualidade nutricional e sensorial do pão enriquecido com chia. A metodologia incluiu técnicas analíticas complementares, como FTIR, GC-MS e RMN de ^{1}H, para determinar o índice de acidez, o índice de peróxidos, o índice de saponificação, a composição de ácidos graxos e o teor de polifenóis totais. Ambos os óleos apresentaram baixos índices de acidez e altos índices de iodo, indicando boa qualidade e grau de insaturação. O óleo mexicano apresentou maior teor de ácido α-linolénico (ω-3), enquanto o óleo brasileiro apresentou maior proporção de ácidos graxos saturados, contribuindo para maior estabilidade oxidativa. A avaliação sensorial mostrou que o pão enriquecido com 5% de óleo mexicano apresentou a maior aceitação (7,02/9). A análise de componentes principais (PCA) revelou associações positivas entre as concentrações de 5 e 7,5% e atributos sensoriais e texturais. Os resultados demonstram que ambos os óleos são ingredientes funcionais adequados para a fortificação do pão, com potencial para aplicação em alimentos funcionais e biotecnologia alimentar.

Palavras-chave: óleo de chia, potencial antioxidante, aplicação biotecnológica, análise sensorial, enriquecimento do pão

1. Introduction

Vegetable oils are key raw materials in multiple sectors owing to their complex composition, which provides a wide range of technological applications in food, biofuels, green oleochemistry, cosmetic, and industrial

formulations, such as surfactants and emollients (Dijkstra, 2013). They constitute the world's primary renewable source of lipids, surpassing animal fats in both consumption and economic importance.

Salvia hispanica L. (chia), a member of the Lamiaceae family, is an annual herbaceous plant originally cultivated by ancient Central American civilizations. While wild and domesticated forms share morphological similarities, only *S. hispanica* is commercially exploited (Reales et al., 2004). Its remarkable adaptability to arid environments makes chia a valuable crop for regions facing water scarcity.

Chia seeds possess an outstanding nutritional profile, containing 15-25% protein, 30-39% fat, 26-41% carbohydrates, and 18-30% dietary fiber, along with vitamins, minerals, and a dry matter content of 90-93% (Ixtaina et al., 2008). Their abundance in antioxidants further supports their classification as functional foods. Since their approval as a novel food by the European Union in 2020 (European Commission, 2020), chia seeds and their oil have attracted increasing interest as functional food ingredients. Chia oil has been incorporated into bakery products to enhance their ω-3 fatty acid content, improve nutritional quality, and develop functional foods while maintaining acceptable sensory properties. Today, chia is widely cultivated in Mexico, Bolivia, Argentina, Ecuador, Australia, and Guatemala, underscoring its strategic role in global food security.

Chia oil content may reach 39%, with α-linolenic acid (omega-3) representing up to 68% of total fatty acids (Ayerza, 1995), surpassing the 57% found in flaxseed oil (Sultana, 1996). This makes chia oil one of the richest plant-based sources of omega-3, particularly suitable for food fortification. Chia seed oil has gained increasing attention as a functional edible oil because of its exceptionally high content of α-linolenic acid (ω-3), which accounts for approximately 60-65% of its total fatty acids (Tak et al., 2022). In addition to its favourable fatty acid profile, chia oil contains natural antioxidants, including tocopherols and phenolic compounds, that contribute to its nutritional value and oxidative stability. These characteristics have promoted its incorporation into functional foods, particularly bakery products, to improve their nutritional quality while maintaining desirable technological and sensory properties (Chen, Wu, et al., 2024; Mondor, 2024).

Despite growing scientific interest in chia oil, comparative studies assessing oils from different geographic origins remain scarce. Such investigations are essential for optimizing cultivation practices and guiding industrial applications. Accordingly, the present study compares chia seed oils from Mexico, the center of origin and one of the principal producers of *Salvia hispanica* L., and Brazil, an important emerging producer in South America. These two origins were selected because differences in genotype, environmental conditions, and agronomic practices may influence the physicochemical characteristics and fatty acid composition of chia oil, thereby affecting its nutritional quality and technological performance in bakery applications. The comparative evaluation provides valuable information for selecting suitable chia oils for bread fortification and the development of functional foods (Grancieri et al., 2019).

2. Materials and Methods

2.1 Biological Materials

Two commercially available chia seed (*Salvia hispanica* L.) samples were purchased from the Algerian retail market. One commercial package originated from Mexico and the other from Brazil, as indicated on the product labels. Each sample consisted of a single commercial batch and was used as a representative sample of its geographical origin. Upon purchase, the seeds were kept in their original sealed packages and stored in a cool, dry place protected from light until oil extraction and subsequent physicochemical, spectroscopic, chromatographic, and baking analyses (Figure 1).

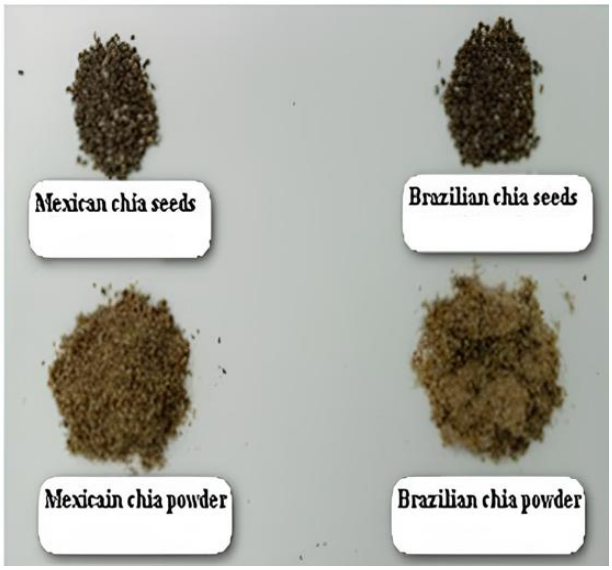

Figure 1. Chia seeds and chia powders

2.2 Chia Seed Oil Extraction

For analysis, the cold solvent extraction of chia oil was carried out according to the method described by Timilsena et al. (2016). The ground chia seeds were mixed with n-hexane in a ratio of 1:10 (chia seeds: solvent). To optimize the yield of the cold extraction, the sample was pre-treated for 15, 30, and 60 minutes in an ultrasonic bath. The container was covered with aluminum foil to minimize the effect of light on lipid oxidation, then mechanically stirred on a shaking plate for 4 hours at room temperature. The suspension was then centrifuged (Sigma, Sigma 2-16PK, Germany) at 4,000 × g for 10 minutes at 4 °C. The solvent phase (supernatant) containing the oil extracted from the chia seeds was filtered through filter paper, and the solvent was then recovered by distillation using a rotary evaporator (IKA-Werke GmbH & Co. KG, model RV10 basic, Germany) at 45 °C.

For bread preparation, chia oil was obtained separately by mechanical pressing of chia seeds using an oil press (Vevor, model KYL-380, China). The extracted oil was collected, filtered to remove residual seed particles, and stored in sealed amber containers under 4 °C conditions until incorporation into the bread formulations (Figure 2). The mechanically pressed oils from Brazilian and Mexican chia seeds were used directly for bread preparation and sensory evaluation.

