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Chia Seed Powder as a Functional Ingredient in Wheat Bread: Nutritional Enrichment, Dough Functionality, and Quantitative Structure–Function Relationships

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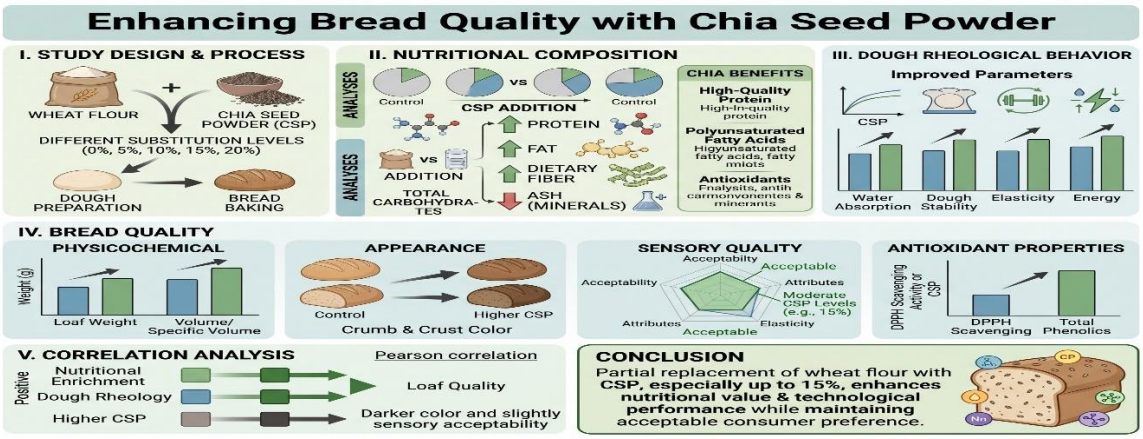
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Abstract:

The addition of nutrient-rich plant ingredients to wheat bread is a way to improve its nutritional value while keeping the technological and sensory properties acceptable. This study investigated the effect of chia seed powder (CSP) as a multifunctional ingredient in Balady bread by replacing wheat flour at levels of 0, 7.5, 10 and 15% (w/w). The nutritional, phytochemical, antioxidant, rheological, physical, color and sensory properties of the developed breads were investigated with a special emphasis on the quantitative relationships between quality parameters. Chia seed powder exhibited a nutrient-dense composition characterized by high protein, lipid, dietary fiber, mineral, and phenolic contents, together with a favorable essential amino acid profile and high α-linolenic acid content. Increasing CSP incorporation progressively increased protein, lipid, dietary fiber, and ash contents of bread while decreasing its relative carbohydrate proportion. CSP incorporation also increased water absorption, dough stability, maximum resistance, and dough energy, accompanied by increases in loaf weight, loaf volume, and specific volume. These rheological modifications were strongly and quantitatively correlated to loaf development, especially for dough stability and maximum resistance. Increasing CSP levels decreased crust and crumb lightness, and sensory evaluation showed good acceptance of all formulations, with 15% CSP remaining within the acceptable sensory range. Correlation and hierarchical clustering analyze further revealed coordinated relationships linking nutritional enrichment, dough functionality, loaf development, color and sensory response, revealing a nutritional-technological versus appearance-sensory trade-off. The results suggest that CSP can be used as a multifunctional ingredient in the production of nutritionally enriched Balady bread with good technological performance and sensory acceptability.

Key words: *chia; bread; rheology; nutrition; antioxidants; sensory*

Graphical abstract



1. Introduction

Recently, there has been an increased development of functional cereal products enriched with bioactive ingredients, due to the consumers' interest for foods with health benefits beyond the basic nutrition. Bread is one of the world's most consumed staple foods because it is affordable, accessible and makes a major contribution to daily energy intake. Bread is the most consumed food around the world, and hence, it is a good matrix to improve the diet quality by adding nutrient-dense ingredients, increasing the nutritional and functional value of bread, while maintaining the desired processing properties and panel acceptance [1–3].

become an important strategy for producing healthier bakery products. Various raw materials, including legumes, cereal fractions, dairy-derived ingredients, and edible seeds, have been investigated as partial substitutes for wheat flour to improve the nutritional profile of bread. Among these alternatives, oilseeds such as chia, flaxseed, sunflower, and pumpkin have attracted considerable attention because they provide high levels of protein, dietary fiber, minerals, essential fatty acids, and naturally occurring antioxidant compounds that may simultaneously improve the nutritional quality and technological performance of baked products (Adamczyk et al., 2021; Ceglińska et al., 2018).

Chia (*Salvia hispanica* L.), a member of the Lamiaceae family, has emerged as one of the most promising functional ingredients for cereal-based foods. Its seeds are characterized by a unique nutritional composition that includes high-quality protein with a favorable amino acid balance, abundant dietary fiber, substantial concentrations of α-linolenic acid (ω-3), essential minerals, and numerous phenolic compounds with antioxidant activity (Fernandes et al., 2023; Madruga et al., 2020; Sana et al., 2024 ;Din et al., 2021; Knez Hrnčič et al., 2019; Rahman et al., 2017)). In addition to their nutritional importance, chia seeds contain a soluble mucilage capable of absorbing large quantities of water, giving them hydrocolloid-like functionality that can modify dough hydration, mixing behavior, gas retention, and the structural characteristics of bread (Capitani et al., 2012; Kulczyński et al., 2019; Lazaro et al., 2018) .

Beyond their technological functionality, chia seeds have been associated with several health-promoting properties attributed to their bioactive constituents. Their high levels of polyunsaturated fatty acids, dietary fiber, and phenolic compounds have stimulated increasing interest in their incorporation into cereal-based foods intended to provide improved nutritional quality and additional physiological benefits. Therefore, chia has been approved for use in different food products, and several studies have investigated the use of chia in bread and other bakery products to improve nutritional density while maintaining acceptable product quality (Adamczyk et al., 2021; Romankiewicz et al., 2017). Previous studies have shown that chia supplementation can improve bread by increasing protein, dietary fiber, essential fatty acids, minerals and antioxidant capacity. However, the extent of such improvements is highly dependent on the degree of substitution as over-incorporation can alter the rheological properties of the dough, loaf structure, crumb properties and sensory attributes (Miranda-Ramos et al., 2020). Most of the published studies have been directed toward the study of individual quality parameters, but relatively few studies have been directed toward the simultaneous study of the interactions among the nutritional composition, dough rheology, bread quality, antioxidant characteristics and panel acceptance in a single experimental study.

Therefore, the present study evaluated the potential of chia seed powder (CSP) as a multifunctional ingredient for wheat bread by partially replacing wheat flour at four substitution levels (0, 7.5, 10, and 15%). Rather than assessing CSP enrichment solely through conventional comparisons of bread quality attributes, the study integrated nutritional composition, phytochemical and antioxidant characteristics, dough rheology, loaf properties, instrumental color, and sensory response to elucidate quantitative structure–function relationships across the bread-making system. Targeted correlation and regression analyzes and hierarchical clustering were carried out to understand how CSP-associated compositional changes related to dough functionality, loaf development and colour-sensory responses. The integrated approach provides a quantitative framework to evaluate the functional contribution of CSP and to identify a substitution level balancing nutritional enrichment, technological performance and sensory acceptability. The novelty of this work is in addressing CSP enrichment as a structure–function problem, rather than as a fortification strategy, thus providing a quantitative link between the compositional features of CSP and dough functionality, loaf development and sensory response.

