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Original Research

Enhancing the Quality and Shelf-Life of Beef Burgers With Chia Seed (*Salvia hispanica*) Extract During Refrigerated Storage

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Abstract

Background: The growing demand for clean-label meat products has intensified interest in plant-derived antioxidants as alternatives to synthetic preservatives. **Objective**: This study aimed to evaluate the potential of chia seed (*Salvia hispanica*) extract (CSE) as a natural antioxidant to enhance quality attributes and extend the refrigerated shelf-life of beef burgers stored at 4 °C for 15 days. **Methods**: Five beef burger formulations were prepared: a negative control without antioxidants (T0), a positive control containing a synthetic antioxidant (SET), and three treatments incorporating CSE at 0.5% (T1), 1.0% (T2), and 1.5% (T3). Chia seed extract was characterized using high-performance liquid chromatography (HPLC), which identified gallic acid, caffeic acid, and quercetin as major phenolic constituents. Burgers were assessed for lipid oxidation (TBARS), water-holding capacity (WHC), cooking yield, shrinkage, texture profile attributes, and color stability throughout storage. **Results**: CSE supplementation markedly reduced lipid oxidation, with the T3 formulation showing the lowest TBARS value (0.28 mg MDA/kg) at day 15 compared with the control (1.27 mg MDA/kg) ($p < 0.05$). CSE-treated burgers exhibited improved WHC, reflected in higher cooking yield (91.27%) and reduced shrinkage. Texture profile analysis revealed better retention of hardness, springiness, cohesiveness, and resilience across storage. Additionally, CSE preserved color stability by mitigating reductions in L* and a* values. These enhancements are attributed to the antioxidant activity of chia phenolics, which inhibit lipid peroxidation and protein degradation. **Conclusions**: This study provides the first comprehensive assessment of chia seed extract as a natural antioxidant in beef burgers. CSE—particularly at 1.5%—effectively improved oxidative stability, texture, and overall storage quality, demonstrating its potential as a clean-label alternative to synthetic antioxidants in meat preservation.

Keywords: chia seed extract; *Salvia hispanica*; beef burger; TBARS; lipid oxidation; natural antioxidant; texture profile; shelf-life; refrigerated storage; clean-label meat product

1. Introduction

The growing consumer awareness of food safety, nutrition, and health has driven the global meat industry to reformulate traditional products with functional and natural additives. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), although effective in delaying oxidative spoilage, have raised concerns regarding their potential toxicological effects and long-term health risks [1]. As a result, the focus has shifted toward natural antioxidant sources derived from plants, herbs, and seeds, which are rich in polyphenols and bioactive compounds that align with clean-label demands [2,3]. Lipid oxidation is among the most critical challenges affecting the quality, safety, and shelf-life of meat and meat products. The oxidative degradation of unsaturated fatty acids leads to rancid flavors, discoloration, texture deterioration, and the formation of potentially harmful compounds [4]. Such reactions begin during meat processing and continue throughout storage, especially under aerobic and refrigerated conditions. Consequently, controlling lipid peroxidation is essential to preserve key quality attributes such as color, texture, flavor, and nutritional value [1,5]. Among natural antioxidants investigated, chia seeds (*Salvia hispanica* L.), a member of the Lamiaceae family, have gained significant attention due to their health-promoting properties and multifunctional roles in food applications. Native to Central and South America, chia seeds are a rich source of dietary fiber, polyunsaturated fatty acids—particularly α-linolenic acid (ω-3)—plant proteins, phenolic acids, and flavonoids [6,7]. Their phenolic profile includes compounds such as rosmarinic acid, caffeic acid, quercetin, and chlorogenic acid, which exhibit strong antioxidant and antimicrobial activities [8,9].

Several studies have confirmed the potential of chia (*Salvia hispanica*) to inhibit lipid and protein oxidation in food systems. For example, chia extract significantly reduced thiobarbituric acid reactive substances (TBARS) values in chicken patties during refrigerated storage [10], while chia-derived hydrocolloids enhanced water-holding capacity, minimized cooking losses, and improved texture stability in processed meats [11]. These findings underscore the dual role of chia derivatives as natural antioxidants and functional binders. Beef burgers, widely consumed processed meat products, are particularly susceptible to oxidative spoilage due to their high fat content and oxygen exposure during grinding and shaping. Rising consumer

demand for healthier formulations with reduced synthetic additives has prompted exploration of natural alternatives that preserve quality and sensory attributes [12,13]. In this context, chia seed extract offers a promising strategy for reformulating burgers, providing both antioxidant protection and functional benefits. Despite extensive research on the nutritional and health effects of chia seeds, few studies have systematically investigated the impact of concentrated chia seed extracts on the physicochemical, textural, and oxidative stability of beef burgers during refrigerated storage. Prior work has primarily focused on whole or ground seeds, leaving a research gap in evaluating chia extracts as functional additives for meat preservation [14,15].

Furthermore, chia seed extract exhibits multifunctional properties that can simultaneously influence various quality attributes of meat products. The hydrophilic nature of chia mucilage improves moisture retention, cooking yield, and structural resilience during thermal processing, while its abundance of phenolic antioxidants suppresses lipid oxidation, thereby prolonging shelf-life and preserving desirable color and flavor. These combined effects align with the increasing trend of incorporating plant-based ingredients to enhance both the technological performance and functional quality of processed foods [16,17]. Accordingly, the present study was designed to evaluate the effect of chia seed powder extract on the physicochemical, oxidative, and textural properties of beef burgers stored under refrigerated conditions for 15 days. Key parameters assessed included cooking yield, shrinkage, color stability (L*, a*, b*), texture profile (hardness, springiness, cohesiveness, chewiness, resilience), and lipid oxidation as indicated by TBARS values. The outcomes of this investigation are expected to contribute to the development of functional meat products with enhanced storage stability and consumer acceptability, while supporting the application of chia as a natural preservative and quality enhancer in meat processing.

2. Materials and Methods

2.1 Materials

Fresh ground beef (80% lean, 20% fat) was obtained from a local meat processing facility in Taif, Saudi Arabia. Chia seeds (*Salvia hispanica* L.) were purchased from Al-Dawaa Pharmacy (Taif, Saudi Arabia). Seeds were manually cleaned to remove foreign materials and stored in airtight containers at ambient temperature until further use. All analytical-grade reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA), including thiobarbituric acid (TBA; cat. no. T5500), trichloroacetic acid (TCA; cat. no. T6399), sodium chloride (NaCl; cat. no. S9888), butylated hydroxytoluene (BHT; cat. no. W218405), and malondialdehyde (MDA) standard for TBARS analysis (cat. no. MDA325). Microbiological media were obtained from Merck (Darmstadt, Germany), including plate count agar (PCA; cat. no. 105463), MacConkey agar (cat. no. 105465), and potato dextrose agar (PDA; cat. no. 110130). Distilled water was used for the preparation of all solutions and for equipment cleaning.

2.2 Preparation of Chia Seed Powder Extract

Chia seeds (*Salvia hispanica*) were dried in a hot-air oven at 40 ± 2 °C for 12 h to reduce moisture content. The dried seeds were ground into a fine powder using a laboratory grinder (Kenwood Multi Mill, Model BL335, cat. no. N/A, Kenwood Appliances, Taif, Saudi Arabia) and sieved through a 60-mesh screen to ensure uniform particle size. For extraction, 50 g of chia seed powder was mixed with 500 mL of distilled water (1:10 w/v) and stirred at 45 °C for 2 h using a magnetic stirrer (MS300HS, cat. no. 88880013, Thermo Fisher Scientific, Waltham, MA, USA). The extraction procedure was selected based on food-processing feasibility and previously reported aqueous extraction methods for plant phenolics to ensure practical relevance for potential industrial application. The mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper (cat. no. 1001-110, Cytiva/Whatman, Marlborough, MA, USA). The filtrate was freeze-dried using a bench-top freeze dryer (FreeZone 2.5 L, cat. no. 7670520, Labconco Corporation, Kansas City, MO, USA) to obtain chia seed extract powder, which was stored in airtight containers at 4 °C until further use in burger formulation.

2.3 Determination of the Chemical Composition of Chia Seed Powder

The proximate and bioactive composition of chia seed (*Salvia hispanica* L.) powder was analyzed prior to extract preparation using Association of Official Analytical Collaboration (AOAC) methods [18] and complementary instrumental techniques. All analyses were performed in triplicate, and results were expressed as mean ± standard deviation.

2.3.1 Proximate Composition

The chemical composition of chia seed (*Salvia hispanica*) powder was analyzed before extract preparation to determine its nutritional profile. The analyses were carried out according to AOAC (2005) official methods [18].

- Moisture content was measured by drying approximately 5 g of ground chia seed in a hot air oven at 105 °C until constant weight.
- Crude protein was determined using the Kjeldahl method, and the nitrogen content was converted to protein using a factor of 6.25.
- Crude fat was extracted using petroleum ether in a Soxhlet apparatus for 6 hours.
- Ash content was determined by incineration in a muffle furnace at 550 °C for 6 hours.
- Total carbohydrates were calculated by difference: 100 – (% moisture + % protein + % fat + % ash)

Additionally, the dietary fiber content was analyzed using the enzymatic-gravimetric method [19], and results were expressed as g/100 g dry weight. All analyses were performed in triplicate, and results were reported as mean ± standard deviation.

2.3.2 Fatty Acid Profile

Fatty acid composition of chia seed oil was determined using gas chromatography (GC-FID). Lipids were extracted by cold maceration with hexane (cat. no. 34859, Sigma-Aldrich, St. Louis, MO, USA), and the extracted oil was converted into fatty acid methyl esters (FAMEs) by transesterification using methanolic potassium hydroxide (cat. no. 60325, Sigma-Aldrich, St. Louis, MO, USA). FAMEs were separated and quantified using a gas chromatograph (Model GC-7890A, cat. no. N/A, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector and a capillary column (HP-88, 100 m × 0.25 mm × 0.2 µm, cat. no. 112-8802, Agilent Technologies, Santa Clara, CA, USA). Fatty acids were identified by comparing retention times with known standards (Supelco 37 Component FAME Mix, cat. no. 47885-U, Sigma-Aldrich, St. Louis, MO, USA) and expressed as a percentage of total identified fatty acids.

2.3.3 Mineral Content

Mineral elements such as calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), and zinc (Zn) were determined using atomic absorption spectrophotometry (AAS; PerkinElmer AAnalyst 400, Waltham, MA, USA) after dry ashing of samples at 550 °C followed by dissolution in nitric acid.

2.3.4 Total Phenolic and Flavonoid Content

- Total phenolic content (TPC) was measured by the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g dry weight.
- Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE)/g dry weight.

2.3.5 Phenolic Compound Profiling by HPLC

Individual phenolic compounds were identified and quantified using high-performance liquid chromatography with a diode-array detector (HPLC-DAD). Methanolic extracts of chia seed powder were filtered through a 0.45 µm syringe filter (cat. no. SLHV033RS, Millipore, Burlington, MA, USA) and injected into an HPLC system (Shimadzu LC-20AT, cat. no. N/A, Shimadzu Corporation, Kyoto, Japan) equipped with a reverse-phase C18 column (250 mm × 4.6 mm, 5 µm, cat. no. 99101, Phenomenex, Torrance, CA, USA). The mobile phase consisted of solvent A (0.1% formic acid in water, cat. no. 94318, Sigma-Aldrich, St. Louis, MO, USA) and solvent B (acetonitrile, cat. no. 34967, Sigma-Aldrich, St. Louis, MO, USA), applied using a gradient elution over 60 minutes. Detection was performed at 280 nm, and phenolic compounds were identified by comparison with authentic standards (gallic acid, cat. no. G7384; caffeic acid, cat. no. C0625; quercetin, cat. no. Q4951; all from Sigma-Aldrich, St. Louis, MO, USA).

2.3.5.1 HPLC Analysis of Phenolic Compounds in Chia Seed Extract.

High-performance liquid chromatography (HPLC) was employed to identify and quantify individual phenolic compounds present in chia seed (*Salvia hispanica*) extract. The analysis was carried out using a reverse-phase HPLC system (Shimadzu LC-20AT, Japan) equipped with a diode array detector (DAD) and an autosampler. Chia seed powder extract was dissolved in methanol (HPLC grade), filtered through a 0.45 µm PTFE syringe filter, and stored at 4 °C until injection.

Chromatographic Conditions:

- Column: C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size)
- Mobile Phase:
  - Solvent A: 0.1% formic acid in water
  - Solvent B: Acetonitrile
- Gradient Program:
  - 0–10 min: 5% B
  - 10–30 min: 5–40% B
  - 30–40 min: 40–60% B
  - 40–45 min: 60–80% B
  - 45–50 min: return to 5% B
- Flow Rate: 1.0 mL/min
- Injection Volume: 20 µL
- Detection Wavelengths: 280 nm and 320 nm, depending on compound class
- Run Time: 50 minutes

Phenolic compounds were identified by comparing retention times and UV spectra with those of authentic standards, including gallic acid, caffeic acid, chlorogenic acid, ferulic acid, rutin, quercetin, and others. Quantification was based on external calibration curves for each standard. Results were expressed as mg of compound per gram of dry extract (mg/g). All measurements were performed in triplicate and presented as mean ± standard deviation.

2.3.5.2 Quantification of Total Phenolic Content and Antioxidant Activity.

(a) Total Phenolic Content (TPC)

The total phenolic content (TPC) of chia seed extract was determined using the Folin–Ciocalteu method. Briefly, 0.5 mL of extract solution (1 mg/mL) was mixed with 2.5 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water; cat. no. F9252, Sigma-Aldrich, St. Louis, MO, USA) and incubated for 5 min at room temperature. Subsequently, 2 mL of 7.5% sodium carbonate solution (cat. no. S7795, Sigma-Aldrich, St. Louis, MO, USA) was added. After incubation in the dark for 30 min, absorbance was

measured at 765 nm using a UV–visible spectrum spectrophotometer (UV-1800, cat. no. N/A, Shimadzu Corporation, Kyoto, Japan). Gallic acid (cat. no. G7384, Sigma-Aldrich, St. Louis, MO, USA) was used as the calibration standard, and results were expressed as mg gallic acid equivalents (GAE) per g of extract.

(b) Antioxidant Activity (DPPH Assay)

The radical scavenging activity of chia seed extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. A 0.1 mM DPPH solution was prepared in methanol, and 1 mL of extract (1 mg/mL) was added to 2 mL of DPPH solution. The mixture was incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm. The percentage inhibition of DPPH radicals was calculated relative to a blank. Ascorbic acid served as a positive control.

2.4 Quantification of Vitamins in Chia Seed Powder

The vitamin content of chia seed (*Salvia hispanica*) powder was quantified using standardized methods to assess its nutritional contribution as a functional ingredient.

2.4.1 Water-Soluble Vitamins

Water-soluble vitamins, including thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pyridoxine (vitamin B6), and ascorbic acid (vitamin C), were extracted using acid and enzymatic hydrolysis methods. Approximately 2 g of chia seed powder was homogenized with 25 mL of 0.1 M hydrochloric acid (HCl; cat. no. 258148, Sigma-Aldrich, St. Louis, MO, USA) and incubated in a water bath (Model WB-22, cat. no. N/A, Memmert, Schwabach, Germany) at 100 °C for 30 min, followed by enzymatic digestion with takadiastase (cat. no. D0256, Sigma-Aldrich, St. Louis, MO, USA) at 37 °C for 3 h. The extract was filtered through Whatman No. 1 filter paper (cat. no. 1001-110, Cytiva/Whatman, Marlborough, MA, USA) and analyzed by high-performance liquid chromatography with a UV–visible spectrum (HPLC-UV; Shimadzu LC-20AT, cat. no. N/A, Shimadzu Corporation, Kyoto, Japan) using a reverse-phase C18 column (250 mm × 4.6 mm, 5 µm, cat. no. 99101, Phenomenex, Torrance, CA, USA). Mobile phases and detection wavelengths were optimized for each vitamin, and results were expressed as mg/100 g dry weight.

2.4.2 Fat-Soluble Vitamins

Fat-soluble vitamins, including α-tocopherol (vitamin E), vitamin A (retinol), and vitamin K, were extracted using ethanol/hexane solvent extraction. Briefly, 2 g of powdered sample was mixed with ethanol and hexane (4:3 v/v), vortexed, and centrifuged at 4000 rpm for 10 minutes. The supernatant was evaporated under nitrogen, reconstituted in methanol, and filtered through a 0.22 µm syringe filter before injection into the HPLC system. Detection was performed at 292 nm for α-tocopherol and 325 nm for vitamin A. Concentrations were determined using external calibration curves and expressed as mg/100 g dry weight. All vitamin determinations were performed in triplicate, and results were reported as mean ± standard deviation.