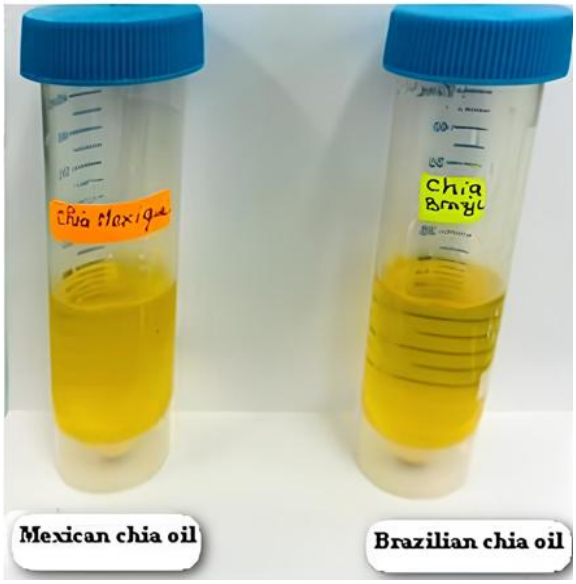

Figure 2. Oil extracted from chia seeds

2.3 Chia Oil Physicochemical Characterization

Physicochemical determinations were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation. Acid value was expressed as mg KOH g^{-1} oil; peroxide value as meq O_2 kg^{-1} oil; saponification value as mg KOH g^{-1} oil; iodine value as g I_2 100 g^{-1} oil; relative density as g cm^{-3} at 20 °C, and specific UV absorbance as extinction coefficients (K232 and K270). Acid, peroxide, saponification, and iodine values were determined according to ISO 660:2020 (International Organization for Standardization [ISO], 2020a), ISO 3960:2020 (ISO, 2020b), ISO 3657:2020 (ISO, 2020c), and ISO 3961:2018 (ISO, 2018), respectively. Relative density was measured at 20 °C using a 25 mL pycnometer (ISO, 2017a), while UV absorbance at 232 and 270 nm was recorded using a Shimadzu UV-1800 UV–Vis spectrophotometer (ISO, 2017b).

2.4 Bioactive Compounds

The extraction of phytochemical compounds from oil was performed according to the method described by Capannesi et al. (2000), with 80/20% methanol/water (v/v). Carotenoids were quantified at 450 nm following the method described by Pacheco et al. (2014). Total chlorophyll and β-carotene contents were determined according to AOCS method Cc 13i-96 (American Oil Chemists' Society, 2017) and Siger et al. (2017), respectively.

The total phenolic compounds (TPC) in oil were determined according to the method described by Costa et al. (2018). Briefly, 30 µL of the extract, 150 µL of Folin-Ciocalteu reagent (1:10), and 120 µL of sodium carbonate solution (7.5% m/V) were mixed and incubated (15 min, 45 °C). After 30 min, the absorbance was read at 765 nm in a UV/Vis spectrophotometer (Shimadzu UV-1800, Japan). A calibration curve was prepared with the standard-gallic acid (5-100 mg/L). The total flavonoids content (TFC) of oil was determined according to the method described by Costa et al. (2018). Briefly, 1 mL of the extract, 4 mL of deionized water, and 300 µL of sodium nitrite (25%) were mixed. After 5 min, 300 µL of aluminum chloride (10%) was added and mixed. After 1 min, 2 mL of sodium hydroxide (1 M) and 2.5 mL of deionized water were added and mixed. The absorbance was read at 510 nm in the same UV/Vis spectrophotometer (Shimadzu UV-1800, Japan) as that used for determining the TPC. A calibration curve was prepared with the standard quercetin (2.5-400 mg/L).

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) inhibition of the oil was analyzed according to the method described by Costa et al. (2018). Briefly, 30 µL of the extract was mixed with 270 µL of freshly prepared DPPH• solution (6×10^{-5} mol/L in ethanol). The absorbance at 525 nm was measured every 2 min to observe the kinetics reaction, with the endpoint at 20 min, in the same UV/Vis spectrophotometer (Shimadzu UV-1800, Japan) as that used for determining the TPC.

2.5 FT-IR Spectroscopy

Fourier-transform infrared (FT-IR) spectra of chia oil were recorded using a Bruker Alpha spectrophotometer over the range of 400-4000 cm^{-1} (64 scans, resolution 4 cm^{-1}). Structural characterization was carried out based on the identification of characteristic functional group absorption bands.

2.6 Fatty Acid Profiling by GC-MS

Fatty acids were converted into their corresponding fatty acid methyl esters (FAMEs) by alkaline transesterification prior to GC-MS analysis, following a previously reported procedure for chia seed oil with minor modifications (Oteri et al., 2022). Briefly, 0.1 g of chia oil was mixed with 2 mL of heptane and agitated. Subsequently, 0.2 mL of 2 N methanolic KOH was added, and the mixture was vigorously agitated for 30 s to promote transesterification. After phase separation, the clear upper organic phase containing the FAMEs was carefully collected and subjected to GC-MS analysis.

GC-MS analysis was performed using an Agilent 7890A gas chromatograph coupled to a 5975C quadrupole mass spectrometer and equipped with a DB-Wax capillary column (30 m × 0.25 mm × 0.25 µm). A 1 µL aliquot of the FAME extract was injected using a split ratio of 15:1. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature was initially set at 100 °C and held for 4 min, then increased to 205 °C at a rate of 4 °C/min, followed by an increase to 230 °C at 1 °C/min and held for 5 min. Fatty acid methyl esters were identified by comparing their retention times and mass spectra with those of external FAME standards and/or reference spectral libraries. Quantification was performed using the C17:0 internal standard, and the results were expressed as mg/g dry matter.

2.7 Positional Distribution by ^{1}H NMR

^{1}H NMR spectra were recorded on a Bruker Ascend™ 400 MHz spectrometer at 25 °C. Samples (200 mg) were dissolved in 1 mL of $CDCl_3$ and 200 µL of DMSO-d_6, then dried over anhydrous sodium sulfate (200 mg). Spectra were acquired with 1024 scans and processed using MestreNova software.

2.8 Baking Test

Bread-making experiments were conducted according to a completely randomized experimental design including six formulations: 0.5, 1.0, 2.5, 5.0, and 7.5% (w/w, based on flour weight), and a control formulation containing no chia oil (0%). These concentrations were selected to cover a progressive range of low to relatively high oil incorporation levels and to assess their effects on bread characteristics and sensory acceptance. The range was designed to identify a suitable incorporation level that could provide the nutritional and functional benefits of chia oil while maintaining acceptable bread quality and sensory properties (Guiotto et al., 2020).