2. MATERIALS AND METHODS

2.1. Materials

Chia seeds (*Salvia hispanica* L.) were obtained from a local market in Tanta City, Gharbiya Governorate, Egypt. Commercial wheat flour (extraction 82%) and instant dry yeast were obtained from the same source and food-grade sodium chloride was used. All chemicals and analytical reagents used for the physicochemical, phytochemical, antioxidant, and instrumental analyses were of analytical grade.

2.2. Preparation of chia seeds powder and blends

Whole chia seeds were milled using a laboratory grinder (Retsch, Germany) equipped with a 20-mesh sieve to produce a uniform chia seed powder (CSP). The powder obtained was packed in airtight polyethylene bags and stored at 5 ± 2°C until analyzed according to the procedure of (Mihafu et al., 2020).

2.3. Bread formulation

Four bread formulations were prepared by partially replacing wheat flour with chia seed powder (CSP) at 0, 7.5, 10, and 15% (w/w). The control formulation contained wheat flour without CSP, whereas the remaining formulations contained CSP at the corresponding substitution levels. Instant dry yeast and sodium chloride were maintained at 0.5 and 1.5 g/100 g flour basis, respectively, in all formulations. A fixed amount of water (60 g/100 g flour basis) was used for all formulations. The detailed formulation of the bread samples is presented in Table 1. For bread preparation, the wheat flour–CSP blend was mixed manually with sodium chloride, yeast, and the predetermined amount of water for 6 min. The resulting dough was bulk fermented at 30°C and 85% relative humidity for 1 h. The dough was divided into 100 g portions after fermentation and shaped. The shaped dough pieces were rested for 45 min at 30°C and proofed for 15 min at 30-35°C and 85% relative humidity. The proofed dough pieces were then baked at 400°C for 2 min. After baking, the loaves were cooled at room temperature for approximately 1 h before further physical, chemical, color, and sensory analyses.

Table 1. Formulation of Balady bread supplemented with different levels of chia seed Powder (CSP)

Ingredient (g/100 g flour basis)	**Control (0% CSP)**	**7.5% CSP**	**10% CSP**	**15% CSP**
Wheat flour (82% extraction)	100.0	92.5	90.0	85.0
Chia seed powder (CSP)	0.0	7.5	10.0	15.0
Dry yeast	0.5	0.5	0.5	0.5
Sodium chloride	1.5	1.5	1.5	1.5
Water	60.0	60.0	60.0	60.0
Total formulation	**162.0**	**162.0**	**162.0**	**162.0**

Where: CSP = Chia seed powder.

2.4. Water addition

A constant water addition of 60 g/100 g flour basis was used during bread preparation for all formulations to maintain identical processing conditions and allow direct comparison among CSP substitution levels. This fixed water level was independent of the farinograph water-absorption requirement. Farinograph water absorption was determined separately for each wheat flour–CSP blend as an instrumental rheological parameter according to the applicable AACC International method Fig 1.

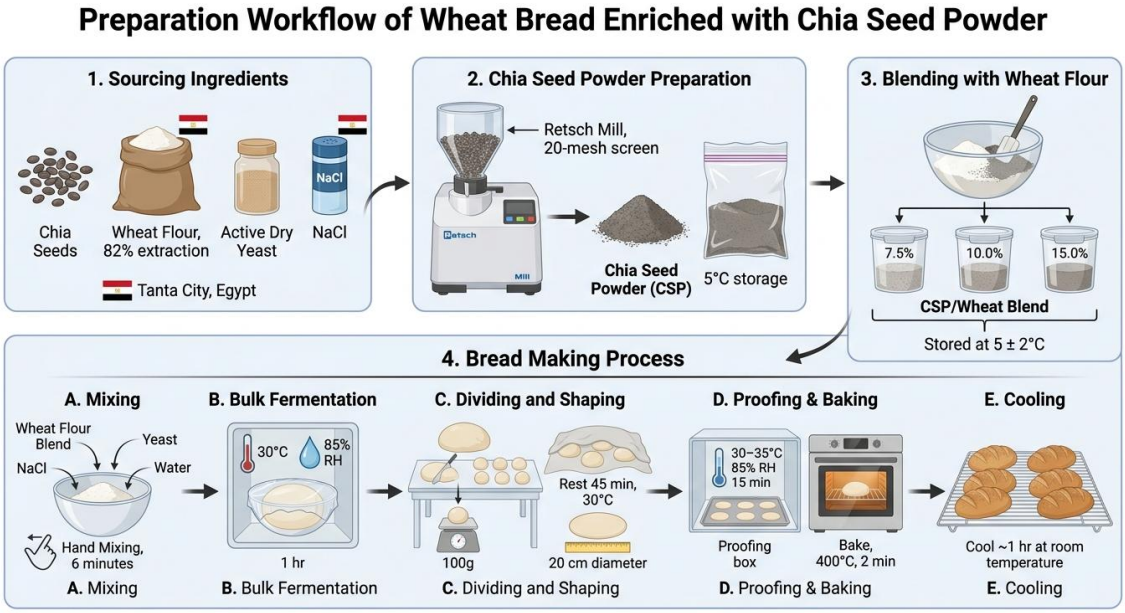


Figure 1. Preparation workflow of bread enriched with chia seed powder

2.5. Chemical analysis

The proximate composition of wheat flour (WF), chia seed powder (CSP), and bread samples was determined according to the official methods of the Association of Official Analytical Chemists [4] . Moisture, crude protein, crude fat (ether extract), crude fiber, and ash contents were measured using the corresponding standard procedures.

Total carbohydrate content was calculated by difference using the following equation:

Carbohydrates (%) =100 − (Moisture+ Protein+ Fat+ Ash+ Crude fiber)

The energy value was estimated according to the Atwater conversion factors described by (AOAC, 2000) using the following equation:

Energy (kcal/100 g) = (4 ×Protein) + (4 ×Carbohydrate) +(9 ×Fat)

2.6. Mineral Analysis: Mineral composition was determined following the [4] procedure. Briefly, 0.5 g of each sample was digested using a sulfuric acid–water digestion mixture. The concentrations of magnesium (Mg), calcium (Ca), sodium (Na), potassium (K), copper (Cu), manganese (Mn), iron (Fe), phosphorus (P), and zinc (Zn) were quantified using an atomic absorption spectrophotometer (AA-6650, Shimadzu Corporation, Kyoto, Japan). All measurements were performed in triplicate.

2.7. Amino Acid Analysis: The amino acid profile was determined according to the procedure described by [5]. Approximately 100 mg of sample was hydrolyzed with 10 mL of 6 N HCl containing 0.1% mercaptoethanol in sealed hydrolysis tubes at 110°C for 24 h. After cooling to room temperature, the hydrolysate was filtered through Whatman No. 1 filter paper and diluted to 25 mL with distilled water. A 5 mL aliquot evaporated to dryness under vacuum in the presence of potassium hydroxide. The residue was dissolved in 1 mL

sodium citrate buffer (pH 2.2), stored at 4°C, and analyzed using an automated amino acid analyzer (Beckman Model 119 CL, Beckman Instruments, Fullerton, CA, USA).

2.8. Determination of Nutritional Parameters of Protein

The nutritional quality of chia seed protein was assessed using the Amino Acid Score (AAS), Computed Protein Efficiency Ratio (C-PER), Biological Value (BV), Essential Amino Acid Index (EAAI), and Nutritional Index (NI).