2.5 Determination of Radical Scavenging Activity ($ABTS^+$, DPPH, and FRAP)

The antioxidant capacity of chia seed (*Salvia hispanica*) powder extract was evaluated using three complementary *in vitro* assays: $ABTS^+$ radical scavenging activity, DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP). All measurements were performed in triplicate, and results were expressed as µmol Trolox equivalents (TE) per gram of extract.

2.5.1 $ABTS^+$ Radical Scavenging Activity

The $ABTS^+$ assay was conducted with slight modifications using the method described previously [19] . The $ABTS^+$ radical cation was produced by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 hours. Before use, the $ABTS^+$ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 100 µL of chia extract (1 mg/mL) was added to 1 mL of diluted $ABTS^+$ solution and incubated for 6 minutes. Absorbance was measured at 734 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800,Shimadzu Corporation, Kyoto, Kyoto Prefecture, Japan). The percentage inhibition was calculated and compared against a Trolox standard curve.

2.5.2 DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed as described by [20]. A 0.1 mM DPPH solution was prepared in methanol. Then, 1 mL of chia extract was mixed with 2 mL of the DPPH solution and incubated in the dark for 30 minutes at room temperature. Absorbance was recorded at 517 nm. The percentage inhibition of DPPH radicals was calculated using the formula:

DPPH inhibitory (%) = (Abs. C – Abs. S)/(Abs. C) × 100

Where Abs. C is the absorbance of the control and Abs. S is the absorbance of the sample.

2.5.3 Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was conducted according to [21] Benzie and Strain (1996). The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 (v/v/v). Then, 100 µL of chia extract was added to 3 mL of the FRAP reagent and incubated at 37 °C for 30 minutes. Absorbance was measured at 593 nm. A standard curve was constructed using Trolox, and results were expressed as µmol Trolox equivalents (TE)/g extract.

2.6 Determination of Tannic Acid Contents (TTC)

The tannic acid content of chia seed (*Salvia hispanica*) powder extract was determined spectrophotometrically using the Folin–Denis method, as described by [18,19] with minor modifications. An aliquot of 1 mL of chia seed extract (1 mg/mL) was mixed with 1 mL of Folin–Denis reagent (cat. no. F9252, Sigma-Aldrich, St. Louis, MO, USA), followed by 2 mL of saturated sodium carbonate solution (cat. no. S7795, Sigma-Aldrich, St. Louis, MO, USA). The mixture was diluted to 10 mL with distilled water and incubated at room temperature for 30 min in the dark. The absorbance of the resulting blue color was measured at 760 nm using a UV–Vis spectrophotometer (UV-1800, cat. no. N/A, Shimadzu Corporation, Kyoto, Japan). Tannic acid (cat. no. T0377, Sigma-Aldrich, St. Louis, MO, USA) was used as the standard, and a calibration curve was prepared using concentrations ranging from 0 to 100 µg/mL. The tannic acid content was calculated from the standard curve and expressed as milligrams of tannic acid equivalents per gram of extract (mg TAE/g extract).

2.7 Application of Chia Seed Extract in Burger Formulation

2.7.1 Burger Preparation

Fresh ground beef (80% lean, 20% fat) was used for burger preparation. The meat was sourced from a local processing facility in Taif, Saudi Arabia, and transported under chilled conditions (4 °C) to the laboratory. Visible fat and connective tissue were manually trimmed upon arrival. The burger formulation consisted of 90% ground beef, 5% cold water, 2% corn starch (cat. no. 33292, Sigma-Aldrich, St. Louis, MO, USA), 1.5% salt (cat. no. S9888, Sigma-Aldrich, St. Louis, MO, USA), 0.5% mixed spices (black pepper, coriander, garlic powder; sourced from a local supplier, Taif, Saudi Arabia), and 0.1% sodium tripolyphosphate (STPP; cat. no. 22103, Sigma-Aldrich, St. Louis, MO, USA). All ingredients were thoroughly mixed in a food mixer (Kenwood Chef XL, Model KVL4100S, cat. no. N/A, Kenwood Appliances, Havant, UK) for 5–7 minutes to form a uniform emulsion. The mixture was then shaped into patties of approximately 80 g and 1.5 cm thickness using a stainless steel mold (cat. no. N/A, local supplier, Taif, Saudi Arabia). Each patty was individually wrapped in polyethylene film (cat. no. 1098-100, Sigma-Aldrich, St. Louis, MO, USA) and stored at 4 °C prior to chia extract incorporation (Table 1).

2.7.2 Treatment Groups with Chia Seed Extract

The prepared burger patties were divided into four groups as follows:

- SET = Positive control with synthetic antioxidant (sodium tripolyphosphate).
- T0 = Negative control with no antioxidant.
- T1: 0.5% chia seed extract (w/w)
- T2: 1.0% chia seed extract (w/w)
- T3: 1.5% chia seed extract (w/w)

The chia seed extract was homogenized into the meat mixture during mixing, before shaping into patties. All treated and control patties were packed in low-density polyethylene (LDPE) bags, labeled, and stored at 4 ± 1 °C. Sampling was carried out on days 0, 3, 6, 9, and 12 to evaluate chemical, microbiological, and sensory changes during refrigerated storage.

2.8 Analytical Evaluation of Burger Samples During Refrigerated Storage

The physicochemical, microbiological, and sensory properties of burger samples treated with chia seed extract were evaluated at regular intervals (0, 3, 6, 9, and 12 days) during refrigerated storage at 4 ± 1 °C, according to methods described below.

2.8.1 pH Measurement

Approximately 10 g of each burger sample was homogenized with 90 mL of distilled water using a laboratory homogenizer (Ultra-Turrax T25, cat. no. 00700354, IKA, Staufen, Germany). The pH of the homogenate was measured using a digital pH meter (Model MP220, cat. no. 30200222, Mettler-Toledo, Greifensee, Switzerland) calibrated with standard buffer solutions at pH 4.0 and 7.0.

2.8.2 Thiobarbituric Acid Reactive Substances (TBARS)

Lipid oxidation was evaluated by the TBARS assay as described by [21], with modifications. A 10 g sample was blended with 50 mL of distilled water and 2.5 mL of 4% HCl. The mixture was distilled, and 5 mL of the distillate was reacted with 5 mL of 0.02 M thiobarbituric acid in a boiling water bath for 35 minutes. Absorbance was measured at 532 nm using a UV-Vis spectrophotometer. TBARS values were expressed as mg malondialdehyde (MDA)/kg of sample.

2.8.3 Sensory Evaluation

Sensory evaluation was conducted to determine the effects of chia seed extract (CSE) on the organoleptic quality of beef burger patties during refrigerated storage.

2.8.4 Panel Composition and Training

A semi-trained panel consisting of 15 members (aged 22–45 years), including postgraduate students and staff from the Department of Food Science, Taif University, participated in the study. All panelists were regular consumers of meat products and received orientation on the evaluation criteria and use of the hedonic scale prior to testing sessions.

2.8.5 Sample Preparation and Presentation

Burger patties were pan-fried to an internal temperature of 72 °C using a calibrated digital thermometer, cooled to approximately 40 °C, and cut into uniform pieces (~25 g). Samples were served warm on white plates coded with

Table 1. Formula for burger ingredients with chia seed extract (g/100 g).

Ingredient	SET (Positive Control)	T0 (Negative Control)	T1 (0.5% CSE)	T2 (1.0% CSE)	T3 (1.5% CSE)
Ground beef	90.00	90.00	89.50	89.00	88.50
Cold water	5.00	5.00	5.00	5.00	5.00
Corn starch	2.00	2.00	2.00	2.00	2.00
Salt	1.50	1.50	1.50	1.50	1.50
Mixed spices*	0.50	0.50	0.50	0.50	0.50
Sodium tripolyphosphate (optional)	0.10	–	0.10	0.10	0.10
Chia seed extract powder	–	–	0.50	1.00	1.50
Total	99.10	99.00	99.10	99.10	99.10

* Mixed spices include black pepper, coriander, and garlic powder in equal proportions.

• SET = Positive control with synthetic antioxidant (sodium tripolyphosphate).

• T0 = Negative control with no antioxidant.

• T1–T3 = Experimental treatments with increasing levels of chia seed extract (CSE).

random three-digit numbers to ensure unbiased evaluation. The serving order was randomized to minimize positional effects. Panelists were provided with water and unsalted crackers to cleanse the palate between samples.

2.8.6 Evaluation Attributes and Scale

○ Sensory parameters included:

- Appearance
- Color
- Odor
- Texture
- Juiciness
- Flavor
- Overall acceptability

Panelists rated each attribute on a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Mean scores and standard deviations were calculated.

2.8.7 Cooking Yield and Shrinkage

Cooking yield was determined by weighing the beef burgers before and after cooking and expressed as a percentage:

$$\text{Cooking Yield (\%)} = \frac{\text{Weight after cooking (g)}}{\text{Weight before cooking (g)}} \times 100$$

- Shrinkage was calculated as the percentage reduction in diameter and/or thickness after cooking.
- The procedures were adapted from [20,21] to ensure reproducibility and comparability.

2.9 Statistical Analysis

All experimental data were analyzed using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). Results are expressed as mean ± standard deviation (SD) of three independent replicates (n = 3). One-way analysis of variance (ANOVA) was performed to evaluate differences among treatments and storage periods. When significant effects ($p < 0.05$) were detected, Duncan's multiple range test was applied for post hoc pairwise comparisons. Sensory evaluation data were analyzed using the non-parametric Kruskal–Wallis test, with the Mann–Whitney U test conducted for pairwise comparisons where appropriate. Graphs and figures were generated using GraphPad Prism (version 9; GraphPad Software Inc., San Diego, CA, USA). A probability level of $p < 0.05$ was considered statistically significant for all tests.

3. Results and Discussion

3.1 Fundamental Extraction Tests of Chia Seed (Salvia hispanica) Powder

The proximate composition of chia seed powder demonstrates its exceptional nutritional value, supporting its use in functional food systems (Table 2). Moisture content was low (5.84 ± 0.12%), consistent with effective drying protocols, which enhance shelf-life and protect against microbial and enzymatic deterioration. Protein content was 20.31 ± 0.24%, within the reported range for chia (16–23%), and rich in essential amino acids such as arginine and leucine, contributing to cardiovascular health, tissue repair, and muscle metabolism [20,22]. Lipid content was high (31.66 ± 0.45%), predominantly polyunsaturated fatty acids (PUFAs), with α-linolenic acid (ALA, ω-3) comprising 62.3% of total fatty acids, providing a plant-based alternative for omega-3 fortification [23,24]. Dietary fiber (23.40 ± 0.33%) included soluble (7.12 ± 0.21%) and insoluble (16.28 ± 0.26%) fractions, supporting gastrointestinal health, glycemic regulation, and satiety [24,25]. Essential minerals were abundant, particularly calcium (590.4 ± 10.6 mg/100 g), magnesium (335.7 ± 8.4 mg/100 g), potassium (722.1 ± 12.3 mg/100 g), and phosphorus (744.5 ± 9.8 mg/100 g), promoting bone, neuromuscular, and metabolic functions [25].

Chia seeds were also rich in bioactive compounds: total phenolic content (TPC) was 38.72 ± 1.34 mg GAE/g, flavonoids 22.18 ± 0.97 mg QE/g, and tannins 12.94 ± 0.88

Table 2. Chemical composition, antioxidant activity, mineral and vitamin content of chia seed powder (*Salvia hispanica* L.).

Parameter	Value (Mean ± SD)
Proximate composition (g/100 g)	
Moisture	5.84 ± 0.12
Crude protein	20.31 ± 0.24
Crude fat	31.66 ± 0.45
Ash	4.56 ± 0.15
Total carbohydrates*	37.63 ± 0.28
Crude fiber	23.40 ± 0.33
Soluble dietary fiber	7.12 ± 0.21
Insoluble dietary fiber	16.28 ± 0.26
Fatty acid composition (% of total fat)	
α-Linolenic acid (C18:3, ω-3)	62.3
Linoleic acid (C18:2, ω-6)	19.5
Oleic acid (C18:1)	10.7
Palmitic acid (C16:0)	4.2
Stearic acid (C18:0)	2.1
Bioactive compounds	
Total phenolic content (mg GAE/g)	38.72 ± 1.34
Total flavonoid content (mg QE/g)	22.18 ± 0.97
Tannic acid content (mg TAE/g)	12.94 ± 0.88
Antioxidant activity	
DPPH inhibition (%)	75.61 ± 1.22
$ABTS^+$ inhibition (%)	81.47 ± 1.08
FRAP (µmol Trolox equivalents/g)	62.53 ± 1.17
Phenolic compounds by HPLC (mg/g)	
Gallic acid	8.37 ± 0.24
Caffeic acid	4.15 ± 0.17
Ferulic acid	3.89 ± 0.21
Chlorogenic acid	2.91 ± 0.15
Rutin	2.46 ± 0.12
Quercetin	1.74 ± 0.11
Mineral content (mg/100 g)	
Calcium (Ca)	590.4 ± 10.6
Magnesium (Mg)	335.7 ± 8.4
Potassium (K)	722.1 ± 12.3
Phosphorus (P)	744.5 ± 9.8
Sodium (Na)	8.3 ± 0.5
Iron (Fe)	7.62 ± 0.41
Zinc (Zn)	4.27 ± 0.19
Copper (Cu)	0.89 ± 0.04
Vitamin content	
Vitamin C (mg/100 g)	7.28 ± 0.18
Thiamine – B1 (mg/100 g)	0.82 ± 0.05
Riboflavin – B2 (mg/100 g)	0.27 ± 0.02
Niacin – B3 (mg/100 g)	5.63 ± 0.14
Pyridoxine – B6 (mg/100 g)	0.48 ± 0.03
Vitamin A (µg/100 g)	142.5 ± 4.2
Vitamin E – α-Tocopherol (mg/100 g)	12.84 ± 0.37
Vitamin K (µg/100 g)	3.51 ± 0.19

*Carbohydrates calculated by difference: 100 – (protein + fat + ash + moisture).

All values are expressed as mean ± standard deviation (mg/g dry extract), n = 3.

mg TAE/g, conferring strong antioxidant potential [26,27]. Antioxidant assays confirmed this activity: DPPH radical scavenging was 75.61 ± 1.22%, $ABTS^+$ inhibition 81.47 ± 1.08%, and ferric reducing antioxidant power (FRAP) 62.53 ± 1.17 µmol TE/g. HPLC analysis revealed abundant phenolics such as gallic acid (8.37 mg/g), caffeic acid (4.15 mg/g), and ferulic acid (3.89 mg/g), compounds known for anti-inflammatory, antimicrobial, and anti-aging effects [27,28].

The high bioactivity of chia seed extract, demonstrated through multiple assays and a diverse phenolic profile, highlights its dual functionality: it can retard lipid oxidation and enhance oxidative stability in lipid-rich foods, including ground beef, while contributing nutritional and health-promoting benefits. Incorporation of chia extract into meat products thus represents a promising natural alternative to synthetic antioxidants, aligning with consumer demand for clean-label, functional ingredients [29].

Regarding vitamin composition, chia seed powder exhibited a diverse profile, providing both water- and fat-soluble vitamins. Vitamin C content was 7.28 ± 0.18 mg/100 g, supporting its contribution to immune defense and collagen synthesis. Among B-complex vitamins, niacin (5.63 mg/100 g) was particularly abundant, complementing energy metabolism and neurological function. Additionally, the seeds were a rich source of vitamin E (12.84 ± 0.37 mg/100 g), a lipid-soluble antioxidant that protects cellular membranes and unsaturated fatty acids from peroxidation. The coexistence of vitamin C and E suggests synergistic antioxidant interactions, further enhancing chia's functional role in stabilizing food systems. The compositional richness of chia seed powder—characterized by its high omega-3 fatty acids, substantial dietary fiber, mineral density, and abundant polyphenolic compounds—confirms its value as a multifunctional ingredient. Its strong antioxidant activity, combined with essential micronutrients, makes it an ideal candidate for incorporation into health-oriented food formulations. Potential applications span bakery, dairy, and particularly meat products, where chia can enhance shelf-life, improve nutritional labeling, and increase consumer appeal. Importantly, its ability to replace synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) aligns with clean-label trends and regulatory shifts toward natural preservation strategies.