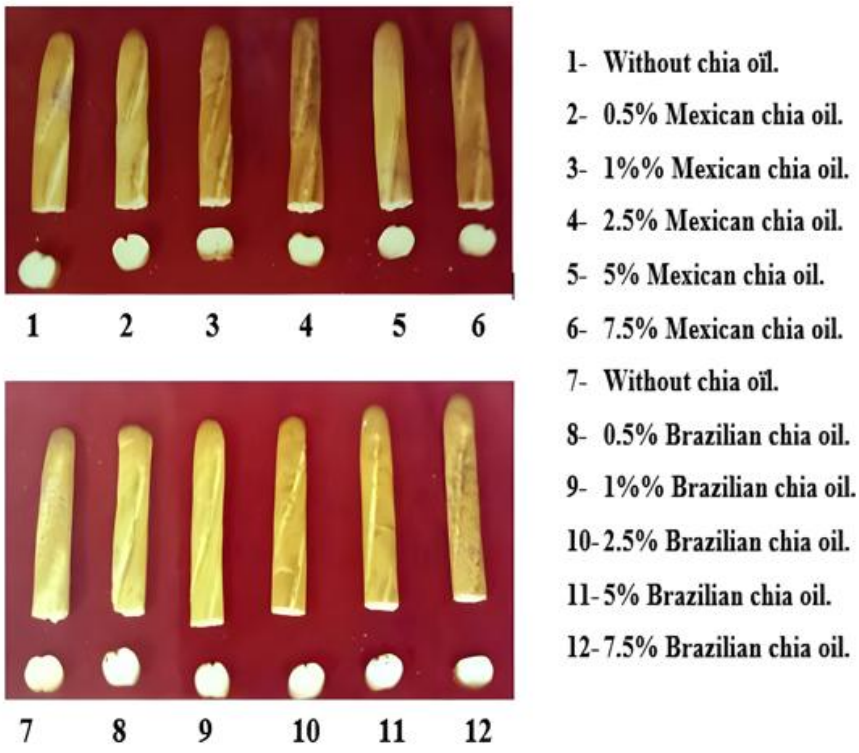

Figure 3. Bread enhanced with chia oil

The control dough formulation (0% chia oil) consisted of wheat flour (500 g), instant dry yeast (2.5%, flour basis), sodium chloride (1.6%, flour basis), ascorbic acid (0.01%, flour basis), and tap water adjusted to obtain a dough consistency of 500 Brabender Units. Chia oil was incorporated at 0.5, 1.0, 2.5, 5.0, and 7.5% (flour weight basis) by replacing an equivalent amount of flour (Sanz-Penella et al., 2009). Ingredients were mixed for 4 min, rested for 10 min, divided into 100 g dough pieces, kneaded, and allowed to rest for an additional 15 min. Dough pieces

were manually shaped, proofed at 28 °C and 85% relative humidity until optimum expansion, and baked in an electric oven at either 190 °C for 18 min or 170 °C for 23 min, depending on the formulation, following the method described by Sanz-Penella et al. (2009). After baking, loaves were cooled at room temperature for 75 min before sensory evaluation (Figure 3).

Sensory evaluation was performed using the baked loaves from each treatment. Bread samples were sliced immediately before serving and evaluated by nine untrained panelists. The mean sensory scores obtained for each loaf were used for statistical analysis.

2.9 Sensory Analyses

Sensory evaluation was performed by a panel of nine untrained volunteers recruited from the Department of Biological Sciences. Panelists were regular bread consumers, aged between 22 and 45 years, with no known allergies or intolerances to the ingredients used in the formulations. Individuals presenting olfactory or gustatory impairments or any health condition likely to influence sensory perception were excluded from the study. Prior to evaluation, the objective of the test and the scoring procedure were explained to all participants. Bread samples (2 cm thick) were coded with random three-digit numbers and presented in a randomized order under identical tasting conditions. Panelists evaluated crust color, crumb color, pore structure, chewiness, taste, and overall acceptability using a 15-point hedonic scale ranging from 1 (“extremely disliked”) to 15 (“extremely liked”), according to the method described by Kihelberg et al. (2006).

2.10 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) of three independent determinations (n = 3). Statistical analyses were performed using XLSTAT 2019 (Addinsoft, Paris, France). One-way analysis of variance (ANOVA) was used to evaluate the effect of chia oil incorporation level (0, 0.5, 1.0, 2.5, 5.0, and 7.5%, flour basis) on the physicochemical and sensory parameters. When significant differences were detected ($p < 0.05$), mean values were compared using Tukey's honestly significant difference (HSD) test. Principal component analysis (PCA) was performed using the sensory variables, namely crust color, crumb color, pore structure, chewiness, taste, and overall acceptability, to identify the attributes contributing most to the discrimination of bread formulations. Hierarchical cluster analysis (HCA) was subsequently applied to the same sensory dataset to classify bread samples according to their overall sensory similarity.

3. Results and Discussion

3.1 Physicochemical and Bioactive Compound Analysis of Chia Oils

The physicochemical characterization of chia seed oils from Brazil and Mexico was first performed to evaluate their quality and suitability for food applications. The measured parameters, including acid value, peroxide value, iodine value, saponification value, relative density, and UV absorbance, provide valuable information on oil freshness, oxidative stability, and degree of unsaturation. The results obtained for both oils are presented in Table 1 and discussed in relation to international quality standards and previously published data.

The physicochemical profiles of Brazilian and Mexican chia seed oils indicate high quality and stability. The acid value, which reflects the concentration of free fatty acids resulting mainly from triglyceride hydrolysis, increases under poor storage conditions (Ben Tekaya & Hassouna, 2007). Acid values were, respectively, lower than those reported for other chia and flaxseed oils (Ixtaina et al., 2011; Segura-Campos et al., 2014). The peroxide value, an indicator of primary lipid oxidation and oil freshness (Chimi, 2005), was 10.90 meq O_2/kg oil (Mexico) and 13.61 meq O_2/kg oil (Brazil). These values remain within the acceptable limits for cold-pressed oils, suggesting limited primary oxidation (Chimi, 2005).

Table 1. Physicochemical analysis of chia oils*

Parameters	Brazilian chia oil	Mexican chia oil	Significance (P<)
Acid value (mg KOH/g oil)	1.55±0.13$^{(d.e.f)}$	1.54±0.11$^{(e.f)}$	0.05
Peroxide (meqO_2/Kg oil)	13.61±0.006$^{(c)}$	10.90±0.002$^{(d)}$	0.001
Saponification value (mg KOH/g oil)	184.66±2.1$^{(a)}$	143.52±2.92$^{(b)}$	0.001
Iodine value (gI_2/100g oil)	188.21±2.31$^{(a)}$	188.29±2.44$^{(a)}$	0.001
Relative density (Kg/m^3)	0.886±0.001$^{(e)}$	0.901±0.002$^{(f)}$	0.001
K232	1.741±0.191$^{(e.f)}$	1.685±0.1$^{(e.f)}$	0.01
K270	0.387±0.043$^{(d.e.f)}$	0.402±0.057$^{(f)}$	0.05
Carotenoids (mg/kg)	5.13±1.01$^{(d)}$	4.98±0.09$^{(e)}$	0.01
Chlorophylls (mg /Kg)	4.66±0.06$^{(d)}$	4.01±0.04$^{(e.f)}$	0.01
Total polyphenols (mg EAG/g oil)	3.91±0.21$^{(d.e)}$	3.89±0.19$^{(e.f)}$	0.05
Flavonoids (mg EQ/g oil)	0.162±0.03$^{(f)}$	0.159±0.008$^{(f)}$	0.01
DPPH radical scavenging (%)	48.43±2.50$^{(b)}$	45.11±2.32$^{(c)}$	0.001

* Values are expressed as mean ± standard deviation (n = 3). Means within the same row followed by different lowercase letters differ significantly according to Tukey's HSD test ($p < 0.05$). AV: acid value; PV: peroxide value; SV: saponification value; IV: iodine value; K232 and K270: specific UV extinction coefficients.

The saponification index estimates the average molecular weight of fatty acids, with lower values suggesting longer chain lengths. Saponification values were 143.52 mg KOH/g oil (Mexico) and 184.66 mg KOH/g oil (Brazil), both lower than those reported for Argentine and Guatemalan chia oils (193 mg KOH/g oil) and for conventional oils such as sunflower, soybean, and safflower, indicating the presence of longer-chain fatty acids (Ivanova et al., 2022). The iodine values of the Brazilian and Mexican chia seed oils were 188.21 and 188.29 g I_2/100 g oil, respectively, indicating a high degree of unsaturation characteristic of chia oil. These values are consistent with those previously reported for *Salvia hispanica* oils, which generally range from approximately 180 to 200 g I_2/100 g oil, depending on cultivar, geographical origin, environmental conditions, and extraction method (Timilsena et al., 2017). The similarity observed between the two oils suggests a comparable degree of unsaturation despite their different geographical origins.