2.8.1. Amino Acid Score (AAS)

The amino acid score was calculated according to the FAO/WHO [6] reference amino acid pattern as follows:

$$\text{AAS (\%)} = \frac{mg\ of\ essential\ amino\ acid\ in\ 1g\ test\ protein}{mg\ of\ the\ same\ amino\ acid\ in\ \text{FAO/WHO}\ reference\ protein}\ x\ 100$$

The amino acid with the lowest score was considered the first limiting amino acid.

2.8.2. Computed Protein Efficiency Ratio (C-PER)

The Computed Protein Efficiency Ratio was estimated according to [7] using the following equation

C-PER = - 0.468 + 0.454 (Leucine) – 0.105 (Tyrosine)

where leucine and tyrosine are expressed as g/100 g protein.

2.8.3. Biological Value (BV)

Biological value was calculated according to [8] using the following equation:

$$\text{BV} = 49.09 + 10.53(\text{C} - \text{PER})$$

2.8.4. Essential Amino Acid Index (EAAI)

The Essential Amino Acid Index (EAAI) was calculated according to[9], using egg protein as the reference standard:

$$\mathbf{EAAI} = \left(\prod_{i=1}^{n} \frac{AA_i}{AA_{ri}}\right)^{1/n} \times 100$$

Where,

- n = number of essential amino acids
- AA_i = concentration of the *i*th essential amino acid in the test protein
- AA_{ri} = concentration of the corresponding amino acid in the reference (egg) protein.

2.8.5. Nutritional Index (NI)

The Nutritional Index (NI) was calculated as follows:

$$NI = EAAI \times \frac{\text{Protein content}(\%)}{100}$$

2.9. Phytochemical Composition and Antioxidant Activity

The phytochemical profile of chia seed powder (CSP) was characterized by determining total phenolic content (TPC), tannins, phytic acid, oxalate, antioxidant activity, and individual phenolic compounds using established analytical methods.

2.9.1. Extraction of Phytochemicals

Phenolic compounds were extracted according to the method of [10] with slight modifications. Briefly, 1.0 g of finely ground CSP was mixed with 20 mL of 80% methanol and shaken at room temperature for 2 h. The suspension was centrifuged at 5,000 × g for 15 min, and the supernatant was collected for the determination of total phenolic content and antioxidant activity.

2.9.2. HPLC Analysis of Individual Phenolic Compounds

Individual phenolic compounds in chia seed powder (CSP) were identified and quantified by high performance liquid chromatography (HPLC) according to the method of [11] with slight modifications. Compound identification was based on comparison of the retention times and UV spectra of the detected compounds with those of authentic reference standards. The concentrations of the identified phenolic compounds were expressed as mg/100 g dry weight. All determinations were performed in triplicate, and the results were reported as mean ± standard deviation (SD).

2.9.3. Antioxidant Activity

The antioxidant activity of CSP extracts was measured by the DPPH radical-scavenging assay [12] and the ABTS radical-cation decolorization assay [13]. The results are reported as antioxidant activity/equivalent units where appropriate for each assay. All determinations were performed in triplicate and expressed as mean ± standard deviation (SD).

2.10. Rheological Properties of Dough

Dough rheological properties were evaluated using a Brabender Farinograph and Brabender Extensograph (Brabender GmbH & Co. KG, Duisburg, Germany) according to the approved methods of the AACC International [14]. Farinograph measurements included water absorption, dough development time, dough stability, degree of softening, and farinograph quality number. Extensograph analysis comprised extensibility, resistance to extension, proportional number, and dough energy.

2.11. Physical Properties of Bread

2.11.1. Loaf weight: After cooling to room temperature, loaf weight was determined using a digital laboratory balance according to [15] procedures.

2.11.2. Loaf volume: Loaf volume was determined using the rapeseed displacement method described by (Mesías et al., 2023). The volume of the empty baking pan was first measured with rapeseed. Following baking and loaf removal, the remaining pan volume was refilled with rapeseed. Bread volume was calculated from the difference between the two measurements.

2.11.3. Specific Volume: Specific volume ($cm^3\ g^{-1}$) was calculated as the ratio of loaf volume to loaf weight according to (Mesías et al., 2023).

2.11.4. Loaf Diameter: Loaf diameter was measured using a graduated ruler following the standard procedure described by [15]

2.11.5. Color Characteristics: Crust and crumb color were measured using a HunterLab ColorFlex EZ spectrocolorimeter (HunterLab, Reston, VA, USA) according to (Feng et al., 2013) . Before measurement, the instrument was calibrated with a standard white calibration tile (*L = 92.46, a* = –0.86, b* = –0.16**). Color values were recorded in the CIELAB system, where L* represents lightness, a* indicates the red–green axis, and b* represents the yellow–blue axis. Three independent measurements were recorded for each loaf, and the results were expressed as mean ± SD.

2.11.6. Sensory Evaluation: Sensory quality was evaluated by a panel of 10 trained assessors from the Department of Food Science and Technology, Faculty of Home Economics, Al-Azhar University, following the procedure of (Divyashree et al., 2016). The panel assessed crust color, crumb color, taste, aroma, texture, chewability, loaf shape, and overall acceptability using a 10-point hedonic scale, where 1 = extremely poor and 10 = excellent. Mean sensory scores were calculated for each attribute.

2.12. Experimental Design: The experiment followed a completely randomized design (CRD) consisting of four bread formulations containing 0 (control), 7.5, 10, and 15% chia seed powder (CSP) as partial replacements for wheat flour. Each formulation was prepared independently in triplicate. Physicochemical, rheological, antioxidant, and sensory analyses were performed on each replicate, and the resulting mean values were subjected to statistical evaluation.

2.13. Statistical Analysis: All analyses were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS software (Version 15.0, SPSS Inc., Chicago, IL, USA). Data were analyzed by one-way analysis of variance (ANOVA), and treatment means were separated using Duncan's Multiple Range Test (DMRT) at $P < 0.05$.

2.14. Multivariate and Correlation Analysis

Pearson's correlation analysis was performed to characterize the direction and strength of associations among the nutritional composition, CSP-derived bioactive contributions, dough rheological properties, loaf characteristics, instrumental color attributes, and sensory responses of the bread formulations. Correlation matrices were visualized as heatmaps to facilitate interpretation of the magnitude and direction of the relationships among variables. Two complementary correlation analyses were conducted. The first evaluated the correlation structure among the measured nutritional, rheological, physical, color, and sensory attributes of the bread formulations. The second integrated the estimated contributions of CSP-derived nutritional and bioactive constituents with the corresponding bread quality attributes at each CSP incorporation level. The contribution of each CSP constituent was estimated from its measured concentration in CSP and its proportional inclusion level in the respective bread formulation. Targeted Pearson correlation and linear regression analyze were also performed to quantify selected structure-function relationships suggested by the experimental results. These included relationships between dough rheological properties and loaf characteristics and between nutritional enrichment, instrumental color and sensory color attributes. Regression equations, coefficients of determination (R^2), Pearson correlation coefficients (r), and corresponding P-values were calculated where appropriate. For treatment-level correlation and regression analyses, the mean value of each of the four CSP formulations (0, 7.5, 10, and 15% CSP) was used as one analytical observation. Accordingly, these analyses were interpreted as exploratory quantitative associations along the CSP substitution gradient rather than as evidence of independent causal relationships. Given the limited number of treatment levels ($n = 4$), correlation and regression statistics were interpreted cautiously. Statistical significance was considered at $P < 0.05$.