3.2 High-Performance Liquid Chromatography (HPLC) Analysis of Phenolic Compounds in Chia Seed Extract

High-performance liquid chromatography (HPLC) was employed to identify and quantify the major phenolic compounds present in chia seed (*Salvia hispanica* L.) extract, providing insights into its antioxidant potential and suitability as a functional food ingredient (Fig. 1; Tables 3,4). The analysis was performed using a Shimadzu LC-20AT HPLC system equipped with a diode array detector (DAD; Shimadzu Corp., Kyoto, Japan), allowing pre-

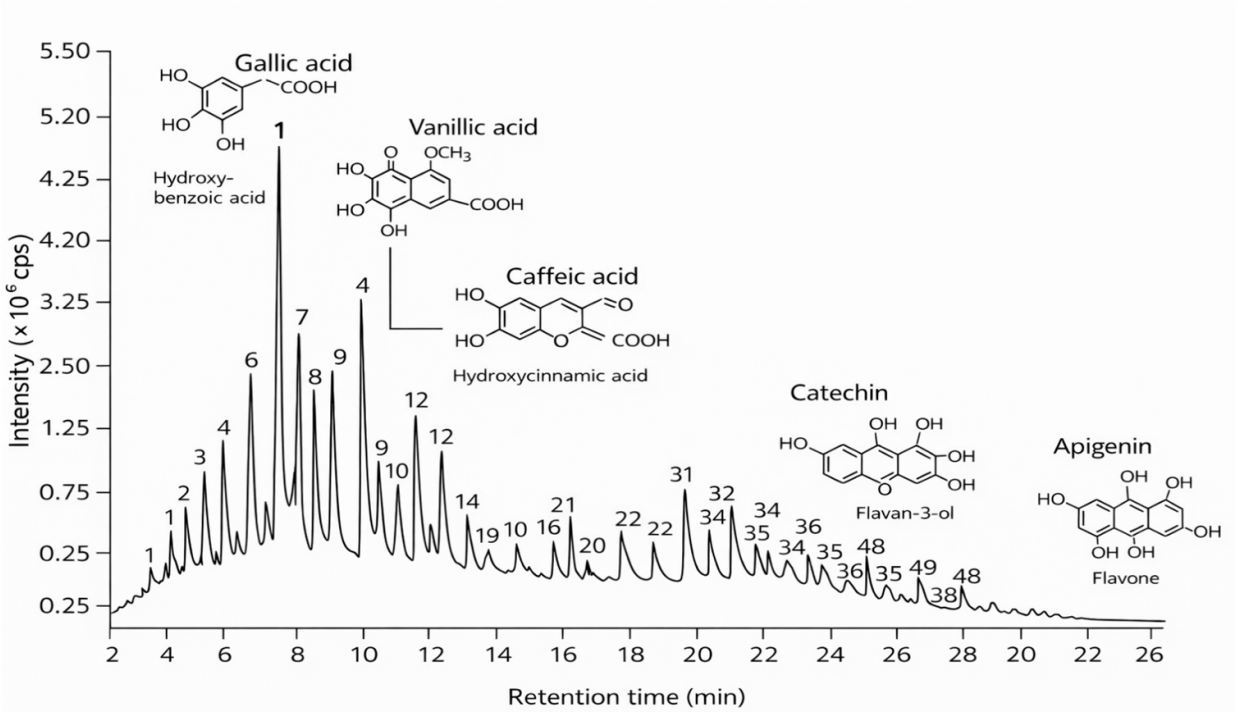

Fig. 1. Base peak chromatogram of chia seed (*Salvia hispanica* L.) extract obtained by high-performance liquid chromatography with diode array detection (HPLC-DAD). The chromatogram exhibits a complex phenolic profile comprising 48 distinct peaks eluting between 2.1 and 26.0 minutes. Early-eluting peaks (2–10 min), including gallic acid, protocatechuic acid, and p-hydroxybenzoic acid, correspond to low-molecular-weight, highly polar hydroxybenzoic acids, while later peaks represent less polar phenolics, including ferulic acid, rutin, and quercetin. The most intense peaks were observed between 22 and 24 minutes, corresponding to high-intensity flavonoid compounds (see Table 4). Peaks 13 and 15 represent to minor early-eluting traces of rutin and quercetin; however, their major quantitative signals were recorded at 32.48 and 39.72 min, respectively. UV absorbance was monitored at 280 nm and 320 nm for optimal detection of phenolic acids and flavonoids. The chromatographic pattern, consistent with Table 3, reflects the diverse composition of hydroxybenzoic, hydroxycinnamic, and flavonol derivatives, which collectively contribute to the potent antioxidant potential of chia seed extract.

cise profiling of phenolic constituents based on retention time and UV-visible spectra. The chia extract was dissolved in HPLC-grade methanol, filtered through a 0.45 µm PTFE syringe filter, and stored at 4 °C until analysis. Separation was achieved on a C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size), providing optimal resolution for plant-derived phenolics. Compound identification was based on comparison with authentic standards, including gallic acid, caffeic acid, chlorogenic acid, ferulic acid, rutin, and quercetin. Quantification was performed using external standard calibration curves, and all determinations were carried out in triplicate. Results were expressed as mg/g dry extract (mean ± standard deviation). The resulting chromatogram (Fig. 1) exhibited a complex phytochemical profile with 48 distinct peaks. Early-eluting peaks (e.g., peaks 4, 6, and 10) corresponded to smaller, more polar phenolic acids, whereas the most intense peaks were observed between 22 and 24 minutes, representing chlorogenic acid, ferulic acid, quercetin, and related flavonoids. This elution pattern aligns well with previously reported chia seed phenolic profiles [29,30].

Quantitative analysis (Table 4) identified gallic acid (8.37 mg/g), caffeic acid (4.15 mg/g), and ferulic acid (3.89 mg/g) as the predominant phenolic compounds, accompanied by appreciable levels of chlorogenic acid (2.91 mg/g), rutin (2.46 mg/g), and quercetin (1.74 mg/g). Overall, hydroxybenzoic acids (e.g., gallic acid) and flavonols (e.g., quercetin, rutin) were the most abundant phenolic classes. These compounds are recognized for their potent antioxidant, anti-inflammatory, and antimicrobial properties. From a functional perspective, gallic and caffeic acids are known inhibitors of lipid peroxidation, while flavonols such as quercetin and rutin contribute cardiovascular, neuroprotective, and anti-carcinogenic effects. The phenolic composition observed in chia seed extract, therefore, underscores its potential as a natural antioxidant source capable of enhancing lipid stability, delaying rancidity, and improving the nutritional and functional quality of processed meat products. These findings highlight chia seed extract's promise as a clean-label alternative to synthetic antioxidants, supporting current trends toward natural preservation strategies in the food industry. High-performance liquid

Table 3. Tentative identification of phenolic compounds in chia seed (*Salvia hispanica* L.) extract based on HPLC retention times.

Peak No.	Retention time (min)	Tentative compound	Compound class/Remark
1	2.1	Gallic acid	Hydroxybenzoic acid
2	2.5	Protocatechuic acid	Hydroxybenzoic acid
3	3.1	p-Hydroxybenzoic acid	Hydroxybenzoic acid
4	3.8	Vanillic acid	Hydroxybenzoic acid
5	4.6	Caffeic acid	Hydroxycinnamic acid
6	5.1	Chlorogenic acid	Hydroxycinnamic acid
7	6.3	Syringic acid	Hydroxybenzoic acid
8	7.0	Ferulic acid	Hydroxycinnamic acid
9	8.1	Sinapic acid	Hydroxycinnamic acid
10	9.2	p-Coumaric acid	Hydroxycinnamic acid
11	10.4	Catechin	Flavan-3-ol
12	11.1	Epicatechin	Flavan-3-ol
13	12.2	Rutin (trace)	Flavonol glycoside (early eluting trace)
14	13.5	Myricetin	Flavonol
15	14.6	Quercetin (trace)	Flavonol (early eluting trace)
16	15.3	Kaempferol	Flavonol
17	16.0	Naringenin	Flavanone
18	17.1	Apigenin	Flavone
19	18.3	Rosmarinic acid	Polyphenolic ester
20	19.0	Luteolin	Flavone
21	19.5	Caffeic acid derivative	Hydroxycinnamic acid conjugate
22	19.9	Ferulic acid derivative	Hydroxycinnamic acid conjugate
23	20.5	Rutin derivative	Flavonol glycoside conjugate
24	21.0	Quercetin-3-O-glucoside	Flavonol glycoside
25	21.3	Kaempferol derivative	Flavonol glycoside
26	21.6	Luteolin-7-O-glucoside	Flavone glycoside
27	21.9	Apigenin-7-O-glucoside	Flavone glycoside
28	22.3	Unidentified phenolic compound	Possible flavonoid derivative
29	22.6	Unidentified compound	Co-eluting phenolic glycoside
30	22.8	Chlorogenic acid derivative	Hydroxycinnamic conjugate
31	23.0	Rosmarinic acid derivative	Polyphenolic conjugate
32	23.4	Rutin (major)	Flavonol glycoside (confirmed in Table 4, 32.48 min)
33	23.6	Quercetin (major)	Flavonol (confirmed in Table 4, 39.72 min)
34	23.8	Myricetin derivative	Flavonol conjugate
35	24.0	Kaempferol derivative	Flavonol conjugate
36	24.2	Rosmarinic acid isomer	Phenolic ester derivative
37	24.4	Luteolin derivative	Flavone conjugate
38	24.6	Apigenin derivative	Flavone conjugate
39	24.8	Chlorogenic acid isomer	Hydroxycinnamic acid isomer
40	25.0	Rutin-like compound	Flavonol glycoside derivative
41	25.1	Quercetin-like compound	Flavonol derivative
42	25.3	Rosmarinic acid conjugate	Polyphenolic compound
43	25.5	Unidentified compound	Tentative phenolic conjugate
44	25.6	Unidentified compound	Tentative flavonoid derivative
45	25.7	Unidentified compound	Tentative phenolic polymer
46	25.8	Unidentified compound	Tentative phenolic polymer
47	25.9	Unidentified compound	Tentative phenolic polymer
48	26.0	Unidentified compound	Tentative phenolic polymer

Notes:

- Peak identification is tentative, based on comparison with authentic standards and previously published chromatographic data.
- UV absorbance was monitored at 280 and 320 nm, wavelengths typical for phenolic acids and flavonoids.
- Peaks 13 and 15 represent early-eluting traces of rutin and quercetin, while their major peaks appeared later at 32.48 min and 39.72 min, respectively, and were used for quantitative analysis (Table 4).
- Peaks 21–48, observed between approximately 19–26 min, likely correspond to flavonoid glycosides, chlorogenic and rosmarinic acid derivatives, and unidentified polymeric phenolics that contribute to the overall antioxidant complexity of the extract.
- Further LC–MS/MS analysis is required for precise structural confirmation of these late-eluting compounds.

Table 4. Phenolic compounds identified in chia seed (*Salvia hispanica* L.) extract by HPLC.

Phenolic compound	Class	Retention time (min)	Concentration (mg/g DW)
Gallic acid	Hydroxybenzoic acid	6.27	8.37 ± 0.24
Caffeic acid	Hydroxycinnamic acid	11.92	4.15 ± 0.17
Ferulic acid	Hydroxycinnamic acid	21.34	3.89 ± 0.21
Chlorogenic acid	Hydroxycinnamic acid	18.76	2.91 ± 0.15
Rutin	Flavonol glycoside	32.48	2.46 ± 0.12
Quercetin	Flavonol	39.72	1.74 ± 0.11

Notes:

- All values are expressed as mean ± standard deviation (mg/g dry extract), n = 3.
- Quantification was performed using external standard calibration curves for each compound.
- The major peaks of rutin (32.48 min) and quercetin (39.72 min) were used for quantification; minor early-eluting traces observed between 12–15 min were excluded from quantitative analysis (see Table 3 and Fig. 1).
- Among the quantified compounds, gallic acid showed the highest concentration, followed by caffeic acid and ferulic acid, indicating that hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic classes in chia seed extract.

chromatography with diode array detection (HPLC-DAD) was employed to characterize and quantify the major phenolic constituents of chia seed (*Salvia hispanica* L.) extract, providing insight into its antioxidant potential and suitability as a natural functional ingredient. The chromatographic analysis revealed a rich and complex phenolic profile, as illustrated in Fig. 1, comprising a total of 48 distinct peaks across the retention range of 2.1–26.0 minutes. The identified compounds were tentatively assigned based on retention times, UV spectral characteristics, and comparison with authentic standards and previously reported chromatographic data (Table 3).

Early-eluting peaks, particularly those appearing between 2 and 10 minutes, corresponded predominantly to low-molecular-weight, highly polar phenolic acids. These included gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, and ferulic acid, among others. Such compounds belong primarily to the hydroxybenzoic and hydroxycinnamic acid classes, which are well known for their strong antioxidant and radical-scavenging activity. Mid-to-late eluting peaks, detected between 15 and 26 minutes, represented larger, less polar compounds such as flavonols and flavones, including myricetin, quercetin, kaempferol, luteolin, and apigenin. The presence of both phenolic acids and flavonoids underscores the chemical diversity of chia seed extract, reflecting its multifunctional bioactivity potential.

Notably, peaks 13 and 15 corresponded to early-eluting traces of rutin and quercetin; however, their major quantitative signals were observed at 32.48 min and 39.72 min, respectively. These later peaks were used for quantification and are presented in Table 4. The chromatogram thus exhibits a bimodal pattern typical of plant extracts, where early peaks indicate polar phenolic acids and later peaks represent high-molecular-weight flavonoids. UV absorbance was monitored at 280 nm and 320 nm, which are optimal wavelengths for detecting phenolic acids and flavonoids, respectively.

Quantitative analysis (Table 4) revealed that gallic acid (8.37 ± 0.24 mg/g dry extract) was the predominant phenolic compound in chia seed extract. This hydroxybenzoic acid is widely recognized for its potent antioxidant, antimicrobial, and antimutagenic properties, and its abundance correlates with the high total phenolic content (TPC) and strong radical scavenging activity observed in DPPH and ABTS assays. Caffeic acid (4.15 ± 0.17 mg/g) and ferulic acid (3.89 ± 0.21 mg/g) were also detected in substantial quantities. These hydroxycinnamic acids are effective in neutralizing free radicals, chelating transition metals, and inhibiting lipid peroxidation, which makes them particularly valuable for extending the shelf-life of lipid-rich foods, including meat products [31–33].

Chlorogenic acid (2.91 ± 0.15 mg/g) was another significant hydroxycinnamic derivative identified, contributing synergistically to antioxidant capacity. It is additionally associated with antidiabetic, metabolic, and neuroprotective effects [34], further enhancing the nutraceutical potential of chia extract. Among the flavonoids, rutin (2.46 ± 0.12 mg/g) and quercetin (1.74 ± 0.11 mg/g) were confirmed as key constituents. Both belong to the flavonol class and are known for their anti-inflammatory, antihypertensive, and cardioprotective actions [35,36]. Notably, rutin—a glycosylated flavonol—exhibits high thermal stability, maintaining its antioxidant capacity during thermal processing, making it suitable for incorporation into cooked or baked products.

The coexistence of hydroxycinnamic acids and flavonols in chia seed extract highlights its multifunctional role in food systems. These compounds not only enhance the extract's antioxidant activity but also contribute to technological benefits, including improved lipid stability, color retention, and moisture preservation. In meat formulations,

for instance, chia seed extract can significantly suppress oxidative deterioration, extend product freshness, and reduce reliance on synthetic antioxidants such as BHT and BHA. Functionally, the extract offers dual benefits—enhancing nutritional value through bioactive phenolics while simultaneously improving product stability by mitigating oxidative processes. Additionally, the phenolic matrix contributes to flavor and color stabilization, both crucial for maintaining consumer acceptability during storage.

The chromatographic and quantitative findings (Tables 3,4) demonstrate that chia seed extract is a potent source of both hydroxybenzoic and hydroxycinnamic acids, complemented by biologically active flavonols. The predominance of gallic acid, together with significant levels of caffeic, ferulic, and chlorogenic acids, ensures robust radical-scavenging activity, while rutin and quercetin further enhance biological efficacy and processing stability. This chemical composition confirms chia seed extract as a natural, clean-label antioxidant with high potential for application in oxidation-sensitive foods, particularly processed meats. Such findings substantiate the extract's dual role as a nutraceutical and natural preservative, supporting the development of healthier and more sustainable food preservation strategies in response to increasing consumer demand for synthetic additive-free products.