Oil density, which depends on composition and temperature and serves as an indicator of purity (Karleskind, 1992), was 0.901 kg/m³ (Mexico) and 0.886 kg/m³ (Brazil) at 20 °C, consistent with values reported in the literature and suggestive of high unsaturation (Alvarado & Aguilera, 2001). UV spectrophotometry (K_{232}, K_{270}) identified primary and secondary oxidation products (Timilsena et al., 2017). Extinction coefficients K_{232} (1.658-1.741) and K_{270} (0.387-0.402) were similar to those of Australian chia oil, confirming moderate oxidative degradation (Timilsena et al., 2017).

Pigment contents were low, with carotenoids ranging from 4.98 to 5.13 mg/kg and chlorophyll from 4.01 to 4.66 mg/kg, which may contribute to shelf-life extension by limiting photooxidation (da Silva et al., 2017; Uzunova et al., 2016). Total phenolic and flavonoid contents were 3.89-3.91 mg GAE/g DM and 0.159-0.162 mg QE/g DM, respectively, confirming the presence of these bioactive compounds in chia oils. However, comparisons with studies conducted on chia seeds or solid by-products should be made cautiously because phenolic and flavonoid contents are strongly affected by the matrix and extraction procedure (Alvarado & Aguilera, 2001; Timilsena et al., 2017). Recent research on chia seed oils has also demonstrated that the extraction method significantly influences the recovery of minor bioactive compounds, including total phenolics, as well as the antioxidant properties of the resulting oils (Chen, Wang, et al., 2024). Therefore, differences among studies should be interpreted in relation to the oil matrix, geographical origin, and extraction conditions rather than through direct quantitative comparisons. Flavonoids are recognized as bioactive compounds that can contribute to antioxidant activity through their free-radical-scavenging properties. However, their contribution to the antioxidant potential of chia oil may vary depending on their concentration, chemical composition, and extraction

efficiency. Recent studies have demonstrated that processing and extraction conditions can influence the recovery of bioactive compounds and the antioxidant properties of chia seed oils (Al-Juhaimi et al., 2024).

DPPH assay results revealed significantly higher radical-scavenging activity in Brazilian chia oil (48.43%) than in Mexican oil (45.11%) ($p \leq 0.05$), indicating differences in antioxidant capacity between the two oils. However, these differences should be interpreted cautiously, as DPPH activity may be influenced by the oil matrix, extraction method, processing conditions, and assay parameters. Previous research on *Salvia hispanica* has demonstrated that processing and extraction conditions can affect the levels of bioactive compounds and the antioxidant activity of chia-derived products (Al-Juhaimi et al., 2024; Chen, Wang, et al., 2024). Therefore, the higher DPPH activity observed for Brazilian oil may be associated, at least in part, with differences in the concentration or composition of minor antioxidant constituents. Further analyses would be required to establish a direct relationship between specific bioactive compounds and DPPH radical-scavenging activity.

From a technological perspective, the greater antioxidant capacity of Brazilian chia oil may improve its resistance to lipid oxidation during processing and storage. In bakery applications, this characteristic is particularly relevant because lipid oxidation can be accelerated by dough mixing, proofing, and baking temperatures. Enhanced oxidative stability may contribute to better preservation of ω-3 polyunsaturated fatty acids, reduce the formation of undesirable oxidation products responsible for rancid flavors, and extend the shelf life of enriched bread. Furthermore, natural antioxidants present in the oil may help maintain sensory quality, color stability, and nutritional value throughout storage, as previously reported for lipid-containing bakery products enriched with natural antioxidants (Ben Moussa et al., 2022).

3.2 Fourier Transform Infrared (FT-IR) Spectral Analysis

The Fourier-transform infrared (FT-IR) spectra of chia oils are shown in Figure 4.

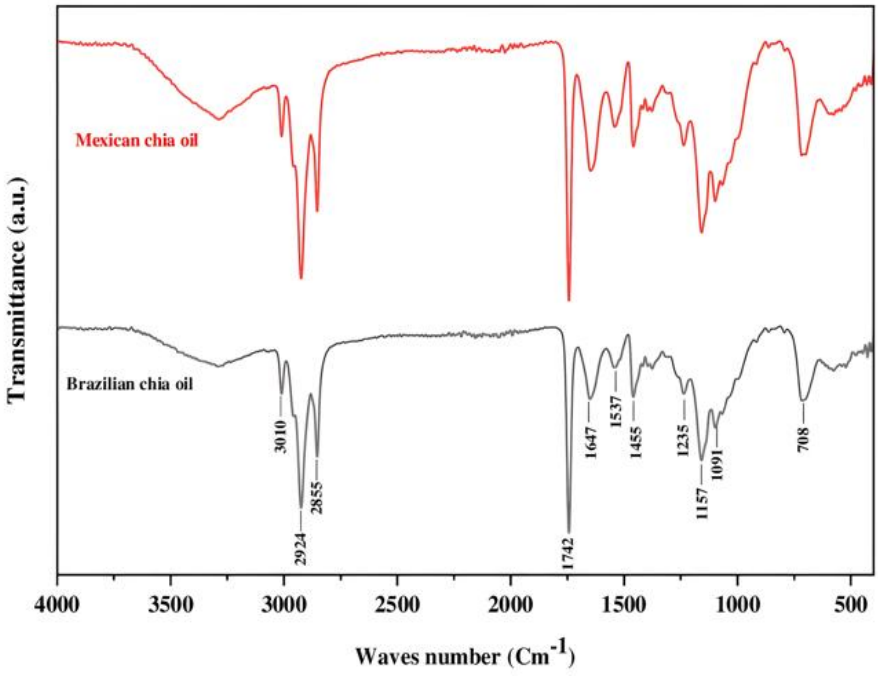

Figure 4. FTIR spectra of chia oils

The ATR-FTIR spectra of Brazilian and Mexican chia oils (Figure 4) showed the characteristic absorption bands of edible vegetable oils, including C–H stretching vibrations at 2928–2853 cm^{-1}, cis olefinic =C–H stretching at approximately 3010 cm^{-1}, ester carbonyl (C=O) stretching at 1742 cm^{-1}, and cis C=C stretching at 1647 cm^{-1}. Additional bands associated with CH_3/CH_2 bending and C–O–C stretching confirmed the predominance of

triglycerides, in agreement with the characteristic FTIR profiles reported for edible vegetable oils (Rohman & Che Man, 2012). The absence of a broad band around 3470 cm^{-1} suggested a low moisture content in both oils.

The spectra of the two oils were highly similar, although slight differences in the relative intensity of the bands at 3010 and 1647 cm^{-1} were observed, consistent with their fatty acid profiles determined by GC-MS. In particular, the Mexican oil contained a higher proportion of α-linolenic acid (68.1%) than the Brazilian oil (54.4%). However, their nearly identical iodine values (188.21 and 188.29 g I_2/100 g oil, respectively) indicated a comparable overall degree of unsaturation. These results confirm the highly unsaturated lipid profile characteristic of *Salvia hispanica* seed oil, despite differences in the relative abundance of individual fatty acids (Marineli et al., 2014).

Overall, the FTIR results complemented the chromatographic and physicochemical analyses, providing additional evidence of the similar triglyceride composition and high degree of unsaturation of the two chia oils. Their oxidative behavior, however, may also depend on endogenous antioxidant compounds, including tocopherols and phenolic constituents, rather than solely on the degree of fatty acid unsaturation (Shahidi & Zhong, 2010). Thus, ATR-FTIR combined with GC-MS and physicochemical analyses provided a complementary assessment of the compositional characteristics of chia oils from different geographical origins (Rohman & Che Man, 2012).