3. Results

3.1. Compositional functionality of chia seed powder

3.1.1. Proximate Composition and Mineral Profile of Chia Seed Powder

The proximate composition showed that chia seed powder (CSP) was significantly richer in nutritional profile than wheat flour (Table 2). The protein content increased to 20.30 g/100 g, almost two times higher than wheat flour (10.52 g/100 g). The difference was higher for lipids, being 35.43 g/100 g in CSP and only 1.10 g/100 g in wheat flour. Similarly, the contents of crude fiber (24.54 g/100 g) and ash (6.92 g/100 g) in CSP were significantly higher than that of wheat flour (1.23 and 1.57 g/100 g, respectively). However, the

percentage of carbohydrates in wheat flour (85.58 g/100 g) was higher than CSP (12.81 g/100 g). The lower moisture content of CSP (7.27 g/100 g) than wheat flour (13.75 g/100 g) might also be responsible for improved storage stability. Moreover, the mineral profile reflected the nutritional value of CSP. Magnesium was the predominant mineral (597.50 mg/100 g), followed by phosphorus (557.56 mg/100 g), potassium (465.00 mg/100 g), calcium (184.85 mg/100 g), sodium (47.15 mg/100 g), iron (5.35 mg/100 g), zinc (4.88 mg/100 g), and manganese (4.16 mg/100 g). Copper was not found under the analytical conditions used. However, these minerals were not detected or determined in the wheat flour samples analyzed. Taken together, the compositional data indicated that CSP was much richer in protein, dietary fiber, lipids and essential minerals compared to wheat flour, suggesting that CSP has potential as a nutrient-rich ingredient for the development of fortified bakery products.

Table 2. Proximate composition and mineral profile of chia seed powder (CSP) compared with wheat flour (82% extraction).

Component	Unit	Chia Seed Powder (CSP)	Wheat Flour (WF 82% Extraction)	Fold change (CSP/WF)	Component	Unit	Fold change (CSP/WF)
Proximate composition					Mineral composition		
Moisture	g/100 g	7.27	13.75	0.53	Magnesium (Mg)	mg/100 g	597.50
Protein	g/100 g	20.30	10.52	1.93	Phosphorus (P)	mg/100 g	557.56
Fat	g/100 g	35.43	1.10	32.21	Potassium (K)	mg/100 g	465.00
Crude fiber	g/100 g	24.54	1.23	19.95	Calcium (Ca)	mg/100 g	184.85
Ash	g/100 g	6.92	1.57	4.40	Sodium (Na)	mg/100 g	47.15
Total carbohydrate[1]	g/100 g	12.81	85.58	0.15	Iron (Fe)	mg/100 g	5.35
-	-	-	-	-	Manganese (Mn)	mg/100 g	4.16
-	-	-	-	-	Copper (Cu)	mg/100 g	ND

Values are expressed on a dry weight basis. Total carbohydrate was calculated by difference (100 − moisture − protein − fat − crude fiber − ash). Not detected (ND).

3.1.2. Amino Acid Composition and Protein Quality of Chia Seed Powder

Amino acid composition and protein quality parameters of chia seed powder (CSP) are presented in Table 3A–C. The total amino acid content was 98.40 g/100 g protein, comprising 35.60 g/100 g protein of essential amino acids (EAA) and 62.80 g/100 g protein of non-essential amino acids (NEAA). Essential amino acids accounted for 36.18% of total amino acids and nonessential amino acids for 63.82% with an EAA/NEAA ratio of 0.57 indicating a well-balanced amino acid composition. When compared with the FAO/WHO reference amino acid pattern, the levels of all quantified essential amino acids were found to be above the recommended reference values. The amino acid scores were highest for

methionine + cysteine (405.88%), followed by threonine (377.78%), leucine (337.50%), lysine (293.75%), valine (269.23%), phenylalanine + tyrosine (268.42%), histidine (256.25%) and isoleucine (192.31%). These results indicate that CSP is a balanced source for essential amino acids for nutritional enrichment.

Among the non-essential amino acids, glutamic acid (16.60 g/100 g protein) was the predominant component, followed by proline (14.30 g/100 g protein), aspartic acid (9.00 g/100 g protein), arginine (8.50 g/100 g protein), alanine (5.30 g/100 g protein), serine (4.60 g/100 g protein), and glycine (4.50 g/100 g protein). The ammonia content was 1.60 g per 100 g of protein. The calculated protein quality indices also showed the nutritional value of CSP protein. The protein efficiency ratio (PER), biological value (BV), essential amino acid index (EAAI), and nutritional index (NI) were found to be 1.81, 68.10%, 0.72 and 14.62, respectively. The leucine/isoleucine ratio was 2.16. The total aromatic amino acid content was 5.10 g/100 g protein. The overall results suggest that chia seed protein has suitable amino acid composition and quality characteristics as a protein ingredient for fortification of cereals and functional bakery products.

Table 3A. Essential amino acid composition and amino acid scores of chia seed protein

Essential amino acid	**Chia seed protein (g/100 g protein)**	**FAO/WHO reference pattern* (g/100 g protein)**	**Amino acid score (%)**
Histidine	4.10	1.60	256.25
Isoleucine	2.50	1.30	192.31
Leucine	5.40	1.60	337.50
Lysine	4.70	1.60	293.75
Methionine + Cysteine	6.90	1.70	405.88
Phenylalanine + Tyrosine	5.10	1.90	268.42
Threonine	3.40	0.90	377.78
Valine	3.50	1.30	269.23
Total essential amino acids (TEAA)	**35.60**	**12.20**	—

Notes: Values are expressed as g/100 g protein. Amino acid scores were calculated according to the FAO/WHO reference amino acid pattern. *Specify the reference used (e.g., FAO/WHO/UNU, 2007 or FAO/WHO, 1991) and use it consistently throughout the manuscript.

Table 3B. Non-essential amino acid composition of chia seed protein

Non-essential amino acid	**Content (g/100 g protein)**
Glutamic acid	16.60
Proline	14.30
Aspartic acid	9.00
Arginine	8.50
Alanine	5.30
Serine	4.60
Glycine	4.50
Ammonia	1.60

Non-essential amino acid	Content (g/100 g protein)
Total non-essential amino acids (TNEAA)	**62.80**
Total aromatic amino acids (TArAA)	**5.10**
Total amino acids (TAA)	**98.40**

Notes: Values are expressed as g/100 g protein. TNEAA = Total non-essential amino acids; TArAA = Total aromatic amino acids; TAA = Total amino acids.

Table 3C. Protein quality indices of chia seed protein

Parameter	Value
TAA	98.40
TEAA	35.60
TNEAA	62.80
TEAA/TAA (%)	36.18
TNEAA/TAA (%)	63.82
TEAA/TNEAA (%)	56.69
EAA/NEAA ratio	0.57
Leu/Ile ratio	2.16
Total aromatic amino acids	5.10
PER	1.81
EAAI	0.72
BV (%)	68.10
Nutritional Index	14.62