This analytical approach enabled a comprehensive characterization of the bioactive composition of chia seed (*Salvia hispanica* L.) extract, forming the foundation for understanding its antioxidant efficacy and potential applications in food systems. The identified phenolic compounds (Table 4) emphasize chia's dual functionality as both a nutraceutical and a natural preservative, supporting its incorporation into functional and shelf-stable food formulations. Among the detected compounds, gallic acid emerged as the most abundant constituent (8.37 $\pm$ 0.24 mg/g dry extract). As a hydroxybenzoic acid, gallic acid is renowned for its strong antioxidant, antimicrobial, and antimutagenic properties. Its predominance within the extract corresponds with the high total phenolic content (TPC) and the elevated radical-scavenging capacity observed in antioxidant assays. Substantial levels of caffeic acid (4.15 $\pm$ 0.17 mg/g) and ferulic acid (3.89 $\pm$ 0.21 mg/g) were also detected. These hydroxycinnamic acids are extensively documented for their ability to neutralize free radicals, chelate pro-oxidant metals, and inhibit lipid peroxidation, thereby contributing significantly to the extension of meat product shelf-life by mitigating oxidative rancidity [32,33]. Chlorogenic acid (2.91 $\pm$ 0.15 mg/g), another abundant hydroxycinnamic derivative, exhibited synergistic antioxidant effects alongside gallic and caffeic acids. Beyond its antioxidative capacity, chlorogenic acid has been linked to metabolic regulation, antidiabetic, and neuroprotective properties [34], enhancing the extract's value as a functional bioactive ingredient in health-oriented food applications. Among the flavonoids identified, rutin (2.46 $\pm$ 0.12 mg/g) and quercetin (1.74 $\pm$ 0.11 mg/g) were particularly prominent. These flavonols are associated with anti-inflammatory, antihypertensive, and cardioprotective effects [35,36]. Rutin, a glycosylated form of quercetin, is noteworthy for its superior stability under heat, maintaining antioxidant integrity during thermal processing—a property that supports its incorporation into cooked or baked food matrices. Together, rutin and quercetin substantially enhance the extract's biological potency and processing resilience.

The co-occurrence of hydroxycinnamic acids and flavonols in chia seed extract underscores its multifunctionality in food systems. These compounds not only provide potent antioxidant protection but also influence technological attributes such as lipid stability, moisture retention, and color preservation. Their presence contributes to the improvement of organoleptic properties and storage stability in formulated products. In meat-based systems, for instance, the addition of chia extract has the potential to suppress oxidative degradation, prolong product freshness, and reduce the dependence on synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). From a formulation perspective, chia extract fulfills a dual role—enhancing nutritional quality through its diverse bioactive compounds, while simultaneously improving oxidative stability through its phenolic matrix. This duality extends to functional outcomes such as flavor protection, color stabilization, and maintenance of sensory quality throughout storage. The synergistic contribution of gallic, caffeic, ferulic, and chlorogenic acids ensures robust radical-scavenging performance, while rutin and quercetin further amplify biological benefits and thermal resilience.

Overall, the diversity and abundance of phenolic acids and flavonoids identified in chia seed extract confirm its potential as a multifunctional, clean-label additive for oxidation-sensitive foods. The compositional profile (Tables 3,4) illustrates a balance between polar phenolic acids and less polar flavonoids, reflecting a phytochemical spectrum capable of broad antioxidant coverage. These findings substantiate chia seed extract's application as a natural preservative and nutraceutical, capable of enhancing both the nutritional and technological quality of food products. In particular, its efficacy in stabilizing lipid systems and suppressing oxidative rancidity positions chia extract as a promising alternative to synthetic antioxidants, aligning with current industry trends favoring healthier and more sustainable preservation strategies.

The proximate composition of beef burger samples enriched with chia seed (*Salvia hispanica* L.) extract was evaluated on day one of refrigerated storage to determine its influence on product nutritional quality. Moisture content ranged from 69.60 to 71.16 g/100 g across all formulations, with no significant differences observed among treatments ($p > 0.05$). Although chia-enriched groups (T1–

Table 5. Proximate composition of beef burgers prepared with chia seed extracts at day 0 of cold storage.

Parameter	SET	T0	T1 (0.5%)	T2 (1.0%)	T3 (1.5%)
Moisture (g/100 g)	71.13 ± 0.30^{a}	71.16 ± 0.50^{a}	69.81 ± 0.59^{a}	69.60 ± 0.79^{a}	70.66 ± 0.61^{a}
Protein (g/100 g)	15.64 ± 0.42^{a}	15.92 ± 0.37^{a}	16.00 ± 0.72^{a}	16.02 ± 1.45^{a}	16.02 ± 0.46^{a}
Lipid (g/100 g)	9.59 ± 0.29^{a}	9.34 ± 0.57^{a}	9.53 ± 0.72^{a}	10.10 ± 0.85^{ab}	10.98 ± 0.70^{b}
Ash (g/100 g)	2.69 ± 0.01^{a}	2.71 ± 0.06^{a}	2.74 ± 0.14^{a}	2.80 ± 0.05^{a}	2.78 ± 0.04^{a}
Carbohydrates (g/100 g)	0.94 ± 0.15^{a}	0.87 ± 0.59^{a}	1.35 ± 0.42^{a}	1.34 ± 1.97^{a}	1.01 ± 0.20^{a}
pH	6.10 ± 0.05^{a}	6.07 ± 0.04^{a}	6.00 ± 0.09^{a}	6.04 ± 0.05^{a}	6.11 ± 0.03^{a}

Note: Values are expressed as mean $\pm$ SD (n = 3). Within each row, values with different superscript letters (e.g., a, b) are significantly different ($p \leq 0.05$) according to Tukey's test.

Table 6. Percent cooking yield and shrinkage of beef burgers prepared with chia seed extracts during cold storage at 4 °C for 15 days.

Parameter	Period (days)	Control	SET	T1	T2	T3
Cooking yield (%)	0	83.61 ± 0.45^{dA}	88.92 ± 0.67^{abA}	86.81 ± 0.41^{cA}	89.45 ± 0.52^{aA}	87.47 ± 0.48^{bcA}
	5	83.66 ± 0.98^{bA}	84.39 ± 0.50^{bB}	86.63 ± 0.66^{abA}	88.26 ± 0.81^{aAB}	86.02 ± 1.43^{abA}
	10	73.80 ± 0.58^{bB}	74.92 ± 0.80^{abC}	78.11 ± 1.41^{abC}	79.81 ± 1.07^{abC}	84.34 ± 1.01^{aA}
	15	83.89 ± 0.72^{bcA}	82.87 ± 1.16^{cB}	82.13 ± 0.32^{cB}	87.11 ± 0.23^{bB}	91.27 ± 0.72^{aA}
Shrinkage (%)	0	12.92 ± 0.84^{aA}	13.35 ± 0.97^{aA}	13.53 ± 1.86^{aA}	13.06 ± 1.07^{aA}	11.35 ± 0.82^{aA}
	5	13.93 ± 0.30^{aA}	13.06 ± 1.15^{aA}	13.46 ± 1.93^{aA}	12.84 ± 1.18^{aA}	13.20 ± 0.86^{aA}
	10	15.97 ± 0.42^{aB}	15.74 ± 0.94^{aA}	15.99 ± 1.39^{aA}	15.16 ± 0.50^{aA}	13.10 ± 2.79^{aA}
	15	13.31 ± 0.99^{aA}	13.30 ± 1.41^{aA}	14.87 ± 1.08^{aA}	14.64 ± 1.03^{aA}	11.35 ± 0.57^{aA}

Note:

- Values are mean $\pm$ SD (n = 3).
- a–d in the same row indicate significant differences ($p \leq 0.05$) between treatments.
- A–C in the same column indicate significant differences ($p \leq 0.05$) over time.
- Cooking Yield: Burgers with chia seed extract, especially T3, consistently exhibited higher cooking yields than the control, even after 15 days of cold storage. This indicates that chia extracts improve water and fat retention during cooking [39] (Ali & Zhang, 2023).
- Shrinkage: There were no significant differences in shrinkage among treatments, but T3 tended to have slightly lower shrinkage percentages, indicating better structural integrity.
- Overall, T3 (the highest level of chia extract) showed the best performance in terms of cooking quality and yield retention during storage.

T3) exhibited marginally lower moisture levels compared to controls, the reduction was not substantial. This slight decrease may be related to the hydrophilic polysaccharides naturally present in chia seeds, which can bind water and stabilize the meat matrix, thereby minimizing free water release [36]. Protein content showed a modest increase in the chia-supplemented formulations relative to the control samples. Treatments T2 and T3 recorded values exceeding 16 g/100 g, suggesting that chia extract can contribute incrementally to the protein fraction of the product. This improvement is consistent with the high-quality protein profile of chia seeds, which contain 20–23% protein enriched with essential amino acids [37]. The incorporation of chia seed extract, therefore, not only preserves the proximate composition of beef burgers but also offers an avenue for nutritional enhancement, particularly in terms of protein contribution. These findings are presented in Table 5 and Fig. 2.

A statistically significant increase in lipid content was observed in treatment T3 (10.98 ± 0.70 g/100 g) compared with both control and SET groups ($p \leq 0.05$). This elevation is likely attributable to the intrinsic lipid fraction of chia seeds, which naturally contain 30–35% oil, particularly rich in omega-3 fatty acids such as α-linolenic acid [38].

Thus, higher levels of chia extract incorporation not only increase total lipid content but may also enhance the fatty acid profile of the product, contributing to its nutritional value. Ash content remained consistent across all formulations, indicating that chia addition did not markedly alter total mineral content. Minor increases in carbohydrate levels were noted in treatments T1 and T2, though these changes were not statistically significant ($p > 0.05$). Such variations may be related to the presence of dietary fiber or residual polysaccharides derived from the chia extract matrix. The pH values of all samples ranged between 6.00 and 6.11, with no significant differences detected ($p > 0.05$). This stability suggests that chia seed extract does not interfere with the initial acidity of the meat matrix, a favorable outcome for maintaining microbial stability during storage.

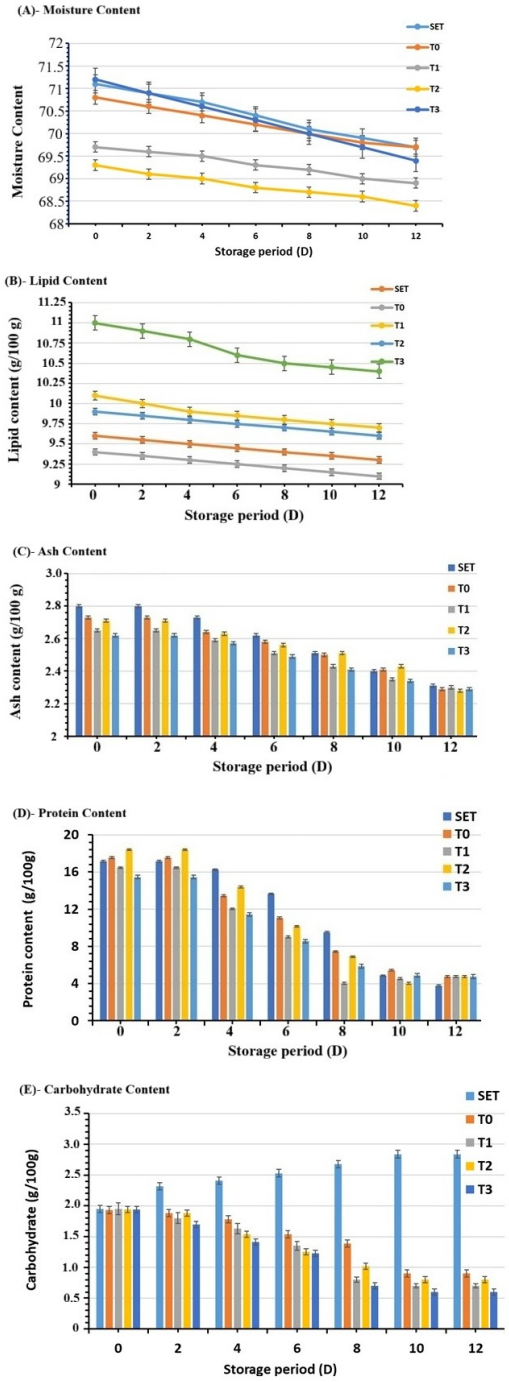

Fig. 2. Proximate composition of beef burgers prepared with chia seed extracts during cold moisture content decreased gradually in all beef burger treatments during refrigerated storage, a typical trend attributed to water migration and evaporation. (A) Moisture content: Shows changes in moisture percentage of control and chia-supplemented beef burgers (T1–T3) during cold storage, reflecting water migration and retention behavior. (B) Protein content: Illustrates the effect of chia seed extract incorporation and storage time on protein levels of beef burgers. (C) Fat content: Demonstrates variations in fat percentage among treatments during refrigerated storage. (D) Ash content: Represents changes in total mineral (ash) content of beef burgers as affected by chia seed extract concentration and storage duration. (E) Carbohydrate content: Displays the influence of chia seed extract addition on calculated carbohydrate content during storage. Chia-supplemented samples (T1–T3) initially exhibited slightly lower moisture values, likely due to the water-binding and gel-forming properties of chia polysaccharides. By day 12, however, T3 (1.5% chia extract) demonstrated superior moisture retention compared with the other chia treatments, suggesting that higher chia incorporation may stabilize water distribution within the meat matrix.

Overall, these findings confirm that chia seed extract can be successfully incorporated into beef burger formulations without negatively impacting proximate composition. On the contrary, its inclusion may enhance the protein fraction and lipid quality while also offering secondary functional benefits—such as improved antioxidant stability, enhanced water-holding capacity, and prolonged shelf-life—which are examined in subsequent storage studies.

Lipid content also declined slowly across all groups throughout storage, most likely as a result of oxidative degradation. T3, which began with the highest lipid content, retained more fat than other treatments during storage. This observation suggests a lipid stabilization of chia seed extract, attributable to its richness in polyphenols, flavonoids, and tocopherols that may inhibit lipid oxidation. Table 6 (Ref. [39]) presents the changes in cooking yield and shrinkage percentage of beef burgers fortified with chia seed extracts (CSEs) over 15 days of refrigerated storage. Cooking yield, an essential technological parameter, reflects the ability of a product to retain water and fat during heat treatment, while shrinkage corresponds to the dimensional and weight losses that occur during cooking. Both parameters are critical indicators of product quality, consumer acceptability, and industrial profitability [40,41]. At day 0, the incorporation of chia seed extracts significantly improved ($p \leq 0.05$) the cooking yield compared with both the control and SET (synthetic extract-treated) samples. Among treatments, T2 (89.45%) and T3 (87.47%) displayed the highest yields, surpassing the control group (83.61%). This initial enhancement can be attributed to the high mucilage and soluble dietary fiber content of chia seeds, which develop a viscous, gel-like network during mixing and cooking. These hydrocolloidal structures effectively bind and entrap free water and lipids within the meat matrix, minimizing drip and fat loss during heat treatment [35,42].

By day 5, a gradual reduction in cooking yield was observed across all samples, likely associated with early protein denaturation and oxidative interactions that reduce the water-holding capacity of myofibrillar proteins. Nonetheless, CSE-treated burgers maintained significantly higher yields compared to the control, confirming the stabilizing role of chia bioactives during short-term storage. The yield values for T2 (88.26%) and T1 (86.63%) remained particularly high, indicating that chia extract effectively mitigates the moisture and fat loss commonly seen during initial storage stages. As storage progressed to day 10, all samples showed a marked decline in cooking yield, with the control and SET dropping to 73.80% and 74.92%, respectively. This reduction can be attributed to protein oxidation, structural weakening, and increased water mobility within the meat matrix. However, chia-supplemented burgers demonstrated a significantly improved performance. Notably, T3 (84.34%) retained substantially higher yield compared with all other treatments ($p \leq 0.05$), suggesting that the antioxidant phenolics and polysaccharides present in chia extracts play a key role in stabilizing protein–water interactions and maintaining emulsion integrity under storage stress.

Interestingly, by day 15, a notable increase in cooking yield was recorded for all chia-supplemented samples, with T3 reaching the highest yield (91.27%), followed by T2 (87.11%), both markedly outperforming the control (83.89%) and SET (82.87%). This apparent recovery may be explained by the water-binding and film-forming capacity of chia mucilage, which may continue to reinforce the meat matrix structure during cold storage. The chia extracts' polyphenolic components, particularly gallic, caffeic, and ferulic acids, may also inhibit oxidative degradation of muscle proteins and lipids, thereby preserving the physicochemical conditions necessary for optimal water retention [43,44]. These interactions likely prevent excessive protein cross-linking and preserve a more hydrated network, contributing to higher yields at the final storage stage.

Overall, the data indicate that chia seed extract supplementation significantly enhances the technological functionality of meat systems by improving cooking yield. The improvement is both immediate (day 0)—due to hydrocolloid formation—and sustained (up to day 15)—through antioxidant protection and protein–polysaccharide interactions. Treatments containing higher concentrations of chia extract (particularly T3) consistently achieved superior performance throughout storage, underscoring a dose-dependent relationship between chia content and yield stability.

In contrast, the control and SET samples exhibited greater fluctuations and overall lower yields, suggesting that chia's combination of hydrophilic fibers and bioactive compounds provides a more comprehensive stabilizing effect than conventional synthetic antioxidants. The ability of chia components to form viscous networks and retain moisture also contributes to better product juiciness, mouthfeel, and consumer acceptability. From a mechanistic perspective, chia's hydrocolloids act as natural water binders, reducing drip loss during cooking, while the antioxidants and phenolic acids (e.g., gallic, ferulic, and chlorogenic acids) counteract oxidative stress, maintaining protein functionality. This synergistic behavior enhances the product's structural integrity, yielding higher cooking recovery rates and lower post-cooking deformation. Collectively, the results demonstrate that chia seed extract supplementation serves a dual functional role in meat systems:

(1) Technological: by improving cooking yield and preserving structural integrity through hydrocolloidal gel formation and moisture entrapment; and

(2) Nutritional: by introducing natural antioxidants and omega-3 fatty acids that mitigate oxidation and improve overall product stability.