3.3 Determination of Fatty Acid Profile by GC-MS

The fatty acid concentration of the two chia varieties is shown in Table 2.

Table 2. Fatty acid composition of chia oil (g/100 g)

Fatty acids	Brazilian chia oil (%)	Mexican chia oil (%)
C14 (Myristic acid)	0.036	0.032
C16 (Palmitic acid)	19.870	18.817
C18:0 (Stearic acid)	0.672	0.030
C18:1 ω_9 (Oleic acid)	24.118	12.860
C18:2 ω_6 (Linoleic acid)	0.711	0.040
C18:3 ω_3 (α-linolenic acid)	54.395	68.125
C20:0 (Arachidic acid)	0.122	0.071
C22:0 (Behenic acid)	0.075	0.026
Reports and sums		
Total saturated fatty acids	20.775	18.976
Total polyunsaturated fatty acids	55.106	68.165
Total monounsaturated fatty acids	24.118	12.860
$(\omega_6)/(\omega_3)$	0.013	0.0006

In the food industry, chia seeds are widely recognized as an important source of edible oil because of their exceptional content of polyunsaturated fatty acids, particularly α-linolenic acid (ALA), which is considered one of the richest plant-derived sources of ω-3 fatty acids (Ayerza, 1995). Previous studies reported total lipid contents ranging from approximately 30 to 35%, with polyunsaturated fatty acids representing nearly 80% of the total fatty acids, whereas saturated and monounsaturated fatty acids occur in much lower proportions. According to Segura-Campos et al. (2013), the ALA content of chia oil generally ranges between 540 and 670 g/kg of oil. Ixtaina et al. (2011), on the other hand, reported linoleic acid contents ranging from 120 to 210 g/kg of oil. These variations in fatty acid composition may depend on the cultivar, geographical origin, and environmental conditions.

The results obtained in the present study are consistent with these reports but also reveal clear compositional differences between the Brazilian and Mexican chia oils. GC-MS analysis showed that the Mexican oil contained

a markedly higher proportion of α-linolenic acid (68.165%) than the Brazilian oil (55.106%), whereas the latter exhibited relatively higher levels of saturated fatty acids. Despite these differences, both oils showed very similar iodine values (188.21 and 188.29 g I_2/100 g oil), confirming their highly unsaturated nature. The higher ALA content of the Mexican oil enhances its nutritional value because ALA is an essential ω-3 fatty acid associated with cardiovascular, anti-inflammatory, and neuroprotective benefits (Saini & Keum, 2018).

Conversely, the slightly lower degree of unsaturation of the Brazilian oil, together with its higher antioxidant activity observed in the present study, may provide greater resistance to lipid oxidation during processing and storage. This characteristic is particularly advantageous for food applications such as bakery products, where thermal processing and prolonged storage can accelerate oxidative deterioration of ω-3-rich lipids (Ayerza & Coates, 2011). Therefore, although the Mexican chia oil appears nutritionally more attractive because of its higher ALA content, the Brazilian oil may offer technological advantages through improved oxidative stability. These findings highlight the importance of geographical origin as a determinant of chia oil quality and suggest that the selection of a specific oil should depend on its intended nutritional or technological application.

3.4 Characterization of Chia Oil by ^{1}H NMR

The ^{1}H NMR spectra of Brazilian and Mexican chia oils displayed characteristic signals in the range of 0.0-5.5 ppm (Table 3, Figure 5), corresponding mainly to functional groups associated with triglycerides and unsaturated fatty acids. In the Mexican oil, signals assigned to stigmasterol (0.68 ppm), terminal methyl groups (0.85 and 0.95 ppm), methylene protons (2.3–2.6 ppm), and vinylic protons (4.5–5.5 ppm) confirmed the characteristic triacylglycerol structure and high degree of unsaturation. The observed signals were consistent with its higher α-linolenic acid (ALA, ω-3) content, while the absence of prominent oxidative degradation signals suggested no substantial oxidation under the conditions of analysis (Harwood, 2023).

The NMR results were consistent with the GC-MS fatty acid profiles, which showed a higher proportion of ω-3 fatty acids in the Mexican oil, whereas the Brazilian oil contained relatively higher levels of saturated fatty acids (SFAs) (Harwood, 2023). These differences may reflect the combined effects of genetic background, cultivar, geographical origin, environmental conditions, and processing factors. Climatic conditions, soil characteristics, agronomic practices, seed maturity, post-harvest handling, storage, and extraction procedures can all influence lipid biosynthesis and the final fatty acid profile. However, since these variables were not experimentally controlled in the present study, their individual contributions cannot be established definitively. Further studies involving multiple cultivars from different geographical locations and standardized cultivation and extraction conditions are therefore needed to clarify the relative influence of genetic, environmental, and processing factors on chia oil composition.

Table 3. Assignment of ^{1}H NMR spectra signals from chia oils and their integrals

Signal (multiplicity)	**Assignment** (Andrade et al., 2012)
a (multiplet)	$-CH_3$ (terminal methyl group of oleic, linoleic, and saturated acids)
b (triplet)	$-CH_3$ (terminal methyl group of α-linolenic acid)
c (multiplet)	$-(CH_2)_n-$ (aliphatic methylene group)
d (multiplet)	$-OCO-CH_2-CH_2-$ (β-carbonyl methylene group)
e (multiplet)	$-CH_2-CH=CH-$ (allylic methylene group)
f (triplet)	$-OCO-CH_2-CH_2-$ (α-carbonyl methylene group)
g (multiplet)	$-CH=CH-CH_2-CH=CH-$ (bis-allylic methylene group)
h (double doublet)	$-CH_2-O-COR$ (glycerol methylene group)
i (double doublet)	$-CH_2-O-COR$ (glycerol methylene group)
j (multiplet)	–CH–O–COR (glycerol methine group)
k (multiplet)	–CH=CH– (olefinic group)

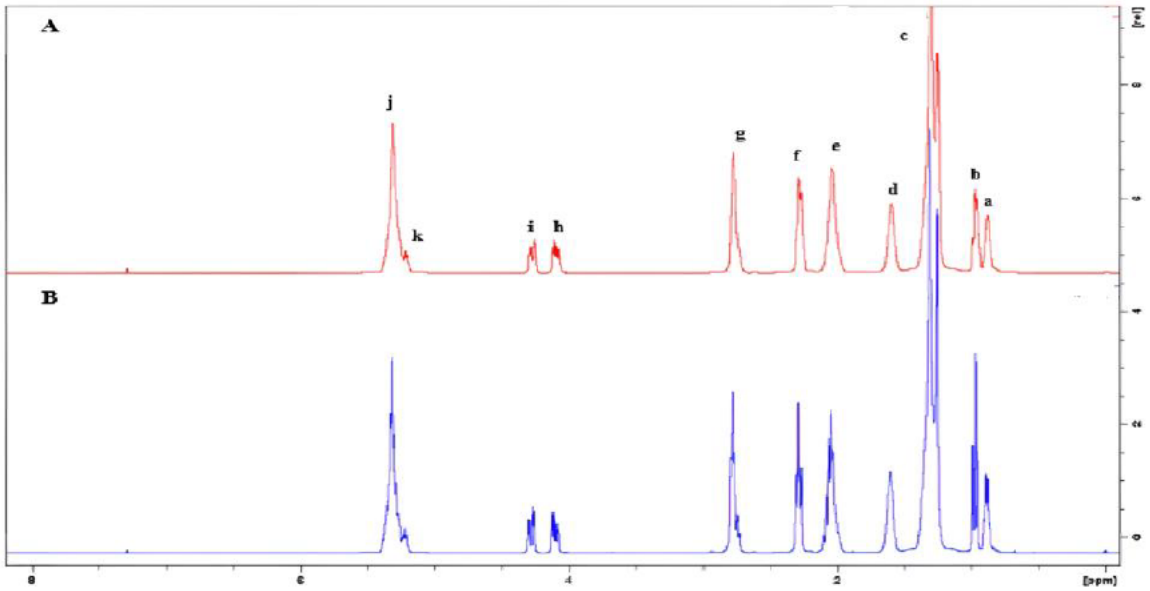

Figure 5. ^{1}H NMR spectra of the chia oils used. A: Mexican oil, B: Brazilian oil

Letters a-k indicate the different ^{1}H NMR signals, with their corresponding chemical assignments provided in Table 3.