3.1.3. Phytochemical Composition and Antioxidant Activity of Chia Seed Powder

Table 4 shows the phytochemical profile and antioxidant potential of chia seed powder (CSP). CSP contained a measurable amount of phenolic compounds with a total phenolic content (TPC) of 1.36 mg GAE g −1 and tannins of 2.60 mg CE g −1 suggesting that chia seeds are natural source of antioxidant phytochemicals. Conversely, the levels of the main antinutritional compounds were relatively low, such as phytic acid (1.79 mg 100 g^{-1}) and oxalate (0.60%), which suggests that these components are unlikely to significantly diminish the nutritional value of CSP. The antioxidant capacity of CSP was assessed by two complementary assays . The sample exhibited 45.87% DPPH radical scavenging activity and ABTS antioxidant capacity of 1.65 mg TE g−1, confirming the significant free radical neutralizing capacity. The antioxidant performance is consistent with the presence of naturally occurring phenolic compounds identified in the seed. By HPLC analysis twenty individual phenolic compounds were identified (Fig. 2). Pyrogallol was the predominant compound (45.85 ppm), followed by caffeic acid (23.20 ppm), ellagic acid (13.91 ppm), benzoic acid (10.27 ppm), catechin (9.43 ppm), and vanillic acid (9.00 ppm). At intermediate concentrations, protocatechuic acid, 3-hydroxytyrosol, p-hydroxybenzoic acid, chlorogenic acid and catechol were found, while ferulic acid, isoferulic acid, gallic acid and α-coumaric acid were detected in relatively smaller amounts. The wide range of phenolic compounds identified by HPLC gives a fair explanation to the antioxidant activity measured in CSP. To summarize, these findings point out chia seed powder as a good source of phenolic antioxidants and bioactive compounds, and provide

justification for its use as a functional ingredient to improve the nutritional quality and oxidative stability of fortified bakery products.

Table 4. Phytochemical composition and antioxidant activity of chia seed powder (dry weight basis).

Parameter	Unit	Value
Total phenolic content (TPC)	mg GAE g^{-1}	1.36
Tannins	mg CE g^{-1}	2.60
Phytic acid	mg 100 g^{-1}	1.79
Oxalate	%	0.60
DPPH radical scavenging activity	% inhibition	45.87
ABTS antioxidant capacity	mg TE g^{-1}	1.65

Abbreviations: GAE = gallic acid equivalents; CE = catechin equivalents; TE = Trolox equivalents.

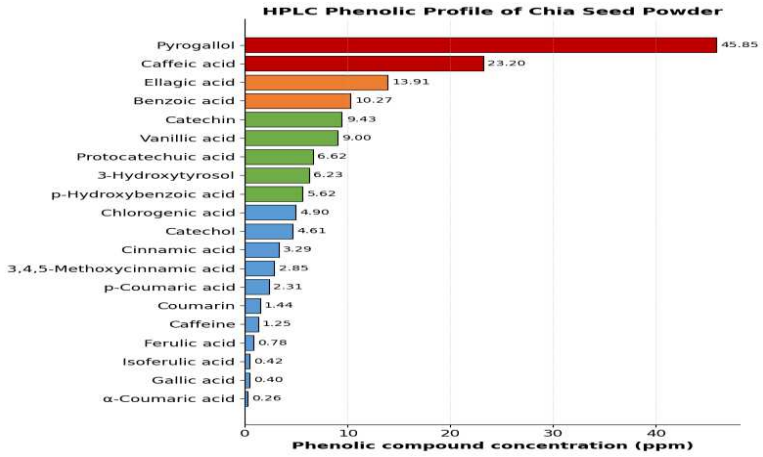


Figure 2. Horizontal Bar Chart illustrating the concentrations of individual phenolic compounds identified in chia seed powder by HPLC. Phenolic compounds are arranged in ascending order of concentration, highlighting pyrogallol, caffeic acid, and ellagic acid as the predominant constituents.

3.2. Quantitative relationships between CSP incorporation, dough functionality and loaf expansion

The regression analysis showed a sequential relationship between incorporation of chia seed powder (CSP) and dough hydration, mixing stability, mechanical resistance and expansion of bread (Fig. 3). Water absorption increased from 62.0% in control to 71.0% at 15% CSP and showed a strong positive linear relationship with CSP level (R 2 = 0.961, P = 0.020). The increase in hydration was closely related to dough stability which increased from 4.0 to 12.0 min and had a strong relationship with water absorption (R^2 = 0.991, P = 0.005). Dough stability was also strongly associated with maximum resistance, which increased from 368 to 710 BU across the CSP treatments (R^2 = 0.995, P = 0.002). Most importantly, the increase

in dough mechanical resistance was associated with greater specific volume and the maximum resistance had a strong positive correlation with specific volume ($R^2 = 0.976$, $P = 0.012$). These relationships offer a quantitative connection between CSP-related hydration behavior, dough mechanical response and loaf expansion. Conversely, extensibility showed a non-linear response, decreasing at 7.5% CSP, returning to the control value at 10% and decreasing further at 15%. Thus, CSP altered the balance between dough resistance and extensibility rather than resulting in a uniform improvement in all rheological properties. Collectively, the regression relationships suggest a sequential hydration–stability–resistance response that was associated with improved loaf expansion.

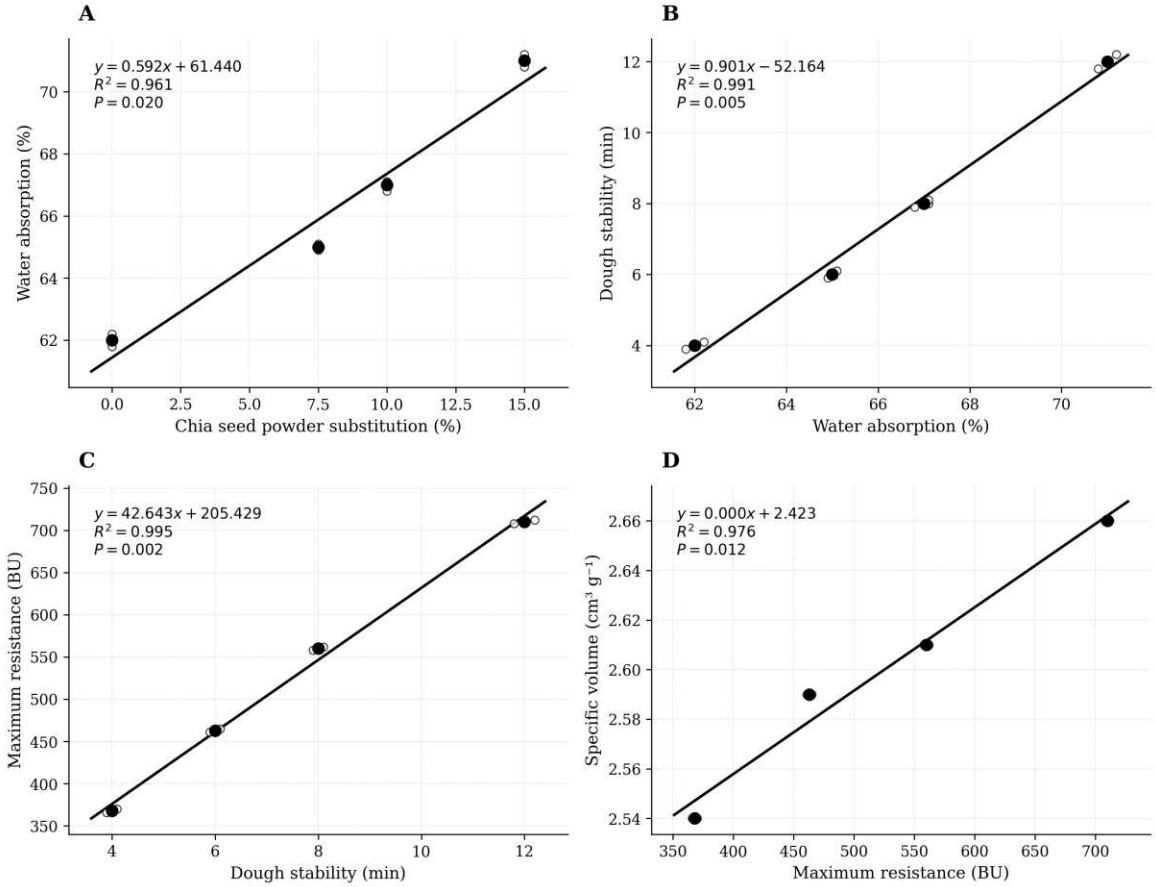


Figure 3. Quantitative relationships linking chia seed powder (CSP) incorporation with dough hydration, stability, mechanical resistance, and loaf expansion. (A) Relationship between CSP level and farinograph water absorption; (B) relationship between water absorption and dough stability; (C) relationship between dough stability and maximum resistance to extension; and (D) relationship between maximum resistance and bread specific volume. Regression equations, coefficients of determination (R^2), and significance levels are shown for each relationship**.**