These outcomes validate chia seed extract as a promising clean-label alternative to synthetic antioxidants (e.g., BHT, BHA) in processed meats. Beyond yield enhance-

Table 7. Color parameters (L*, a*, b*) of beef burgers prepared with chia seed extracts during cold storage at 4 °C for 15 days.

Parameter	Day	Control	SET	T1	T2	T3
L* (Lightness)	0	57.30 ± 3.37 ^{abA}	58.25 ± 1.18 ^{aA}	57.07 ± 1.83 ^{abA}	56.11 ± 1.88 ^{abA}	54.51 ± 2.33 ^{bA}
	5	56.12 ± 1.14 ^{aA}	56.75 ± 1.85 ^{aA}	55.23 ± 1.83 ^{abAB}	53.60 ± 1.59 ^{bB}	54.48 ± 2.60 ^{abA}
	10	55.56 ± 1.66 ^{abA}	56.31 ± 1.85 ^{aA}	54.49 ± 1.94 ^{bcB}	53.09 ± 0.42 ^{cB}	54.58 ± 0.83 ^{bcA}
	15	57.44 ± 0.87 ^{aA}	54.38 ± 1.65 ^{bB}	54.21 ± 1.16 ^{bB}	54.31 ± 0.64 ^{bB}	54.64 ± 1.06 ^{bA}
a* (Redness)	0	6.14 ± 1.17 ^{bA}	7.72 ± 1.09 ^{aA}	5.33 ± 0.84 ^{bcA}	4.18 ± 0.65 ^{cdAB}	3.75 ± 0.97 ^{dA}
	5	6.12 ± 0.42 ^{bA}	7.41 ± 1.17 ^{aA}	4.37 ± 0.72 ^{cAB}	3.12 ± 0.38 ^{cB}	4.06 ± 0.99 ^{cA}
	10	5.48 ± 0.26 ^{bA}	7.40 ± 0.48 ^{aA}	4.83 ± 0.53 ^{bcAB}	4.76 ± 0.76 ^{bcA}	4.29 ± 0.99 ^{cA}
	15	6.62 ± 1.07 ^{aA}	6.71 ± 0.82 ^{aA}	3.94 ± 1.27 ^{bB}	3.96 ± 0.90 ^{bAB}	3.92 ± 0.89 ^{bA}
b* (Yellowness)	0	11.84 ± 2.12 ^{bA}	13.65 ± 1.44 ^{bA}	20.24 ± 0.80 ^{aA}	22.18 ± 2.96 ^{aA}	22.94 ± 2.05 ^{aA}
	5	11.30 ± 1.61 ^{cA}	12.73 ± 0.94 ^{cA}	17.16 ± 1.33 ^{bB}	20.25 ± 2.05 ^{aAB}	22.42 ± 2.24 ^{aA}
	10	10.24 ± 1.64 ^{cA}	12.84 ± 1.98 ^{cA}	17.02 ± 1.99 ^{bB}	19.46 ± 2.94 ^{abAB}	21.95 ± 3.45 ^{aA}
	15	11.65 ± 1.17 ^{cA}	10.16 ± 2.19 ^{cB}	15.47 ± 2.57 ^{bB}	18.80 ± 2.09 ^{aB}	21.00 ± 2.93 ^{aA}

Note:

- Values are presented as mean ± SD (n = 3).
- L* = lightness, a* = redness, b* = yellowness.
- Within each row:
  - Means with different lowercase letters (a–d) indicate significant differences between treatments (Tukey's test, $p \leq 0.05$).
  - Means with different uppercase letters (A–B) indicate significant differences over time within the same treatment.

ment, the presence of chia bioactives contributes to extended shelf-life, improved sensory attributes, and a more sustainable, health-conscious product formulation [44,45]. Future studies exploring the molecular interactions between chia polysaccharides and meat proteins, as well as the sensory perception of CSE-enriched products, will be essential to further optimize formulation levels and validate consumer acceptance. Nonetheless, the present findings clearly establish that chia seed extracts, particularly at higher inclusion levels (T3), offer substantial benefits in terms of cooking performance, product yield, and storage stability—confirming their suitability as multifunctional ingredients in modern meat processing.

3.2.1 Color (Lightness, L*)

Color is a crucial quality parameter for meat products, as it strongly influences consumer perception of freshness and overall acceptability [46]. The lightness (L*) values of beef burgers enriched with chia seed extracts (CSEs) were monitored throughout 15 days of refrigerated storage (Table 7). At day 0, burgers supplemented with CSE, particularly T3 (54.51), showed slightly lower L* values than the control (57.30). This modest reduction may be attributed to the presence of chia pigments and phenolic compounds, which impart a natural brownish tint and reduce surface reflectance [35,42].

Despite these initial differences, no significant decline in lightness was observed during storage across all treatments ($p > 0.05$). By day 15, both control and chia-treated samples exhibited comparable L* values, with T3 maintaining stable brightness similar to its baseline. The absence of notable darkening suggests that chia extract incorporation did not accelerate oxidative discoloration. On the contrary, its antioxidant and moisture-binding properties may have contributed to stabilizing surface appearance over time [47]. Overall, the inclusion of chia extract slightly reduced initial product brightness but maintained consistent color stability throughout storage, indicating that CSEs do not compromise visual quality and may support oxidative protection of meat color (Table 7).

3.2.2 Redness (a*)

The a* parameter, reflecting the intensity of red coloration, decreased progressively in all samples during refrigerated storage, consistent with the oxidative conversion of oxymyoglobin to metmyoglobin. At day 0, the control (6.14) and SET (7.72) samples exhibited significantly higher a* values compared to chia-enriched formulations, with T3 showing the lowest initial redness (3.75). This reduction may result from partial dilution of meat pigments by chia extract or interactions between chia-derived antioxidants and myoglobin chemistry. Despite their lower initial redness, chia-containing treatments displayed improved color stability during storage. Particularly after day 10, T2 and T3 exhibited less pronounced declines in a* compared to the control, suggesting that bioactive constituents in chia, including polyphenols, flavonoids, and rosmarinic acid, helped slow pigment oxidation. This stabilization effect implies that chia seed extract contributes to preserving meat redness over time, even if the initial intensity appears reduced.

3.2.3 Yellowness (b*)

The b* values were strongly influenced by chia incorporation. At day 0, chia-enriched burgers showed significantly higher yellowness, with T3 reaching 22.94 compared

to 11.84 in the control. This increase is attributable to the natural yellowish-brown pigments in chia extracts, likely derived from phenolic and carotenoid-like compounds [47]. Although a gradual decline in b* values occurred during storage, chia-containing treatments consistently maintained higher yellowness than the control. Overall, T3 demonstrated the most balanced color performance, combining minimal changes in L*, attenuated loss of redness, and sustained higher yellowness throughout storage. This indicates that chia seed extracts not only influence the initial appearance of meat products but also contribute to stabilizing color quality during shelf-life.

Although chia seed extract caused minor initial darkening and reduced redness, it markedly enhanced color stability and oxidative resistance throughout storage. This effect is attributed to the synergistic action of chia-derived antioxidants, including rosmarinic acid, caffeic acid, and tocopherols, which can scavenge reactive oxygen species and stabilize myoglobin pigments. Consequently, chia extract not only modifies the initial appearance of beef burgers but also plays a protective role in maintaining desirable color attributes during refrigerated storage.

Chia seed extract acted as an effective natural color stabilizer in beef burgers, maintaining visual quality during refrigerated storage. Among the instrumental color parameters, b* (yellowness) was most affected by chia addition. At day 0, the control sample showed a b* value of 11.84, while T3 exhibited a significantly higher value of 22.94, reflecting the presence of yellow–brown pigments associated with chia polyphenols and fiber-bound compounds [48]. Unlike the L* and a* values, which declined gradually with storage, the b* values in T2 and T3 remained stable, indicating a protective effect against oxidative discoloration. This sustained color intensity enhances product shelf-life and supports the use of chia extract as a clean-label antioxidant in meat formulations (Table 7).

The incorporation of chia seed extracts (CSEs) noticeably influenced the color stability of beef burgers during refrigerated storage. While initial redness (a*) values were slightly lower in chia-treated samples compared to controls, these formulations exhibited enhanced oxidative stability, maintaining more consistent redness over time. This outcome can be attributed to the antioxidant activity of chia-derived polyphenols, flavonoids, and tocopherols, which delay myoglobin oxidation. Yellowness (b*) values were significantly higher in CSE-enriched samples, particularly T3, reflecting the natural yellow–brown pigments and fiber-bound phenolics inherent in chia. Importantly, this attribute remained stable throughout storage, suggesting a protective effect against oxidative discoloration. Lightness (L*) values showed minimal change, indicating that chia incorporation did not negatively affect product brightness.

Overall, these findings suggest that chia seed extracts serve as effective natural color stabilizers, mitigating oxidative changes and supporting visual quality retention. Such color enhancement may improve the visual distinctiveness of functional meat products, offering a potential marketing advantage in health-conscious product lines. From a technological perspective, the ability of chia extracts to reduce lightness loss and limit color fluctuations positions them as multifunctional, clean-label ingredients in meat product development [47,48]. Further consumer-focused studies are warranted to determine the concentration levels that balance appealing color characteristics with optimal nutritional and oxidative stability.

3.2.4 Texture Attributes

Texture is one of the most critical determinants of meat product quality, influencing both consumer acceptability and industrial performance. In this study, the influence of chia seed extract (CSE) on the texture profile of beef burgers was evaluated through five key parameters—hardness, springiness, cohesiveness, chewiness, and resilience—over 15 days of refrigerated storage at 4 °C. A three-dimensional (3D) response surface methodology (RSM) approach was employed to visualize the interactive effects of CSE concentration and storage duration on texture characteristics (Fig. 3A–E). This analytical approach provided an integrated understanding of how chia extracts, through their hydrocolloid-forming capacity and bioactive compounds, modulate the mechanical behavior of meat matrices during storage (Table 8). Table 8 presents the evolution of texture parameters in beef burgers supplemented with different concentrations of CSE (T1–T3), compared to the control (without additives) and SET (synthetic antioxidant-treated) samples. Texture attributes are vital indicators of the structural integrity, protein–water interactions, and fat-binding capacity of the meat system. These properties can deteriorate during cold storage due to protein denaturation, lipid oxidation, and moisture migration. However, the addition of CSE, rich in phenolics, polysaccharides, and mucilage, may mitigate these changes and improve texture stability through its antioxidant and water-retention capabilities.

3.3 Hardness

Hardness values varied significantly ($p \leq 0.05$) between treatments and across storage periods. At day 0, the control and SET groups exhibited higher hardness (12.41 $\pm$ 0.71 N and 11.73 $\pm$ 1.02 N, respectively), while T1 (9.35 $\pm$ 0.54 N) was markedly softer. Treatments T2 and T3 maintained hardness levels (12.09 $\pm$ 0.85 N and 12.43 $\pm$ 0.91 N) comparable to the control, suggesting that moderate-to-high concentrations of chia extract support an appropriate structural firmness. Over time, hardness generally increased, reflecting the natural tendency of meat proteins to crosslink and lose moisture during refrigeration. However, the extent of hardening was attenuated in CSE treatments, particularly in T3 (11.49 $\pm$ 0.79 N) after 15 days, compared to the control (13.43 $\pm$ 0.93 N). This indicates that chia's hydrocolloid fraction and phenolic antioxidants preserve juiciness

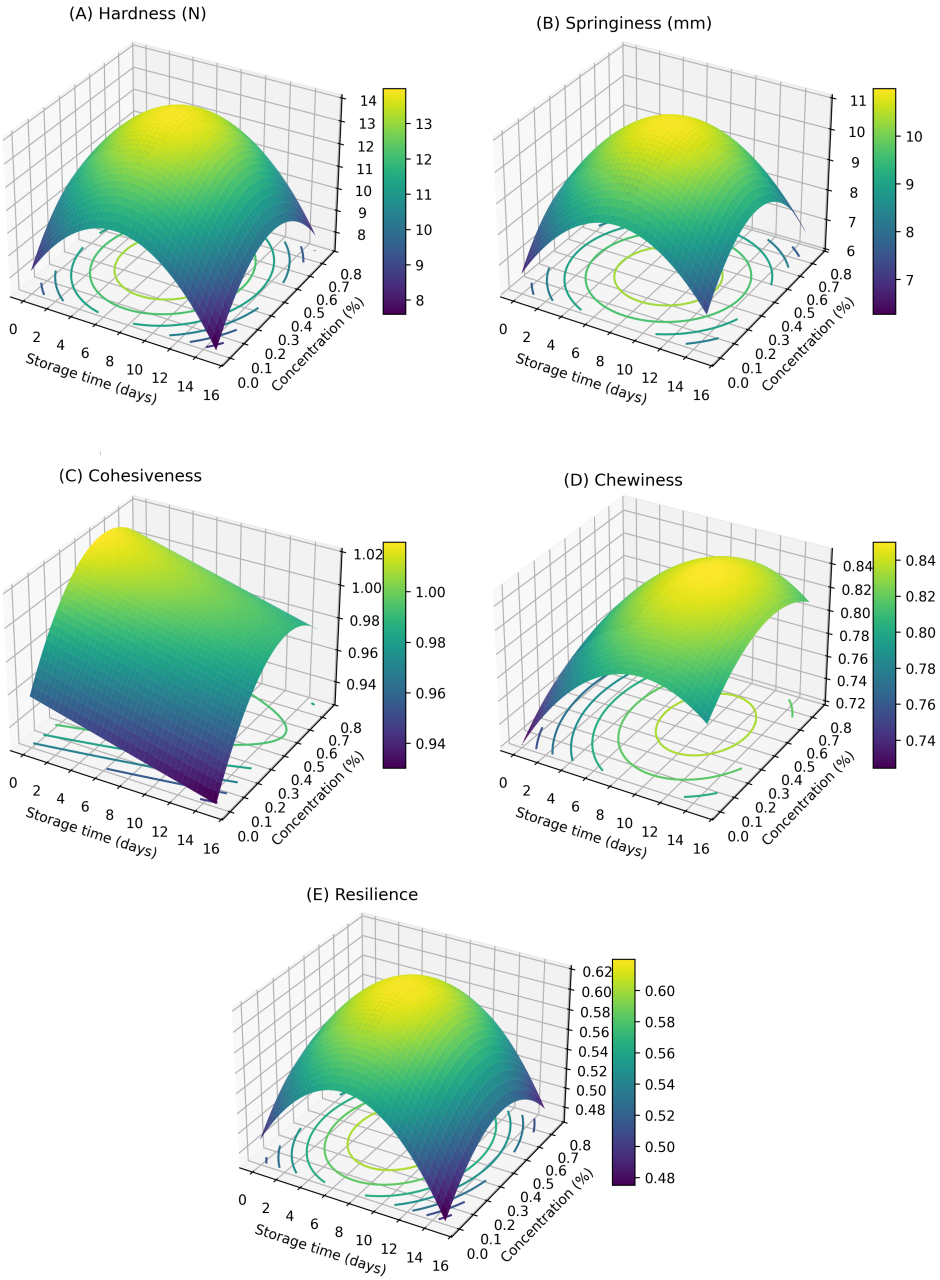

Fig. 3. Three-dimensional (3D) response surface plots from texture profile analysis of beef burgers prepared with chia seed extracts during 15 days of cold storage at 4 °C. (A) Hardness: Illustrates the interactive effects of CSE concentration and storage duration on burger firmness. (B) Springiness: Shows how CSE concentration and storage time influence the elastic recovery of burgers. (C) Cohesiveness: Depicts the combined effects of CSE and storage on internal structural integrity. (D) Chewiness: Represents the influence of CSE and storage duration on the energy required to chew the burgers. (E) Resilience: Displays the effect of CSE and storage on the ability of burgers to recover their shape after deformation.

and delay protein aggregation, maintaining desirable texture stabilit-y (Table 8).

Fig. 3A, the three-dimensional response surface plot for hardness illustrates the interactive effects of chia seed extract (CSE) concentration and storage duration on the firmness of beef burgers during 15 days of refrigerated storage at 4 °C. A gradual decline in hardness was observed in all formulations over time, reflecting typical softening associated with protein denaturation, moisture migration, and weakening of the myofibrillar network during cold storage [35,42]. Notably, burgers fortified with moderate CSE levels (T2: 1.0%) maintained hardness values comparable to the control, indicating good structural preservation. In contrast, higher inclusion (T3: 1.5%) produced a slightly softer

Table 8. Texture profile analysis (TPA) of beef burgers prepared with chia seed extracts during cold storage at 4 °C for 15 days.