3.5 Sensory Analysis

The sensory analysis of pan bread formulations enriched with Mexican and Brazilian chia oils is shown in Figures 6 and 7. Overall, chia oil-enriched bread achieved 95% acceptability, with a mean hedonic score of 7.02, and was characterized by a mild nutty flavor (Bayer et al., 2012). Sensory responses varied among the formulations, indicating that the level of chia oil incorporation influenced overall acceptability and sensory perception. The lower and intermediate oil levels generally maintained favorable sensory characteristics, whereas higher incorporation levels may have intensified the characteristic flavor of chia oil and affected consumer perception. Differences between breads prepared with Mexican and Brazilian chia oils may also be related to variations in their fatty acid composition and minor bioactive constituents. Overall, these findings suggest that chia oil can be incorporated into bread while maintaining good consumer acceptance, supporting its potential as a functional ingredient in reformulated bakery products.

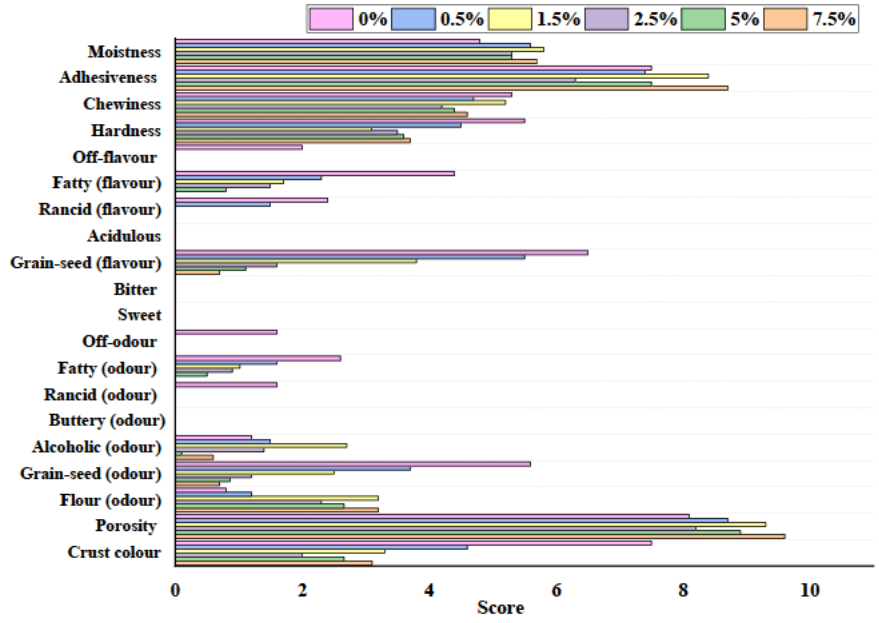

Figure 6. Histogram showing the evaluation of the bread supplied with Mexican chia oil

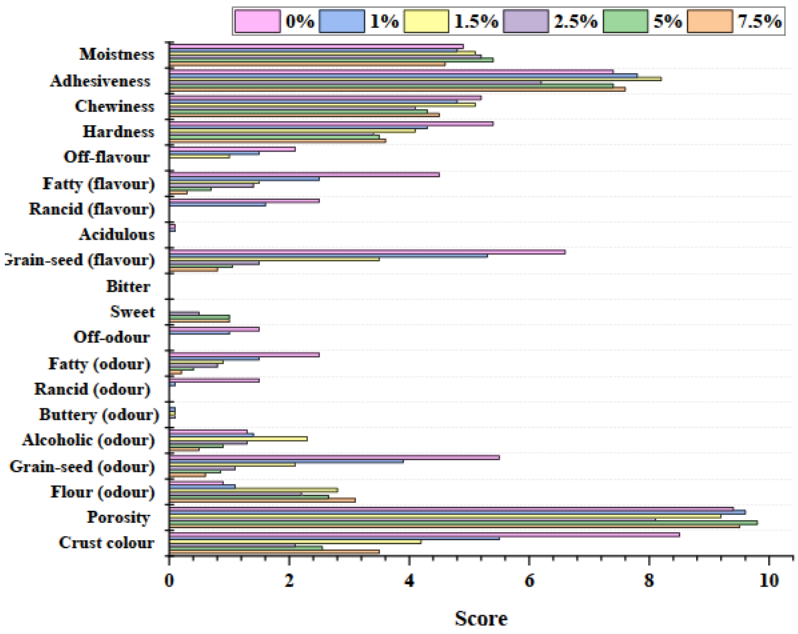

Figure 7. Histogram showing the evaluation of the bread supplied with Brazilian chia oil

3.6 Statistical Analyses

The results of the principal component analysis (PCA) are presented in Figure 8. The first two components, F1 and F2, explained between 98.46% and 98.91% of the total variance, with F1 accounting for approximately 91% and F2 for about 7.5% (Figures 8A and 8B).

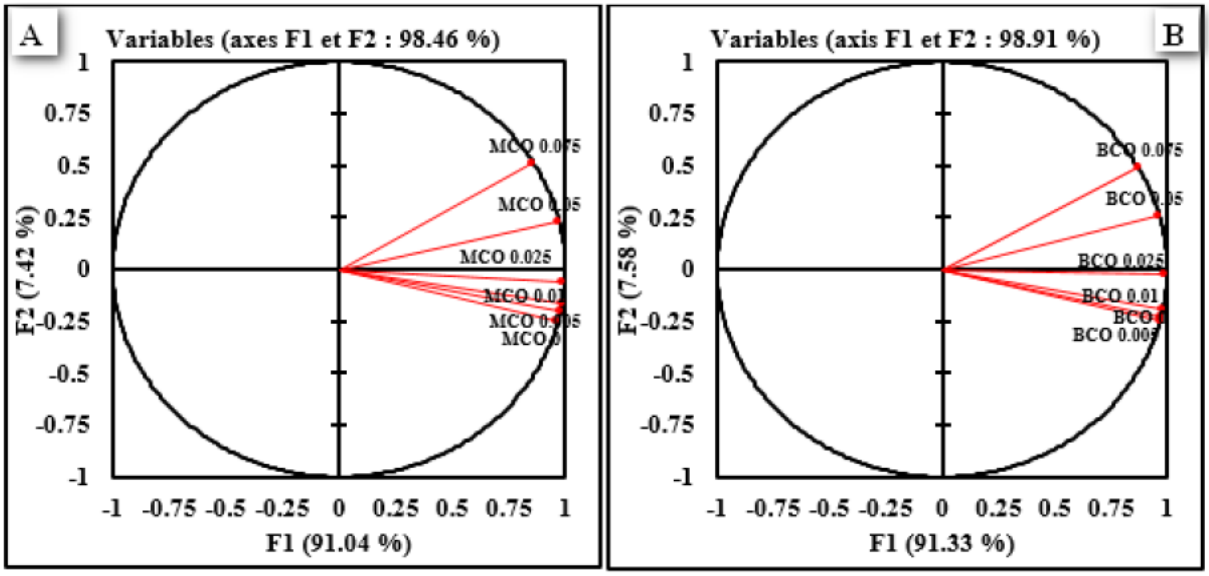

Figure 8. Correlations circle. A: with added Mexican chia oil (MCO), B: with added Brazilian chia oil (BCO)

Axis F1 primarily reflected correlations among sensory attributes, whereas axis F2 represented the effect of chia oil concentration on these parameters. Along F1 (left to right), the strength of correlations among attributes increased, while along F2 (bottom to top), the influence of chia oil concentration became more pronounced (Figures 9A and 9B).