3.3. Transfer of dough functionality to loaf expansion

The physical performance of the resulting bread closely mimicked the rheological response induced by incorporation of CSP (Fig. 4). A significant positive correlation was observed between the loaf volume and dough stability (R 2 = 0.993; P = 0.004) (A): loaf volume increased from 280.40 to 310.50 cm 3 with increase in dough stability from 4.0 to 12.0 min. Similarly, a high correlation was observed between maximum resistance and loaf volume (R2 = 0.987, P = 0.007), which suggests that the gradual increase in resistance of the dough was accompanied by a greater expansion of the loaf. Maximum resistance was also highly correlated to specific volume (R2 = 0.990, P = 0.005) and related the mechanical response of the dough to volume development in relation to the mass of the loaf. Similarly, a non-significant positive trend was observed between dough energy and loaf volume (R2 = 0.893, P = 0.055). Thus, although energy showed a strong positive trend, stronger statistical relationships were found for dough stability and maximum resistance. These results provide a quantitative link between the changes in dough rheology associated with the addition of CSP and the expansion of the loaf, and indicate that the hydration and mechanical responses identified in the rheological analysis are reflected in the physical development of the bread

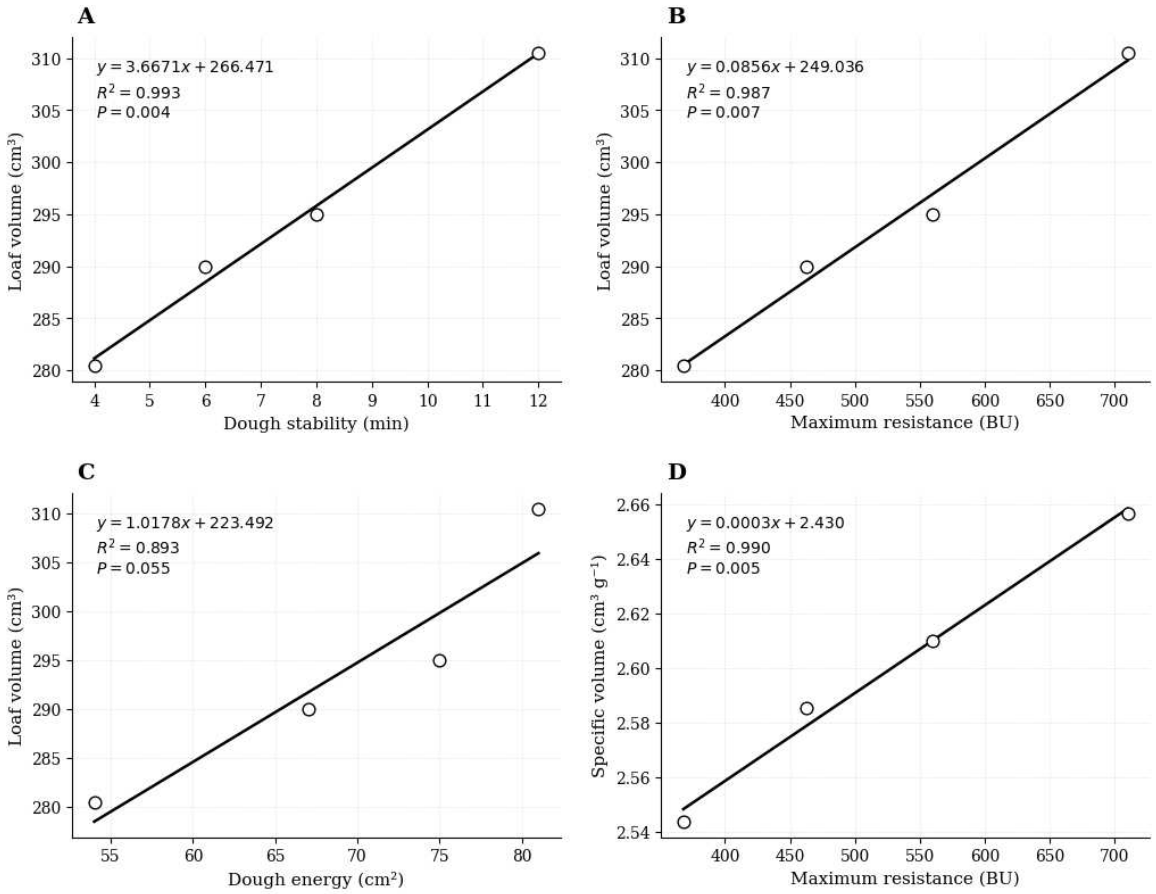


Figure 4. Quantitative relationships between dough rheological functionality and loaf performance of wheat bread supplemented with chia seed powder (CSP). (A) Relationship between dough stability and loaf volume; (B) relationship between maximum resistance and loaf volume; (C) relationship between dough energy and loaf volume; and (D) relationship

between maximum resistance and specific volume. Regression equations, coefficients of determination (R^2), and P-values are shown for each relationship.

3.4. Effect of Chia Seed Powder on the Chemical Composition of Bread

Increasing the substitution level of chia seed powder (CSP) significantly ($P \leq 0.05$) modified the chemical composition of bread (Table 5). The moisture content decreased gradually from 13.97 to 13.02 g/100 g with the increasing amount of CSP, indicating slight reduction of residual moisture during baking. With an increase in the proportion of CSP, the protein content increased from 10.58 to 12.16 g/100 g, which is due to the higher protein concentration of chia seed powder than wheat flour. Similar increases of crude fat, crude fiber and ash contents were also observed reaching 6.83, 4.98 and 2.49 g/100 g, respectively, at the 15% substitution level. These changes are in line with the naturally high levels of lipids, dietary fiber and minerals in chia seeds. The carbohydrate content exhibited the reverse trend, being significantly reduced (85.35 to 73.54 g/100 g) as the level of wheat flour was progressively replaced by CSP. Carbohydrate reduction is due to the reduced starch content and increased percentages of protein, lipids, fiber and minerals from chia seed powder. Because of the increased lipid fraction, the calculated energy value showed a slight increase from 393.98 to 404.27 kcal/100 g. The compositional modifications observed in the fortified bread closely reflected the chemical characteristics of CSP described in Section 3.1. Enrichment with up to 15% CSP increased the concentrations of protein, dietary fiber, lipids, and mineral matter while reducing the relative carbohydrate content, thereby improving the overall nutritional value of the bread.