Parameter	Day	Control	SET	T1	T2	T3
Hardness (N)	0	12.41 ± 0.71 ^{abA}	11.73 ± 1.02 ^{abA}	9.35 ± 0.54 ^{cA}	12.09 ± 0.85 ^{abA}	12.43 ± 0.91 ^{aA}
	5	15.62 ± 0.99 ^{aA}	12.91 ± 0.83 ^{bA}	9.26 ± 0.66 ^{cA}	14.93 ± 1.00 ^{aA}	12.86 ± 1.32 ^{bA}
	10	14.90 ± 0.91 ^{aA}	13.21 ± 0.76 ^{abA}	10.01 ± 0.68 ^{cA}	14.43 ± 1.31 ^{abA}	13.86 ± 0.88 ^{abA}
	15	13.43 ± 0.93 ^{aA}	12.99 ± 1.05 ^{aA}	10.35 ± 0.61 ^{bA}	13.42 ± 0.96 ^{aA}	11.49 ± 0.79 ^{bA}
Springiness (mm)	0	6.30 ± 0.11 ^{aA}	6.42 ± 0.17 ^{aA}	6.40 ± 0.09 ^{aA}	6.48 ± 0.14 ^{aA}	6.35 ± 0.16 ^{aA}
	5	6.36 ± 0.15 ^{aA}	6.40 ± 0.13 ^{aA}	6.32 ± 0.12 ^{aA}	6.33 ± 0.17 ^{aA}	6.38 ± 0.08 ^{aA}
	10	6.34 ± 0.18 ^{aA}	6.43 ± 0.15 ^{aA}	6.36 ± 0.11 ^{aA}	6.10 ± 0.19 ^{aA}	6.37 ± 0.10 ^{aA}
	15	6.29 ± 0.13 ^{aA}	6.28 ± 0.10 ^{aA}	6.41 ± 0.13 ^{aA}	6.40 ± 0.12 ^{aA}	6.39 ± 0.09 ^{aA}
Cohesiveness	0	0.87 ± 0.05 ^{bB}	1.00 ± 0.04 ^{aA}	0.99 ± 0.02 ^{aA}	0.93 ± 0.03 ^{abA}	0.96 ± 0.02 ^{abA}
	5	0.91 ± 0.03 ^{bAB}	0.99 ± 0.03 ^{aA}	0.94 ± 0.03 ^{abA}	0.95 ± 0.04 ^{abA}	0.95 ± 0.04 ^{abA}
	10	0.94 ± 0.02 ^{aA}	0.96 ± 0.02 ^{aA}	0.95 ± 0.03 ^{aA}	0.96 ± 0.04 ^{aA}	0.94 ± 0.05 ^{aA}
	15	0.96 ± 0.03 ^{aA}	0.99 ± 0.03 ^{aA}	0.94 ± 0.03 ^{abA}	0.97 ± 0.02 ^{aA}	0.94 ± 0.04 ^{abA}
Chewiness (N·mm)	0	68.24 ± 3.02 ^{aA}	75.12 ± 3.22 ^{aA}	61.18 ± 2.45 ^{aA}	72.46 ± 2.97 ^{aA}	73.94 ± 3.10 ^{aA}
	5	70.12 ± 2.88 ^{aA}	73.33 ± 3.12 ^{aA}	62.43 ± 2.58 ^{aA}	75.58 ± 2.74 ^{aA}	72.11 ± 2.61 ^{aA}
	10	72.40 ± 3.17 ^{aA}	71.36 ± 2.89 ^{aA}	65.29 ± 2.91 ^{aA}	74.11 ± 2.65 ^{aA}	70.34 ± 2.93 ^{aA}
	15	71.31 ± 2.96 ^{aA}	72.05 ± 3.01 ^{aA}	66.52 ± 2.73 ^{aA}	71.87 ± 3.10 ^{aA}	68.74 ± 2.88 ^{aA}
Resilience	0	0.44 ± 0.02 ^{abA}	0.46 ± 0.01 ^{aA}	0.43 ± 0.01 ^{abA}	0.48 ± 0.02 ^{aA}	0.42 ± 0.01 ^{bA}
	5	0.45 ± 0.02 ^{aA}	0.47 ± 0.01 ^{aA}	0.44 ± 0.01 ^{abA}	0.45 ± 0.02 ^{aA}	0.41 ± 0.02 ^{bA}
	10	0.43 ± 0.02 ^{bA}	0.45 ± 0.01 ^{abA}	0.42 ± 0.01 ^{bA}	0.44 ± 0.02 ^{abA}	0.38 ± 0.01 ^{cA}
	15	0.44 ± 0.01 ^{abA}	0.46 ± 0.01 ^{aA}	0.43 ± 0.01 ^{abA}	0.45 ± 0.01 ^{aA}	0.41 ± 0.01 ^{bA}

Notes:

- Values are expressed as mean ± SD (n = 3).
- Different lowercase letters (a–c) in the same row indicate significant differences among treatments at a specific storage day ($p \leq 0.05$, Tukey's test).
- Different uppercase letters (A–B) in the same column indicate significant differences across storage periods within the same treatment ($p \leq 0.05$, Tukey's test).
- T1–T3 represent different concentrations of chia seed extract. SET, synthetic antioxidant-treated group (positive control).

Table 9. Lipid oxidation (mg malonaldehyde/kg sample) in beef burgers with chia seed extracts during cold storage (4 °C, 15 days).

Treatment	0th day	5th day	10th day	15th day
Control	0.47 ± 0.02 ^{aB}	0.51 ± 0.20 ^{aB}	2.52 ± 0.17 ^{aA}	1.27 ± 0.64 ^{aB}
SET	0.27 ± 0.01 ^{bB}	0.28 ± 0.05 ^{aB}	0.38 ± 0.07 ^{bA}	0.33 ± 0.01 ^{bAB}
T1 (Low CSE)	0.27 ± 0.03 ^{bA}	0.40 ± 0.09 ^{aA}	0.48 ± 0.04 ^{bA}	0.62 ± 0.34 ^{abA}
T2 (Med CSE)	0.23 ± 0.02 ^{bcB}	0.42 ± 0.05 ^{aAB}	0.45 ± 0.06 ^{bAB}	0.59 ± 0.28 ^{abA}
T3 (High CSE)	0.21 ± 0.03 ^{cC}	0.35 ± 0.01 ^{aAB}	0.36 ± 0.05 ^{bA}	0.28 ± 0.01 ^{bB}

Notes:

- CSE, Chia Seed Extract; SET, synthetic antioxidant (positive control).
- Results expressed as mean ± standard deviation (n = 4).
- Different lowercase letters (a–c) in the same column denote significant differences between treatments at a given time ($p \leq 0.05$).
- Different uppercase letters (A–C) in the same row indicate significant differences across storage days within the same treatment ($p \leq 0.05$, Tukey's test).

texture ($p < 0.05$) at extended storage times, likely due to the water-binding and gel-forming properties of chia mucilage, which impart greater moisture retention and elasticity to the meat matrix. These results demonstrate that CSE concentration modulates texture in a controllable manner, allowing formulation flexibility for different product profiles.

This firmness retention effect is mainly linked to the hydrocolloid-forming capacity of chia mucilage and its high dietary fiber content, which together generate a gel-like network within the meat matrix. This structure improves water-holding capacity (WHC) and minimizes structural disintegration during storage [35,42,48]. Furthermore, chia-derived phenolics—particularly gallic, ferulic,

and caffeic acids—are likely to form non-covalent and covalent interactions with muscle proteins and connective tissue, contributing to cross-linking that reinforces matrix integrity and resists oxidative or enzymatic degradation [35,42].

Conversely, hardness in the control and SET samples decreased more rapidly, reflecting the absence of hydrocolloid reinforcement and limited oxidative protection. These findings agree with previous reports indicating that plant extracts rich in polyphenols and soluble fibers synergistically preserve textural stability and extend the sensory shelf-life of meat products during refrigerated storage [49]. Collectively, the enhanced hardness retention in CSE-enriched burgers underscores the multifunctional role of chia extract as both an antioxidant and a natural hydrocolloid, thereby improving product quality and consumer acceptability throughout cold storage.

3.4 Springiness

Springiness values were relatively consistent across treatments and days (6.10–6.48 mm), showing no significant differences ($p > 0.05$). This stability implies that chia extract incorporation did not disrupt the product's ability to regain its shape after compression. The presence of soluble fiber and mucilage in chia may have reinforced the elastic gel network, preserving the structural recovery of the protein matrix even under cold storage. Such findings agree with previous reports indicating that hydrocolloids from plant sources enhance elasticity and water retention in emulsified meat systems (Table 8).

The response surface plot for springiness (Fig. 3B) shows that no significant differences ($p > 0.05$) were observed among treatments throughout the 15 days of refrigerated storage at 4 °C. This overall stability indicates that the elastic properties of beef burgers were effectively maintained during storage, despite the minor, non-significant downward trends typically associated with protein denaturation, moisture redistribution, and reduced muscle fiber elasticity during cold storage [35,42,50]. Although the differences were not statistically significant, burgers containing chia seed extract (CSE) at 1.0 % (T2) and 1.5 % (T3) tended to show slightly higher springiness values than the control and synthetic antioxidant-treated (SET) samples. This observation suggests that CSE may exert a stabilizing effect on the meat matrix, likely due to its mucilage and polyphenol content, which enhance water retention and matrix elasticity. Overall, the inclusion of CSE contributed to maintaining textural resilience and uniformity during refrigerated storage.

This effect can be attributed to the hydrophilic polysaccharides in chia mucilage, which form a gel-like network within the meat matrix, entrapping water and reinforcing protein interactions, thereby minimizing structural collapse [44,45,51]. Additionally, the presence of polyphenolic and flavonoid compounds such as quercetin, rutin, and chlorogenic acid may inhibit protein oxidation, preserving the integrity of myofibrillar proteins responsible for elasticity [35,42]. The stable springiness observed during storage highlights chia seed extract's multifunctional ability to sustain mechanical strength and enhance the perceived juiciness and freshness of beef burgers during cold storage.

3.5 Cohesiveness

Cohesiveness describes the internal bonding strength of the product's matrix. At day 0, the control exhibited the lowest cohesiveness (0.87 ± 0.05), whereas SET and chia-treated samples (T1–T3) displayed higher values (0.93–1.00), demonstrating improved internal integrity due to chia components. Cohesiveness remained stable (0.94–0.99) throughout the 15-day period, suggesting that chia extracts provided a protective effect against oxidative and enzymatic degradation of structural proteins. This may be attributed to the phenolic–protein interactions and the formation of hydrated networks by chia mucilage, both of which enhance binding within the muscle fiber matrix (Table 8).

The response surface plot for cohesiveness (Fig. 3C) demonstrates that the changes in all treatments during 15 days of refrigerated storage were not statistically significant ($p > 0.05$). This suggests that the internal binding strength and structural integrity of the burgers remained relatively stable throughout storage. Nonetheless, burgers fortified with chia seed extract (CSE) at 1.0 % and 1.5 % (T2 and T3) consistently showed higher cohesiveness values than the untreated control and synthetic antioxidant-treated (SET) samples, indicating that CSE contributed to maintaining the internal structure of the meat matrix.

Cohesiveness in meat systems primarily depends on protein–protein and protein–water interactions, which can deteriorate under oxidative or enzymatic stress [45,52]. The enhanced cohesiveness observed in CSE-fortified burgers can be attributed to the dual functionality of chia seed components: (i) phenolic acids such as gallic, ferulic, and caffeic acids protect against protein oxidation by minimizing carbonyl formation and preserving sulfhydryl groups [39,46], and (ii) chia mucilage, rich in soluble polysaccharides, forms hydrocolloid-like gels that fill interstitial spaces, enhance water binding, and stabilize the protein network [47]. Overall, the maintenance of cohesiveness during storage underscores chia seed extract's structural and antioxidative roles, improving the texture, juiciness, and handling properties of beef burgers without significant degradation over time.

3.6 Chewiness

Chewiness, a function of hardness, cohesiveness, and springiness, reflects the energy required to masticate the sample. Initially, chewiness values ranged from 61.18 N·mm (T1) to 75.12 N·mm (SET), with minimal variation ($p > 0.05$) among groups. Across storage, chewiness remained relatively stable for all treatments, indicating that

chia supplementation did not negatively affect mouthfeel or chew resistance. After 15 days, chewiness values were 71.31 N·mm (control), 72.05 N·mm (SET), and 66.52–71.87 N·mm for chia-treated samples. These results show that CSE inclusion maintained mechanical integrity while possibly improving juiciness perception. The hydrocolloid matrix formed by chia's polysaccharides likely contributed to a smoother, less brittle texture by retaining water within the protein network, counteracting the toughening effects of oxidation and dehydration typically observed in untreated burgers (Table 8 and Fig. 3D).

3.7 Resilience

Resilience measures the product's ability to recover energy after deformation, thus reflecting structural elasticity. Values ranged between 0.38 and 0.48 across treatments. T2 displayed the highest resilience (0.48 $\pm$ 0.02 at day 0), indicating a more elastic and compact network. Conversely, slightly lower values were recorded for T3 (0.42 $\pm$ 0.01), suggesting that higher chia levels may increase moisture retention but reduce elastic energy recovery due to a softer matrix. Despite minor fluctuations, resilience remained comparable to or higher than the control during storage, confirming chia's ability to stabilize the structural framework of the meat product (Table 8). The response surface plot for resilience (Fig. 3E) and the corresponding data in Table 8 demonstrated no significant variation ($p >$ 0.05) across storage time, indicating that the ability of the meat matrix to recover its shape after deformation remained largely stable throughout the 15 days of refrigerated storage. Resilience reflects the elastic recovery and internal cohesion of the protein gel network after compression, parameters that depend on protein–protein interactions, water-binding capacity, and the integrity of the myofibrillar matrix.

Across all treatments, resilience values ranged narrowly between 0.38 and 0.48, with no statistically significant changes over time (same uppercase letter "A" in each column). Burgers supplemented with 1.0% CSE (T2) consistently exhibited slightly higher resilience values compared to the control and other treatments, suggesting that moderate levels of chia seed extract enhanced the elastic recovery of the meat structure. The 1.5% CSE (T3) group showed comparable resilience stability, indicating that higher chia inclusion did not compromise textural integrity.

This relative stability can be attributed to the hydrocolloid-forming polysaccharides present in chia mucilage, which interact with the meat matrix to strengthen the gel network and help retain water. Additionally, the antioxidant phenolic compounds identified in the extract—particularly gallic, ferulic, caffeic, and quercetin—may have contributed to maintaining protein functionality by limiting oxidative damage to myofibrillar proteins [49–51]. The combined hydrocolloid and antioxidant mechanisms likely prevented excessive protein cross-linking or degradation, thereby preserving textural elasticity during cold storage.

From a technological standpoint, the stability of resilience across all formulations indicates that chia seed extract effectively supports the mechanical structure of beef burgers without inducing textural deterioration. This is particularly relevant for refrigerated meat systems, where protein oxidation and moisture loss typically reduce elasticity over time. Therefore, the inclusion of CSE not only maintains the natural rebound capacity of the meat matrix but also contributes to the overall textural and structural quality of the product.

Collectively, these findings confirm that chia seed extract functions as a natural stabilizer, preserving resilience and ensuring that the final product retains desirable firmness and elasticity throughout storage. Such stability reinforces its potential as a clean-label antioxidant and texture-enhancing agent in reformulated meat products aimed at extending shelf-life while maintaining consumer-acceptable texture profiles.