Figure 8 presents the sensory responses of the different bread formulations, whereas Figure 9 provides a multivariate PCA-based interpretation of these results, highlighting the relationships among sensory attributes and the discrimination of the formulations.

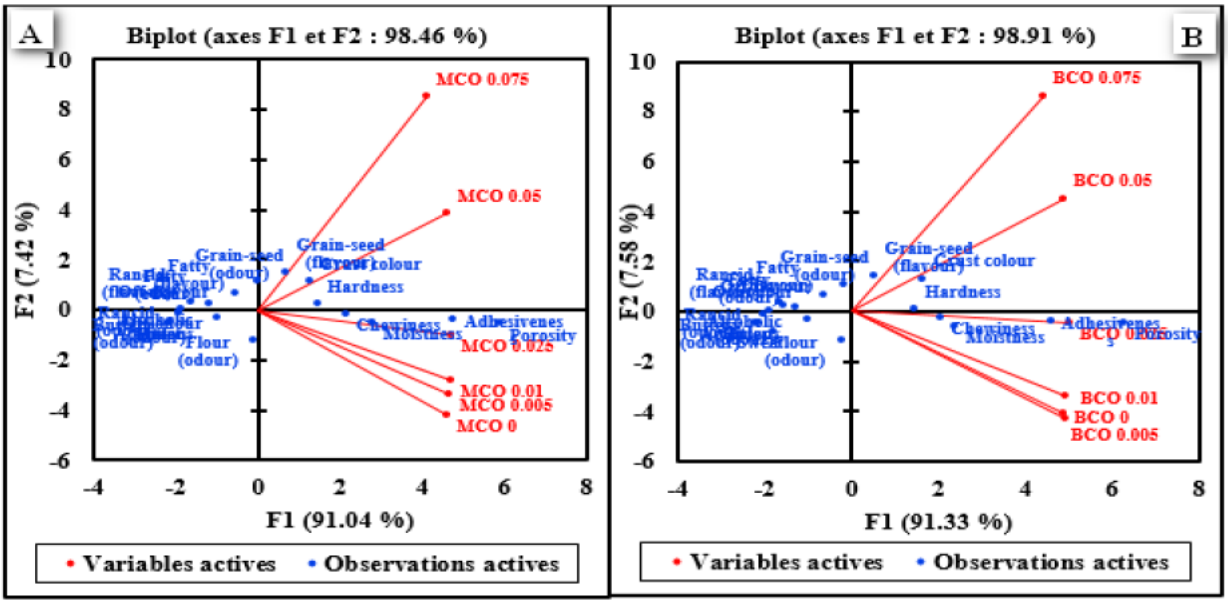

Figure 9. Observations graph. A: with added Mexican chia oil, B: with added Brazilian chia oil

The PCA results (Figure 9) further clarified the relationship between chia oil concentration and sensory attributes. For both Mexican (MCO) and Brazilian (BCO) chia oils, the first two principal components explained most of the total variance (98.46% and 98.91%, respectively), indicating a strong representation of the sensory dataset. In both cases, the formulations containing 5% and 7.5% chia oil were clearly separated from those containing lower concentrations (0-2.5%), suggesting that increasing oil incorporation substantially influenced the sensory profile of the bread. The high-concentration formulations (MCO 0.05 and MCO 0.075; BCO 0.05 and BCO 0.075) were positively associated with grain-seed flavor, grain-seed color, and flavor, indicating that higher chia oil levels enhanced the characteristic sensory perception of chia-enriched bread. In contrast, formulations containing lower oil concentrations were positioned in the opposite direction and showed closer associations with several textural attributes, including crispiness, moistness, adhesiveness, and porosity. Hardness was positioned closer to the intermediate region of the factorial space, suggesting a less pronounced association with the highest oil concentrations. Overall, the similar PCA patterns obtained for MCO and BCO indicate that the concentration of chia oil was a major factor influencing the sensory characteristics of the bread, while the differences in the exact positioning of the formulations may reflect the distinct chemical and fatty acid profiles of the two oil sources. These results suggest that higher chia oil incorporation enhances the characteristic flavor and color of the enriched bread, whereas lower incorporation levels may better preserve the sensory characteristics associated with the bread matrix.

The F1 × F2 score plot revealed three distinct clusters: (i) quality parameters with strong interrelations located on the left of F1, (ii) bread samples with 5% and 7.5% chia oil showing moderate correlations, and (iii) the remaining formulations, which exhibited intermediate relationships.

Hierarchical ascending classification (Figure 10) further distinguished the sensory profiles into separate classes.

Notably, Classes 1 and 3 exhibited the most pronounced sensory characteristics across both chia oils (Table 4). The sensory classification observed in the present study is consistent with previous research on chia-derived ingredients in bakery products. dos Santos et al. (2024) identified three distinct clusters among gluten-free bread formulations containing partially defatted chia flour, demonstrating that the incorporation level of chia-derived ingredients can influence consumer perception and sensory characteristics. Similarly, the PCA in the present study revealed a clear concentration-dependent separation of bread formulations enriched with chia oil. The higher oil levels (5 and 7.5%) were more closely associated with grain-seed flavor, grain-seed color, and flavor attributes, whereas lower concentrations were positioned closer to several textural attributes, including

crispiness, moistness, adhesiveness, and porosity. These observations are consistent with Bartkiene et al. (2023), who reported that the incorporation of chia seeds significantly affected the color, odor, flavor, texture, and overall acceptability of wheat bread, while intermediate chia levels maintained favorable consumer acceptance.