Table 5. Effect of chia seed powder (CSP) on the proximate composition and energy value of bread (dry weight basis)

Parameter	Unit	Control (0% CSP)	7.5% CSP	10% CSP	15% CSP
Moisture	g/100 g	13.97 ± 0.02^{a}	13.52 ± 0.20^{b}	13.33 ± 0.30^{bc}	13.02 ± 0.02^{c}
Protein	g/100 g	10.58 ± 0.50^{b}	11.38 ± 0.70^{ab}	11.61 ± 0.05^{a}	12.16 ± 0.06^{a}
Crude fat	g/100 g	1.14 ± 0.04^{d}	3.90 ± 0.50^{c}	4.82 ± 0.02^{b}	6.83 ± 0.05^{a}
Crude fiber	g/100 g	1.27 ± 0.03^{c}	3.15 ± 0.60^{b}	3.75 ± 0.03^{b}	4.98 ± 0.90^{a}
Ash	g/100 g	1.66 ± 0.06^{b}	2.12 ± 0.02^{ab}	2.24 ± 0.30^{a}	2.49 ± 0.40^{a}
Total carbohydrate[1]	g/100 g	85.35 ± 0.30^{a}	79.45 ± 0.05^{b}	77.58 ± 0.50^{c}	73.54 ± 0.04^{d}
Energy	kcal/100 g	393.98 ± 0.07^{d}	398.42 ± 0.09^{c}	400.14 ± 0.04^{b}	404.27 ± 0.60^{a}

Values are expressed as mean ± standard deviation (n = 3). Within each row, means followed by different superscript letters are significantly different according to one-way ANOVA followed by Duncan's multiple range test at $P \leq 0.05$. [1] Calculated by difference.

3.5. Nutritional enrichment, color and sensory trade-offs associated with CSP incorporation

The nutritional enrichment related to CSP incorporation was correlated with consistent changes in bread color and sensory perception of color (Fig. 5). Protein content was negatively correlated with crust lightness (R^2 = 0.976, P = 0.012) and crust L* decreased from 69.69 to 53.63 with increasing protein content from 10.58 to 12.16 g/100 g. This relationship quantitatively correlates the increase in protein content with the addition of CSP and the gradual darkening of the crust. Crude fiber content and crumb lightness had a similar inverse relationship (R^2 = 0.898), but the association was not significant (P = 0.053). Hence, the data exhibits a strong negative trend between fiber enrichment and crumb lightness, but the evidence was marginal at the selected significance level.

Instrumental measurement of color was also closely related to sensory perception. A strong positive relationship was observed between crust lightness and the sensory crust-color score (R^2 = 0.961, P = 0.020), indicating that darker crusts received lower sensory color scores. In contrast, the relationship between crumb lightness and the sensory crumb-color score was positive but not statistically significant (R^2 = 0.824, P = 0.092). Despite the absence of statistical significance, the positive direction was consistent with the expected relationship between instrumental lightness and perceived crumb-color preference. These relationships indicate that CSP incorporation generated a nutritional–color–sensory trade-off: increasing protein and fiber enrichment was accompanied by reduced instrumental lightness, while changes in crust color were sufficiently reflected in sensory evaluation to produce a significant quantitative relationship. Importantly, the non-significant associations were retained to describe the direction and strength of the observed responses rather than being interpreted as confirmed effects. This integrated analysis therefore extends the nutritional and rheological findings by showing that the functional benefits associated with CSP incorporation were accompanied by measurable, although not uniformly significant, changes in bread appearance and sensory color perception.

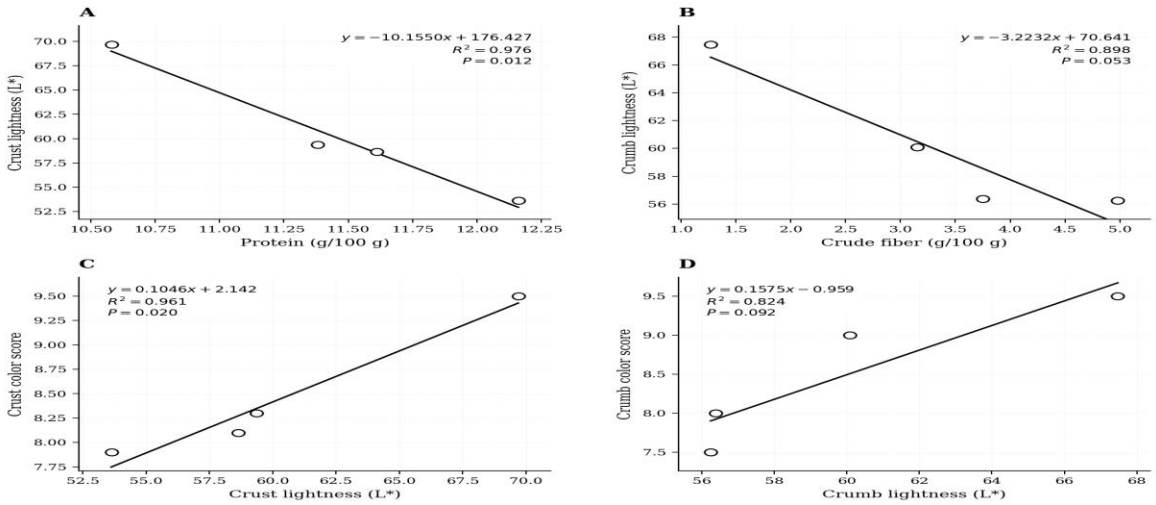


Figure 5. Quantitative relationships between nutritional enrichment, instrumental color, and sensory color attributes of wheat bread supplemented with chia seed powder (CSP). (A) Relationship between protein content and crust lightness (L); (B) relationship between crude fiber content and crumb lightness (L); (C) relationship between crust lightness (L*) and sensory crust-color score; and (D) relationship between crumb lightness (L*) and sensory crumb-color score. Regression equations, coefficients of determination (R^2), and P-values are presented for each relationship.

3.6. Quantitative structure function relationships across the bread making system

The interrelationship between the composition of CSP, the functionality of the dough, the development of the loaf and the sensory response showed a coherent structure-function pattern throughout the bread-making system Fig 6. The compositional characteristics of CSP firstly reflected in the hydration and mechanical behavior of dough, with the increasing incorporation of CSP leading to the progressive increases in water absorption, dough stability, maximum resistance and energy. Among these rheological attributes, dough stability and maximum resistance showed particularly strong positive relationships with loaf volume ($R^2 = 0.993$ and 0.987, respectively; $P < 0.01$), while maximum resistance was also strongly associated with specific volume ($R^2 = 0.990$, $P = 0.005$). These relationships indicate that the rheological response to CSP incorporation was quantitatively connected with subsequent loaf expansion rather than representing an isolated change in dough behavior. The nutritional response followed a similar pattern. The addition of increasing levels of CSP increased protein, fat, crude fiber and ash contents and decreased relative carbohydrate proportion of the bread. These compositional changes were accompanied by systematic decreases in lightness of both crust and crumb. Crust L* was strongly negatively correlated with protein content ($R^2 = 0.976$, $P = 0.012$) and crumb L* showed a strong negative trend with crude fiber ($R^2 = 0.898$, $P = 0.053$). Instrumental crust lightness was also positively correlated with the sensory crust color score ($R2 = 0.961$, $P = 0.020$) suggesting that the instrumental color response was perceived by the sensory panel. The corresponding relationship for crumb color showed the same direction but did not reach statistical significance ($R^2 = 0.824$, $P = 0.092$). Taking together, these relationships describe a sequential response in which CSP incorporation altered the composition of the bread matrix, modified dough hydration and mechanical functionality, and was subsequently reflected in loaf expansion and visual attributes. The sensory results indicate that these technological and nutritional gains were accompanied by a measurable color-related sensory trade-off, although overall acceptability remained high across the tested formulations. The strongest relationships were therefore not limited to individual quality attributes but extended across successive stages of bread development, providing a quantitative framework linking CSP composition; dough functionality; loaf development; color response; sensory perception.