Overall Evaluation: The incorporation of chia seed extracts effectively enhanced or preserved the texture of beef burgers during refrigerated storage. Treatments containing moderate concentrations (T2) achieved the most favorable balance between firmness, elasticity, and cohesiveness, whereas high concentrations (T3) resulted in slightly softer but juicier products. Chia's physicochemical properties—especially its soluble fiber and mucilage—contribute to improved water-holding capacity and gel-forming potential, preventing excessive hardening and moisture loss over time. Furthermore, chia's abundant phenolic antioxidants likely inhibited protein oxidation and crosslinking, thus maintaining a stable texture profile during storage. From a technological standpoint, these findings demonstrate that chia seed extract functions as a natural texture stabilizer comparable to synthetic antioxidants, with added benefits of water retention and oxidative protection. This aligns with the growing demand for clean-label, plant-derived additives in processed meat formulations. The ability of chia components to maintain consistent hardness, chewiness, and cohesiveness over 15 days indicates their synergistic action between hydrocolloid and antioxidant properties. Overall, the inclusion of chia seed extracts, particularly at intermediate levels (T2), provided significant texture preservation without compromising sensory attributes. This suggests potential application in reformulated meat systems aimed at improving shelf-life, juiciness, and structural stability using natural alternatives to synthetic stabilizers. Future work should focus on correlating these textural changes with microstructural and rheological analyses to elucidate the mechanisms of chia–protein interactions responsible for the observed improvements. These 3D RSM plots clearly demonstrate that chia seed extract (CSE) not only enhances oxidative stability but also plays a pivotal role in preserv-

ing the textural integrity of beef burgers during refrigerated storage. The synergistic functionality of chia mucilage, dietary fiber, and polyphenolic compounds underscores its value as a multifunctional, clean-label additive for meat processing. Among the tested inclusion levels, 1.0% CSE (T2) provided the most balanced texture profile, maintaining the highest resilience and overall textural stability during storage, whereas 1.5% CSE (T3) exhibited slightly lower resilience values but retained desirable firmness and structural integrity. The T1 formulation produced softer textures, suitable for applications requiring enhanced tenderness, while T3 imparted greater hardness and cohesiveness. Cohesiveness and springiness remained largely stable ($p > 0.05$) across storage for all formulations, confirming that chia extracts sustain product integrity without undesirable textural changes. Collectively, these findings emphasize the dual functionality of chia extracts as natural antioxidants and texturizing agents, contributing to improved sensory and technological quality during chilled storage. The application of response surface methodology (RSM) provided an effective visualization of these interactions, supporting optimized formulation strategies for functional, clean-label meat products.

3.8 Lipid Oxidation

Lipid oxidation is one of the most critical factors affecting the quality, safety, and shelf-life of meat products during storage. It leads to the development of rancid flavors, discoloration, and nutritional deterioration. The thiobarbituric acid reactive substances (TBARS) assay was used to assess the extent of lipid oxidation in beef burgers formulated with chia seed extract (CSE) at varying concentrations (T1–T3) and stored at 4 °C for 15 days. The corresponding data are presented in Table 9. At the beginning of storage (day 0), the malonaldehyde (MDA) content in the control burgers was 0.47 mg/kg, which increased sharply to 2.52 mg/kg by day 10 before slightly declining to 1.27 mg/kg at day 15. This pattern reflects the typical progression of lipid oxidation, where primary oxidation products (hydroperoxides) accumulate and subsequently decompose into secondary aldehydes such as MDA. The increase observed in the control samples was statistically significant ($p \leq 0.05$), confirming that the absence of antioxidants allowed extensive oxidative degradation during storage.

In contrast, burgers containing chia seed extract (CSE) or synthetic antioxidant (SET) displayed markedly lower TBARS values across all time points, indicating effective oxidative stabilization. The SET-treated samples showed only minimal changes (0.27–0.38 mg/kg) over 15 days, consistent with its known antioxidant potency. Among the CSE formulations, the T3 treatment (high CSE) exhibited the lowest MDA concentrations throughout storage, ranging from 0.21 to 0.36 mg/kg.

The T2 formulation (medium CSE) also effectively delayed lipid peroxidation, showing only minor fluctuations from 0.23 mg/kg at day 0 to 0.59 mg/kg at day 15. Although there was a slight upward trend, the changes over time were not statistically significant until the final sampling point (A–B superscripts), suggesting that moderate chia extract concentrations are sufficient to retard oxidation over two weeks of refrigerated storage.

Importantly, the T1 formulation (low CSE) maintained statistically similar MDA values throughout storage (all labeled "A"), indicating no significant increase ($p > 0.05$) from day 0 to day 15. This stability implies that even at lower doses, chia seed extract provides substantial antioxidative protection, likely due to its rich phenolic composition and synergistic interaction between polyphenols, fiber, and mucilage components. The bioactive compounds identified in Tables 3,4—such as chlorogenic acid, caffeic acid, ferulic acid, rutin, and quercetin—are known for their radical-scavenging and metal-chelating activities, which collectively suppress peroxidation in lipid-rich matrices.

Among all treatments, the control group exhibited the highest degree of oxidation, while T3 (high CSE) and SET (synthetic antioxidant) performed best in preserving oxidative stability. The low TBARS values in T3 samples across all time points ($\leq$0.36 mg/kg) suggest that chia extract at higher concentrations can effectively maintain lipid integrity during cold storage. These findings align with previous reports demonstrating that chia-derived polyphenols and mucilage compounds act as natural antioxidants by donating hydrogen atoms to neutralize free radicals, stabilizing peroxides, and forming protective barriers that limit oxygen diffusion into the meat matrix.

Mechanistically, the antioxidant activity of CSE can be attributed to both its hydrophilic and lipophilic constituents. The hydrophilic phenolics quench reactive oxygen species (ROS) in the aqueous phase, while the viscous chia mucilage forms a network that restricts lipid mobility and oxygen penetration. This dual mode of action results in a consistent reduction of oxidation products and enhances the overall oxidative stability of the burgers. Additionally, chia seed's high content of α-linolenic acid, while prone to oxidation, may interact with its endogenous antioxidants to create a balanced redox environment, further enhancing lipid protection.

Overall, the results from Table 9 confirm that chia seed extract significantly inhibits lipid oxidation in beef burgers during refrigerated storage. The efficacy increases with extract concentration, with T3 providing the most robust antioxidative effect. The absence of significant differences over time in the T1 and T2 groups underscores the stability and sustained antioxidant potential of chia phenolics even at moderate dosages. From a formulation perspective, CSE offers a promising natural alternative to synthetic antioxidants, aligning with clean-label and functional food trends. The use of chia seed extract not only supports oxidative stability but also complements previous findings (Tables 6,7,8)

showing improvements in cooking yield and textural quality.

Thus, chia seed extract can be considered a multifunctional ingredient—providing both antioxidant protection and technological benefits—making it highly suitable for the development of healthier, more stable meat products.

The observed antioxidant effect can be attributed to chia's rich bioactive composition, including polyphenols (such as gallic, caffeic, and ferulic acids), flavonoids (notably quercetin and rutin), and α-linolenic acid, all of which exert radical-scavenging, metal-chelating, and lipid-protective actions [53–55]. These compounds synergistically stabilize the lipid matrix by neutralizing reactive oxygen species, chelating transition metals that catalyze oxidation, and enhancing membrane integrity through amphiphilic interactions. Notably, while the synthetic antioxidant-treated group (SET) also effectively suppressed lipid peroxidation, its performance was occasionally surpassed by T3, particularly at the later stages of storage. This observation underscores the potent and sustained antioxidant activity of chia seed extract, likely due to its combination of phenolic acids and mucilage compounds that provide both hydrophilic and lipophilic protection. Consequently, CSE not only matches but in some cases exceeds the efficacy of conventional synthetic antioxidants in maintaining lipid stability in meat systems. These results reinforce the potential of chia seed extract as a natural, multifunctional preservative capable of replacing synthetic additives while enhancing both the nutritional and functional quality of processed meats.

These findings are consistent with recent evidence showing that plant-derived antioxidants, when applied at effective concentrations, can rival or even surpass the activity of synthetic preservatives such as BHT and BHA [54,55]. Moderate CSE inclusion levels (T1 and T2) provided intermediate protection, with TBARS values remaining below the rancidity threshold but increasing more gradually over storage. This outcome reinforces the dose-dependent antioxidant capacity of chia extracts. The steady rise in TBARS across all treatments—most pronounced in the control and lower CSE levels—reflects the inherent progression of oxidative reactions in refrigerated meat systems, primarily driven by the abundance of unsaturated fatty acids, oxygen exposure, and iron-catalyzed prooxidant activity [56].

Beyond direct oxidative control, CSE may also enhance water retention and structural stability by forming a hydrophilic fiber–mucilage network that limits oxygen diffusion and delays lipid degradation. This dual protective mechanism highlights CSE's multifunctionality, simultaneously improving oxidative resistance and maintaining textural quality during storage. From a technological and commercial standpoint, these attributes are highly relevant: the incorporation of CSE not only prolongs the shelf-life of meat products but also aligns with the growing demand for clean-label, naturally preserved foods. Its dual role as a preservative and functional ingredient, coupled with its rich nutraceutical profile, positions chia extract as a promising additive for developing healthier meat formulations targeted at quality-driven and health-conscious consumer markets [56–58].

While TBARS analysis provided clear evidence of lipid stabilization by chia seed extract, it primarily reflects secondary oxidation products. Future studies should incorporate complementary endpoints such as protein carbonyl quantification, volatile aldehydes (e.g., hexanal by GC–MS), and metal chelation assays to capture a more comprehensive picture of oxidative processes and confirm the dual lipid–protein protective effects of the extract.

In addition to the observed antioxidative and physicochemical benefits, future research should also include systematic microbiological assessments (total viable counts, psychrotrophs, Enterobacteriaceae, and yeasts/molds) under different storage and packaging systems (aerobic, vacuum, and MAP). This would provide a more comprehensive evaluation of chia seed extract's contribution to both chemical stability and microbiological safety in extending the shelf-life of beef burgers.

It should be noted that polyphenols in *Salvia hispanica* extract may chelate transition metals, potentially influencing non-heme iron bioaccessibility. Although the concentrations applied in this study align with clean-label practices, future research should evaluate iron bioavailability and heme stability to ensure that antioxidant benefits are not accompanied by unintended nutritional trade-offs.

Within the tested inclusion levels, chia seed extract (CSE) proved technologically effective in enhancing oxidative stability, color retention, and textural attributes of beef burgers while maintaining sensory acceptability. These results support its compatibility with clean-label objectives, providing a natural alternative to synthetic antioxidants such as BHT and BHA. Nevertheless, broader considerations are needed to consolidate its application in commercial systems. Standardization of the extract, including marker identification and batch-to-batch reproducibility, will be essential for industrial reliability. Additionally, further studies should define the optimal balance between antioxidant efficacy and sensory thresholds, supported by consumer acceptance testing. Expanding oxidative stability assessments to include protein oxidation and volatile compounds, alongside systematic microbiological monitoring under different packaging conditions, will provide a more complete picture of shelf-life extension. Safety aspects, including potential allergenicity, antinutritional factors, and effects on iron bioavailability, must also be evaluated to ensure nutritional adequacy. Finally, pilot-scale studies across different meat matrices (e.g., poultry, pork) and fat levels will help validate scalability and generalisability. Overall, the present findings highlight CSE as a promising candidate for clean-label meat preservation, with

future work focused on regulatory compliance and industrial feasibility to support broader adoption.

4. Limitations and Future Perspectives

This study provides strong evidence that chia seed extract (CSE) can improve oxidative stability, physicochemical properties, and shelf-life of beef burgers under refrigerated storage. Nonetheless, several limitations and safety considerations should be acknowledged to guide future research and facilitate potential industrial applications [59–61].

First, while thiobarbituric acid reactive substances (TBARS) effectively capture secondary lipid oxidation, this endpoint alone may not fully describe the oxidative dynamics within meat systems. Complementary measurements, such as protein carbonyls, volatile aldehydes (e.g., hexanal) by GC–MS, and metal chelation assays, would strengthen mechanistic interpretation by confirming both lipid and protein stabilization. Incorporating such broader oxidative endpoints across different food matrices will better elucidate the multifunctional role of chia-derived phenolics.

Second, microbiological quality remains a critical determinant of refrigerated shelf-life. Although CSE delayed lipid oxidation, it may not directly influence microbial proliferation. Systematic monitoring of total viable counts, psychrotrophs, Enterobacteriaceae, and yeasts/molds under various packaging systems (aerobic, vacuum, and modified-atmosphere packaging) is essential to define the extent to which CSE contributes to safe shelf-life extension [62,63].

Third, extract preparation and standardization warrant further refinement. In this study, aqueous extraction was employed for food-processing feasibility; however, phenolic profiling by HPLC–DAD provided only tentative identification. Confirmation of marker compounds via LC–MS/MS, alongside reporting of batch-to-batch variation and establishing specifications for total phenolics (mg GAE g^{-1}) and key marker ranges, would improve reproducibility and industrial scalability. Such standardization is essential for ensuring consistent antioxidant efficacy in commercial applications. Dose optimization is another important consideration. While the 1.5% inclusion level yielded the greatest antioxidant benefit, practical application must balance functional efficacy with potential sensory thresholds such as bitterness or astringency. Estimating phenolic intake per serving and conducting larger-scale consumer acceptance studies ($n \geq 50$) will help establish optimal inclusion levels that ensure both technological performance and consumer acceptability. Nutritional interactions also merit careful evaluation. Polyphenols are known to chelate transition metals and may reduce non-heme iron bioaccessibility. While the concentrations used here are typical of clean-label applications, future studies should examine potential effects on iron bioavailability and heme stability to confirm that nutritional quality is not inadvertently compromised.

Safety and allergenicity are further important aspects. Although chia is generally recognized as safe (GRAS) in several jurisdictions, rare allergic reactions to chia proteins have been reported, with possible cross-reactivity to other seeds. Moreover, water-soluble antinutrients such as oxalates, phytates, and tannins may be partially retained in aqueous extracts. Targeted determination of these compounds and estimation of their contribution per burger serving will help refine the safety profile of CSE.

Finally, regulatory and labelling frameworks vary internationally. Chia seed and its derivatives are approved for use in specific categories under “novel food” regulations in the European Union and as GRAS in the United States; however, broader applications in processed meat products may require additional safety assessment and formal approval. Transparent labelling, including potential allergen statements, will be essential to ensure consumer trust and regulatory compliance. In summary, while CSE demonstrates clear promise as a multifunctional, clean-label preservative for meat systems, future research should address these limitations by integrating advanced analytical endpoints, microbiological monitoring, standardized extract specifications, consumer sensory testing, nutritional assessments, and regulatory alignment. Such efforts will support the safe, scalable, and market-ready adoption of chia-based antioxidants in the food industry.

This study confirmed the efficacy of chia seed extract (CSE) in enhancing the oxidative stability, texture, and shelf-life of beef burgers; however, certain limitations should be acknowledged. The findings are based on a single beef burger formulation under aerobic refrigerated storage, which may not fully capture the variability of commercial meat systems. Replication across species (poultry, pork), fat levels, and packaging types (vacuum, MAP), along with pilot-scale trials, is necessary to validate generalisability and industrial feasibility. From a regulatory perspective, chia-derived ingredients are classified as “novel foods” in some regions, with varying approval and labelling requirements, including allergen declarations due to rare cases of cross-reactivity. Standardization of extract composition and further toxicological validation will be essential to support regulatory acceptance. Addressing these aspects will help position CSE as a safe, effective, and clean-label antioxidant for commercial meat preservation.

5. Conclusion

Incorporation of chia seed (*Salvia hispanica* L.) extract significantly enhanced the physicochemical quality, oxidative stability, and shelf-life of beef burgers during refrigerated storage. The 1.5% extract level (T3) was most effective, reducing lipid oxidation, improving texture, and maintaining desirable color and moisture. These effects are attributed to the synergistic action of phenolic acids, flavonoids, and omega-3 fatty acids present in the extract. The findings demonstrate chia seed extract’s potential as

a clean-label, natural alternative to synthetic antioxidants such as BHT and BHA, supporting its use in the development of functional and sustainable meat products.

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

EHA—Conceptualization, methodology, formal analysis, investigation, data curation, writing – original draft, and review & editing. The author read and approved the final manuscript. The author has participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The author declares no conflict of interest.

References

[1] Melo JM de, Melo RN de, Sicheski SJ, Daniel BI, Perissinotto A, Janeczko MU, *et al*. Elaboration and evaluation of produced hamburger with meat of old sheep and pig with added chia seed (Salvia hispanica). International Journal of Nutrition and Food Sciences. 2014; 4: 14–18. https://doi.org/10.11648/j.ijnfs.20150401.13.

[2] Goodpaster BH, Sparks LM. Metabolic Flexibility in Health and Disease. Cell Metabolism. 2017; 25: 1027–1036. https://doi.org/10.1016/j.cmet.2017.04.015.

[3] GBD 2017 Risk Factor Collaborators. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet (London, England). 2018; 392: 1923–1994. https://doi.org/10.1016/S0140-6736(18)32225-6.

[4] Mokgalaboni K, Phoswa WN. *Corchorus olitorius* extract exhibit anti-hyperglycemic and anti-inflammatory properties in rodent models of obesity and diabetes mellitus. Frontiers in Nutrition. 2023; 10: 1099880. https://doi.org/10.3389/fnut.2023.1099880.

[5] Skinner AC, Ravanbakht SN, Skelton JA, Perrin EM, Armstrong SC. Prevalence of Obesity and Severe Obesity in US Children, 1999–2016. Pediatrics. 2018; 141: e20173459. https://doi.org/10.1542/peds.2017-3459.