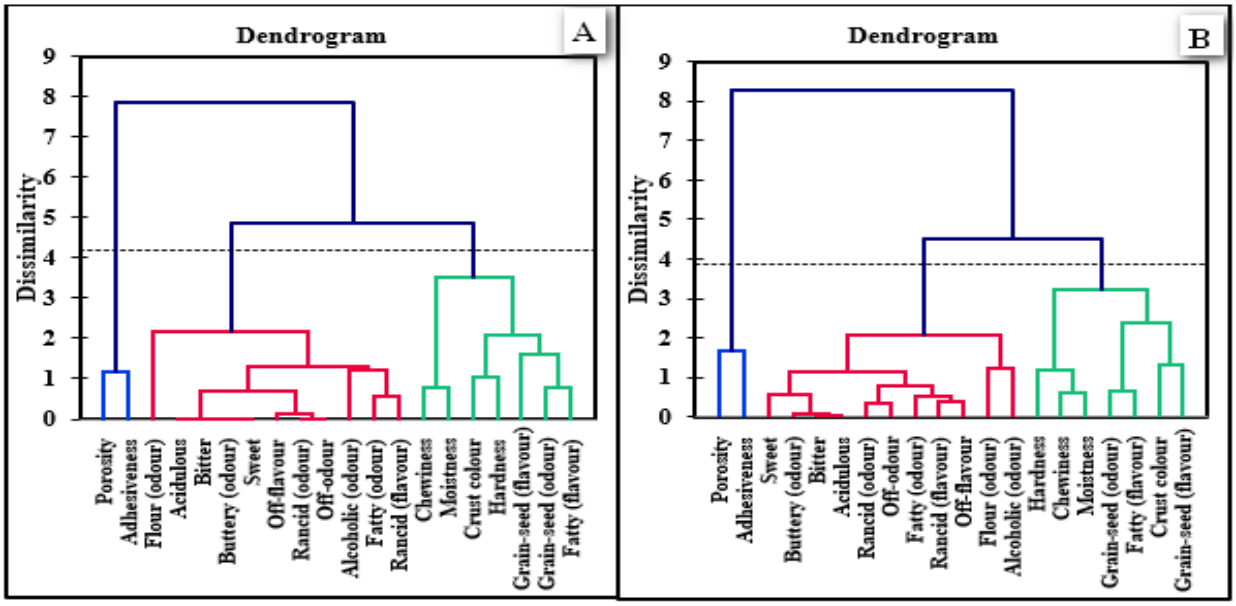

Figure 10. Dendrogram of different characteristics of bread supplied with chia oil. A: with added Mexican chia oil, B: with added Brazilian chia oil

Recent findings on chia seed gum-enriched wheat bread further indicate that the incorporation level of chia-derived ingredients can modify bread appearance, flavor, aroma, texture, and color, supporting the concentration-dependent sensory effects observed in the present study (Al-Ali et al., 2024). Although both dos Santos et al. (2024) and the present study identified three distinct sensory groupings, the nature of the chia ingredient may explain differences in the observed sensory profiles. Partially defatted chia flour contributes proteins, dietary fiber, residual lipids, and other seed components that can influence bread structure and texture, whereas chia oil primarily contributes lipids and volatile compounds that may affect flavor and aroma. Thus, the type and concentration of the chia-derived ingredient, together with the bread formulation, appear to be important determinants of sensory perception and consumer acceptance. Overall, these findings support the use of moderate incorporation levels to achieve a balance between the nutritional or functional benefits of chia and desirable sensory quality.

The hierarchical clustering analysis (Figure 10) provided further insight into the organization of the sensory attributes and complemented the PCA results presented in Figure 9. For both MCO- and BCO-enriched breads, the dendrograms revealed three distinct classes at the selected dissimilarity threshold. Although the overall clustering structure was similar for the two oil sources, differences in the composition and statistical characteristics of the classes were observed (Table 4).

For MCO-enriched bread, Class 1 comprised seven objects and was mainly characterized by attributes related to crust color, grain-seed odor and flavor, fatty flavor, hardness, chewiness, and moistness. Class 2 contained two objects and was associated with porosity and adhesiveness; whereas Class 3 comprised eleven objects and was characterized by several negative or less desirable sensory descriptors, including flour, alcoholic, buttery, rancid, fatty, and off-odors, as well as sweet, bitter, acidulous, rancid, and off-flavors. A similar three-class structure was observed for BCO-enriched bread, although the statistical characteristics of the classes differed between the two oil sources.

Table 4. Hierarchical bottom-up classification of the bread supplied with Mexican chia oils

Bread supplied with Mexican chia oil = Bread supplied with Brazilian chia oil	Class	1	2	3
	Objects	7	2	11
	Sum of weights	7	2	11
Bread supplied with Mexican chia oil ≠ Bread supplied with Brazilian chia oil	Intra-class variance	15.151≠14.382	4.620≠10.610	5.164≠4.597
	Minimum distance to center of gravity	1.686≠1.657	1.520≠2.303	1.139≠1.097
	Average distance to center of gravity	3.388≠3.384	1.520≠2.303	1.906≠1.856
	Maximum distance to center of gravity	5.437≠4.608	1.520≠2.303	4.943≠4.452
Bread supplied with Mexican chia oil = Bread supplied with Brazilian chia oil		Crust color	Porosity	Flour (odor)
		Grain-seed (odor)	Adhesiveness	Alcoholic (odor)
		Grain-seed (flavor)		Buttery (odor)
		Fatty (flavor)		Rancid (odor)
		Hardness		Fatty (odor)
		Chewiness		Off-odor
		Moistness		Sweet
				Bitter
				Acidulous
				Rancid (flavor)
				Off-flavor

The statistical parameters reported in Table 4 provide additional information on the internal structure of these classes. The intra-class variance indicates the degree of heterogeneity among the observations within each class, whereas the minimum, average, and maximum distances to the center of gravity describe the dispersion of observations around the representative center of each class. For example, the relatively high intra-class variance of Class 1 for both MCO and BCO (15.151 and 14.382, respectively) indicates greater heterogeneity than that observed for Class 2, particularly for MCO (4.620). The relatively small average distance to the center of gravity for MCO Class 2 (1.520) indicates a compact group, whereas the corresponding BCO class showed greater dispersion (2.303). These differences suggest that the sensory responses within the clusters were not identical between the two oil sources.

Overall, Figure 10 and Table 4 provide complementary information: the dendrogram visually illustrates the hierarchical relationships and separation of sensory attributes, while Table 4 quantitatively describes the resulting classes and their internal dispersion. Together with the PCA results (Figure 9), these analyses indicate that the sensory profile of chia oil-enriched bread is structured by distinct groups of attributes and that the type of chia oil may influence the organization and variability of these sensory responses.

4. Conclusions

The present study supports the incorporation of chia oil into bread as a promising strategy for improving its nutritional value while maintaining consumer acceptability. Based on the sensory evaluation, an incorporation level of 7.5% chia oil (flour basis) can be considered the most appropriate among the levels investigated, as it provided high overall acceptability without causing undesirable sensory characteristics. The sensory profile was mainly associated with a mild nutty flavor, rather than a salty note, which is consistent with the descriptors identified during the sensory evaluation. From a technological and nutritional perspective, both Mexican and Brazilian chia oils showed potential for bread enrichment. However, Mexican chia oil appears to be the most suitable candidate when priority is given to a higher ω-3 (α-linolenic acid) contribution and oxidative stability, whereas Brazilian chia oil may also be considered when a comparatively higher saturated fatty acid content is

desirable for formulation or stability purposes. Therefore, considering the combined nutritional, technological, and sensory criteria investigated in this study, Mexican chia oil can be regarded as the more promising option for bread enrichment. Overall, the findings indicate that chia oil, particularly Mexican chia oil, can be effectively incorporated into bread at an appropriate level to develop a more nutritionally valuable product without compromising consumer acceptance. Further studies should assess the storage stability and nutritional quality of the enriched bread during shelf life to confirm its technological feasibility on a larger scale.

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Transparency of Data

The entire data set that supports the results of this study was published in the article itself.

Author Contribution Statement

	M Djeziri	K Alileche	S Bougherara	S Bouguerroumi	N Ouissal	MZ Messaoud Boureghda
Conceptualization	■			■		
Formal analysis	■	■				
Investigation	■	■	■	■		
Methodology	■					
Project administration	■					
Software					■	
Supervision	■		■			■
Validation					■	
Writing – original draft	■			■		
Writing – review and editing	■	■		■		■

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