[6] Pintado T, Herrero AM, Ruiz-Capillas C, Jiménez-Colmenero F. Chia (Salvia hispanica L.) products as ingredients for reformulating frankfurters: Effects on quality properties and shelf-life. Meat Science. 2019; 156: 139–145. https://doi.org/10.1016/j.meatsci.2019.05.028.

[7] WHO CVD Risk Chart Working Group. World Health Organization cardiovascular disease risk charts: revised models to estimate risk in 21 global regions. The Lancet. Global Health. 2019; 7: e1332–e1345. https://doi.org/10.1016/S2214-109X(19)30318-3.

[8] Chicco AG, D'Alessandro ME, Hein GJ, Oliva ME, Lombardo YB. Dietary chia seed (Salvia hispanica L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. The British Journal of Nutrition. 2009; 101: 41–50. https://doi.org/10.1017/S000711450899053X.

[9] Kulczyński B, Kobus-Cisowska J, Taczanowski M, Kmiecik D, Gramza-Michałowska A. The Chemical Composition and Nutritional Value of Chia Seeds-Current State of Knowledge. Nutrients. 2019; 11: 1242. https://doi.org/10.3390/nu11061242.

[10] Gómez-Favela MA, Gutiérrez-Dorado R, Cuevas-Rodríguez EO, Canizalez-Román VA, Del Rosario León-Sicairos C, Milán-Carrillo J, *et al*. Improvement of Chia Seeds with Antioxidant Activity, GABA, Essential Amino Acids, and Dietary Fiber by Controlled Germination Bioprocess. Plant Foods for Human Nutrition. 2017; 72: 345–352. https://doi.org/10.1007/s11130-017-0631-4.

[11] Mohammadpour H, Sadrameli SM, Eslami F, Asoodeh A. Optimization of ultrasound-assisted extraction of Moringa peregrina oil using response surface methodology and comparison with Soxhlet extraction. Industrial Crops and Products. 2019; 131: 106–116. https://doi.org/10.1016/j.indcrop.2019.01.030.

[12] Vuksan V, Jenkins AL, Brissette C, Choleva L, Jovanovski E, Gibbs AL, *et al*. Salba-chia (Salvia hispanica L.) in the treatment of overweight and obese patients with type 2 diabetes: A double-blind randomized controlled trial. Nutrition, Metabolism, and Cardiovascular Diseases: NMCD. 2017; 27: 138–146. https://doi.org/10.1016/j.numecd.2016.11.124.

[13] Bacon L, Aphramor L. Weight science: evaluating the evidence for a paradigm shift. Nutrition Journal. 2011; 10: 9. https://doi.org/10.1186/1475-2891-10-9.

[14] Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner CD, *et al*. Efficacy and safety of low-carbohydrate diets: a systematic review. JAMA. 2003; 289: 1837–1850. https://doi.org/10.1001/jama.289.14.1837.

[15] Buckley JD, Howe PRC. Long-chain omega-3 polyunsaturated fatty acids may be beneficial for reducing obesity-a review. Nutrients. 2010; 2: 1212–1230. https://doi.org/10.3390/nu2121212.

[16] Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. JAMA. 2005; 293: 43–53. https://doi.org/10.1001/jama.293.1.43.

[17] Johnston BC, Kanters S, Bandayrel K, Wu P, Naji F, Siemieniuk RA, *et al*. Comparison of weight loss among named diet programs in overweight and obese adults: a meta-analysis. JAMA. 2014; 312: 923–933. https://doi.org/10.1001/jama.2014.10397.

[18] Katsuki A, Urakawa H, Gabazza EC, Murashima S, Nakatani K, Togashi K, *et al*. Circulating levels of active ghrelin is associated with abdominal adiposity, hyperinsulinemia and insulin resistance in patients with type 2 diabetes mellitus. European Journal of Endocrinology. 2004; 151: 573–577. https://doi.org/10.1530/eje.0.1510573.

[19] Post RE, Mainous AG, 3rd, King DE, Simpson KN. Dietary fiber for the treatment of type 2 diabetes mellitus: a meta-analysis. Journal of the American Board of Family Medicine:

JABFM. 2012; 25: 16–23. https://doi.org/10.3122/jabfm.2012.01.110148.
[20] Valdivia-López MÁ, Tecante A. Chia (Salvia hispanica): A Review of Native Mexican Seed and its Nutritional and Functional Properties. Advances in Food and Nutrition Research. 2015; 75: 53–75. https://doi.org/10.1016/bs.afnr.2015.06.002.
[21] Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, *et al*. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. The New England Journal of Medicine. 2009; 360: 859–873. https://doi.org/10.1056/NEJMoa0804748.
[22] Van Gaal L, Scheen A. Weight management in type 2 diabetes: current and emerging approaches to treatment. Diabetes Care. 2015; 38: 1161–1172. https://doi.org/10.2337/dc14-1630.
[23] Vuksan V, Whitham D, Sievenpiper JL, Jenkins AL, Rogovik AL, Bazinet RP, *et al*. Supplementation of conventional therapy with the novel grain Salba (Salvia hispanica L.) improves major and emerging cardiovascular risk factors in type 2 diabetes: results of a randomized controlled trial. Diabetes Care. 2007; 30: 2804–2810. https://doi.org/10.2337/dc07-1144.
[24] El Sheikha AF, Allam AY, Taha M, Varzakas T. How does the addition of biostimulants affect the growth, yield, and quality parameters of the snap bean (*Phaseolus vulgaris L.*)? How is this reflected in its Nutritional Value? Applied Sciences. 2022; 12: 776. https://doi.org/10.3390/app12020776.
[25] Al Jumayi HA, Allam AY, El-Beltagy AED, Algarni EH, Mahmoud SF, El Halim Kandil AA. Bioactive Compound, Antioxidant, and Radical Scavenging Activity of Some Plant Aqueous Extracts for Enhancing Shelf Life of Cold-Stored Rabbit Meat. Antioxidants (Basel, Switzerland). 2022; 11: 1056. https://doi.org/10.3390/antiox11061056.
[26] Grancieri M, Martino HSD, Gonzalez de Mejia E. Chia Seed (Salvia hispanica L.) as a Source of Proteins and Bioactive Peptides with Health Benefits: A Review. Comprehensive Reviews in Food Science and Food Safety. 2019; 18: 480–499. https://doi.org/10.1111/1541-4337.12423.
[27] Zare T, Fournier-Level A, Ebert B, Roessner U. Chia (Salvia hispanica L.), a functional 'superfood': new insights into its botanical, genetic and nutraceutical characteristics. Annals of Botany. 2024; 134: 725–746. https://doi.org/10.1093/aob/mcae123.
[28] Reyes-Caudillo E, Tecante A, Valdivia-López MA. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. Food Chemistry. 2008; 107: 656–663. https://doi.org/10.1016/j.foodchem.2007.08.062.
[29] Ixtaina VY, Martínez ML, Spotorno V, Mateo CM, Maestri DM, Diehl BW, *et al*. Characterization of chia seed oils obtained by pressing and solvent extraction. Journal of Food Composition and Analysis. 2011; 24: 166–174. https://doi.org/10.1016/j.jfca.2010.08.006.
[30] Abbas AA, Al-Zaidi RMK, Thabit ZA. Improving the functional properties and prolonging the shelf life of locally made burgers by adding chia seed gel. Natural and Engineering Sciences. 2025; 10: 490–506. https://doi.org/10.28978/nesciences.1660362.
[31] Capitani MI, Spotorno V, Nolasco SM, Tomás MC. Physicochemical and functional characterization of by-products from chia (Salvia hispanica L.) seeds of Argentina. LWT-Food Science and Technology. 2012; 45: 94–102. https://doi.org/10.1016/j.lwt.2011.07.012.
[32] Choe E, Min DB. Mechanisms and factors for edible oil oxidation. Comprehensive Reviews in Food Science and Food Safety. 2006; 5: 169–186. https://doi.org/10.1111/j.1541-4337.2006.00009.x.
[33] Coorey R, Tjoe A, Jayasena V. Gelling properties of chia seed and flour. Journal of Food Science. 2014; 79: E859–E866. https://doi.org/10.1111/1750-3841.12444.
[34] Domínguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W, Lorenzo JM. A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. Antioxidants (Basel, Switzerland). 2019; 8: 429. https://doi.org/10.3390/antiox8100429.
[35] Motyka S, Koc K, Ekiert H, Blicharska E, Czarnek K, Szopa A. The current state of knowledge on Salvia hispanica and Salviae hispanicae semen (chia seeds). Molecules. 2022; 27: 1207. https://doi.org/10.3390/molecules27041207.
[36] Motyka S, Skała E, Ekiert H, Szopa A. Health-promoting approaches of the use of chia seeds. Journal of Functional Foods. 2023; 103: 105480. https://doi.org/10.1016/j.jff.2023.105480.
[37] Allam AY. Effect of addition loquat (*Eribotrya japonica*) seed powder extract as a natural bioactive compound on the quality characteristics of goat meat nuggets during refrigerated storage–Part I. Journal of Food and Dairy Sciences. 2023; 14: 51–62. https://doi.org/10.21608/jfds.2023.192707.1099.
[38] Kaur N, Kaur S, Agarwal A, Sabharwal M, Tripathi AD. Amaranthus Crop for Food Security and Sustainable Food Systems. Planta. 2024; 260: 59. https://doi.org/10.1007/s00425-024-04490-3.
[39] Ali E, Zhang K. CRISPR-mediated technology for seed oil improvement in rapeseed: Challenges and future perspectives. Frontiers in Plant Science. 2023; 14: 1086847. https://doi.org/10.3389/fpls.2023.1086847.
[40] Allam AY, Khan ZS, Bhat MS, Naik B, Wani SA, Rustagi S, *et al*. Chemical, physical, and technological characteristics of palm olein and canola oil blends. Journal of Food Quality. 2023; 2023: 6503667. https://doi.org/10.1155/2023/6503667.
[41] Sheikha AFE, Allam AY, Oz E, Khan MR, Proestos C, Oz F. Edible Xanthan/Propolis Coating and Its Effect on Physicochemical, Microbial, and Sensory Quality Indices in Mackerel Tuna (*Euthynnus affinis*) Fillets during Chilled Storage. Gels (Basel, Switzerland). 2022; 8: 405. https://doi.org/10.3390/gels8070405.
[42] Allam AY, Tarighat MA, Abdi G, Roumia AF. Utilization of chicory (*Cichorium intybus* L.) and purslane (Portulaca oleracea L.) leaf extracts for the preservation of goat meat sausage. Journal of Food Processing and Preservation. 2024; 1857290. https://doi.org/10.1155/jfpp/1857290.
[43] El Sheikha AF, Allam AY, ElObeid T, Basiouny EA, Abdelaal AA, Amarowicz R, *et al*. Impact of a Carboxymethyl Cellulose Coating Incorporated with an Ethanolic Propolis Extract on the Quality Criteria of Chicken Breast Meat. Antioxidants (Basel, Switzerland). 2022; 11: 1191. https://doi.org/10.3390/antiox11061191.
[44] Sethi S, Joshi A, Arora B, Bhowmik A, Sharma RR, Kumar P. Significance of FRAP, DPPH, and CUPRAC assays for antioxidant activity determination in apple fruit extracts. European Food Research and Technology. 2020; 246: 591–598. https://doi.org/10.1007/s00217-020-03432-z.
[45] Ahmadizadeh M, Rezaee S, Heidari P. Genome-wide characterization and expression analysis of fatty acid desaturase gene family in *Camelina sativa*. Gene Reports. 2020; 21: 100894. https://doi.org/10.1016/j.genrep.2020.100894.
[46] Adegbola PI, Adetutu A, Olaniyi TD. Antioxidant activity of Amaranthus species from the Amaranthaceae family—A review. South African Journal of Botany. 2020; 133: 111–117. https://doi.org/10.1016/j.sajb.2020.07.003.
[47] Muñoz-Tébar N, Carmona M, de Elguea-Culebras GO, Molina A, Berruga MI. Chia seed mucilage edible films with Origanum vulgare and Satureja montana essential oils: Characterization and antifungal properties. Membranes. 2022; 12: 213. https://doi.org/10.3390/membranes12020213.
[48] Özcan MM, Al-Juhaimi FY, Ahmed IAM, Osman MA, Gassem MA. Effect of different microwave power settings on quality

of chia seed oil obtained by cold pressing. Food Chemistry. 2019; 278: 190–196. https://doi.org/10.1016/j.foodchem.2018.11.048.

[49] Coelho MS, Salas-Mellado MM. Chemical characterization of chia (Salvia hispanica L.) for use in food products. Journal of Food and Nutrition Research. 2015; 3: 263–269. https://doi.org/10.12691/jfnr-2-5-9.

[50] Nyingi JW, Mburu M. Chia (Salvia hispanica L.) seeds: Phytochemicals, bioactive compounds, and applications. European Journal of Agriculture and Food Sciences. 2021; 3: 1–12. https://doi.org/10.24018/ejfood.2021.3.6.381.

[51] Özcan MM, Al-Juhaimi FY, Ahmed IAM, Osman MA, Gassem MA. Effect of Soxhlet and cold press extraction on physicochemical characteristics of roasted and non-roasted chia seed oils. Journal of Food Measurement and Characterization. 2019; 13: 648–655. https://doi.org/10.1007/s11694-018-9977-z.

[52] Karrar E, Ahmed IAM, Wei W, Sarpong F, Proestos C, Amarowicz R, *et al*. Characterization of Volatile Flavor Compounds in Supercritical Fluid Separated and Identified in Gurum (*Citrullus lanatus* Var. *colocynthoide*) Seed Oil Using HSME and GC-MS. Molecules (Basel, Switzerland). 2022; 27: 3905. https://doi.org/10.3390/molecules27123905.

[53] Ixtaina VY, Nolasco SM, Tomás MC. Physical properties of Chia (Salvia hispanica L.) seeds. Industrial Crops and Products. 2008; 28: 286–293. https://doi.org/10.1016/j.indcrop.2008.03.009.

[54] Aditya NP, Espinosa YG, Norton IT. Encapsulation systems for the delivery of hydrophilic nutraceuticals: food application. Biotechnology Advances. 2017; 35: 450–457. https://doi.org/10.1016/j.biotechadv.2017.03.012.

[55] Rahman MJ, de Camargo AC, Shahidi F. Phenolic and polyphenolic profiles of chia seeds and their in vitro biological activities. Journal of Functional Foods. 2017; 35: 622–634. https://doi.org/10.1016/j.jff.2017.06.044.

[56] Oliveira-Alves SC, Vendramini-Costa DB, Betim Cazarin CB, Maróstica Júnior MR, Borges Ferreira JP, Silva AB, *et al*. Characterization of phenolic compounds in chia (Salvia hispanica L.) seeds, fiber flour and oil. Food Chemistry. 2017; 232: 295–305. https://doi.org/10.1016/j.foodchem.2017.04.002.

[57] Rokayya S, Li CJ, Zhao Y, Li Y, Sun CH. Cabbage (Brassica oleracea L. var. capitata) phytochemicals with antioxidant and anti-inflammatory potential. Asian Pacific Journal of Cancer Prevention: APJCP. 2013; 14: 6657–6662. https://doi.org/10.7314/apjcp.2013.14.11.6657.

[58] Scapin G, Abaide ER, Nunes LF, Mazutti MA, Vendruscolo RG, Wagner R, *et al*. Effect of pressure and temperature on the quality of chia oil extracted using pressurized fluids. Journal of Supercritical Fluids. 2017; 127: 90–96. https://doi.org/10.1016/j.supflu.2017.03.030.

[59] Constantinou C, Papas A, Constantinou AI. Vitamin E and cancer: An insight into the anticancer activities of vitamin E isomers and analogs. International Journal of Cancer. 2008; 123: 739–752. https://doi.org/10.1002/ijc.23689.

[60] Nesaretnam K, Yew WW, Wahid MB. Tocotrienols and cancer: beyond antioxidant activity. European Journal of Lipid Science and Technology. 2007; 109: 445–452. https://doi.org/10.1002/ejlt.200600212.

[61] Kuksal N, Chalker J, Mailloux RJ. Progress in understanding the molecular oxygen paradox - function of mitochondrial reactive oxygen species in cell signaling. Biological Chemistry. 2017; 398: 1209–1227. https://doi.org/10.1515/hsz-2017-0160.

[62] Segura-Campos MR, Ciau-Solís N, Rosado-Rubio G, Chel-Guerrero L, Betancur-Ancona D. Physicochemical characterization of chia (Salvia hispanica L.) seed oil from Yucatán, Mexico. Agricultural Sciences. 2014; 5: 220–226. https://doi.org/10.4236/as.2014.53025.

[63] Shen Y, Zheng L, Jin J, Li X, Fu J, Wang M, *et al*. Phytochemical and biological characteristics of Mexican chia seed oil. Molecules. 2018; 23: 3219. https://doi.org/10.3390/molecules23123219.