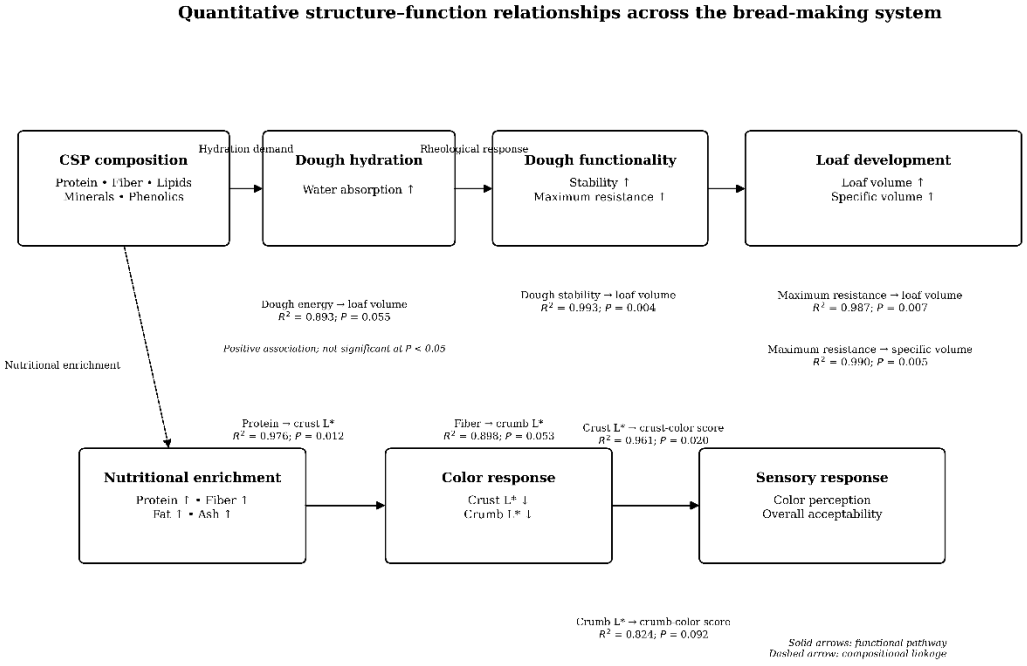


Figure 6. Integrated quantitative framework illustrating the structure function relationships associated with chia seed powder (CSP) incorporation across the bread-making system. The framework links CSP compositional characteristics with dough hydration and rheological functionality, loaf development, instrumental color, and sensory response. Regression statistics are shown for the principal quantitative associations identified in the study. Positive associations are presented without implying direct causality.

3.7 Multivariate Statistical Analysis

3.7.1. Correlation Structure of Bread Quality Attributes

Pearson correlation analysis showed that compositional, rheological, physical, color and sensory properties of CSP-enriched bread were aried systematically across CSP substitution levels (Fig. 7). Water absorption, dough stability, maximum extensibility, loaf volume and specific volume were strongly positively correlated with protein, fat, crude fiber and ash with the correlation coefficients generally ranging from r=0.94 to 1.00. The compositional variables and water absorption were particularly strongly associated with rheological parameters and loaf characteristics. The close relationship of protein and fiber with water absorption and dough stability agrees with the increased hydration requirement and mixing stability noted with CSP incorporation. Similarly, the positive strong correlations of loaf volume and specific volume with maximum elasticity demonstrate the rheological changes accompanying CSP incorporationare in good agreement with loaf development. A contrasting pattern was observed for instrumental color. Crust and crumb lightness (L*) were negatively correlated with most compositional, rheological, and physical attributes, with coefficients ranging from r=−0.83 to −0.99. Thus, the progressive increase in nutritional and technological attributes was accompanied by a reduction in bread lightness.

This pattern agrees with the direct regression relationships presented previously, particularly the inverse association between protein content and crust L*. The heatmap therefore provides complementary evidence that nutritional enrichment and improved dough functionality occurred concurrently with a darker bread appearance. Sensory response showed a different but informative pattern. Overall acceptability was negatively correlated with protein, fat, crude fiber, ash, water absorption, dough stability, maximum elasticity, loaf volume, and specific volume ($r=-0.91$ to -0.96), whereas it was positively correlated with crust and crumb lightness ($r=0.93$ and 0.98, respectively). This pattern indicates that the sensory response was more closely aligned with the color changes than with the improvements in nutritional or rheological characteristics. Importantly, these correlations should be interpreted as coordinated treatment-level associations rather than independent causal relationships, because the variables were evaluated across only four CSP substitution levels. The heatmap therefore serves as an integrative description of the multivariate response pattern, while the targeted regression analyses provide more specific quantitative relationships between selected functional attributes.

Taken together, the correlation structure indicates a consistent nutritional–technological versus appearance–sensory trade-off associated with CSP incorporation. Higher CSP levels were accompanied by greater nutritional density, stronger dough functionality, and improved loaf development, but also by reduced crust and crumb lightness and a corresponding decline in color-related sensory scores. Despite this trade-off, the sensory results demonstrated that the breads remained well accepted at the tested substitution levels. The correlation analysis thus complements the targeted structure–function relationships by showing how the major quality domains changed collectively across CSP incorporation levels.

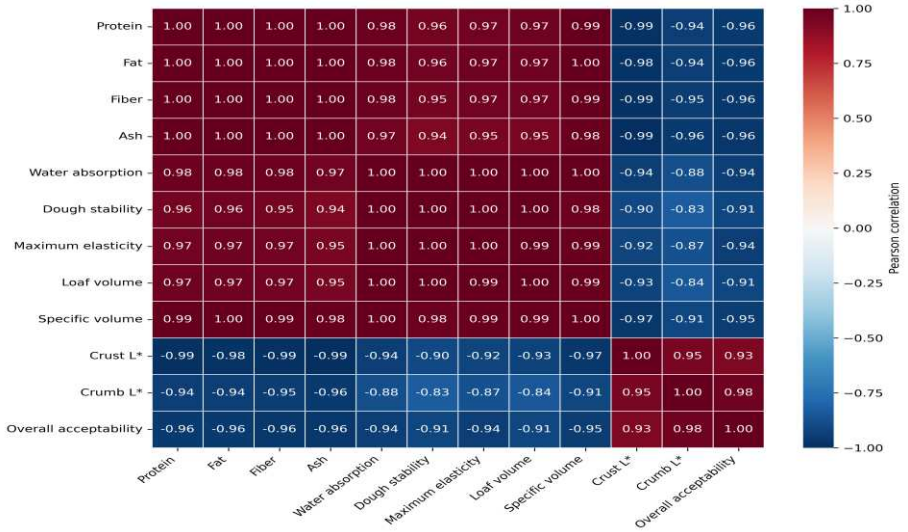


Figure 7. Pearson correlation heatmap illustrating the relationships among nutritional composition, rheological properties, physical characteristics, color attributes, and overall acceptability of bread supplemented with different levels of chia seed powder (CSP). Positive correlations are shown in red and negative correlations in blue, with color intensity proportional to the magnitude of the Pearson correlation coefficient (r).

3.7.2. Correlation heatmap of CSP-derived contributions and bread quality attributes

Correlation heatmap of CSP-derived contributions and bread quality attributes revealed a clear separation between CSP-derived compositional variables and the major bread quality attributes (Fig. 8). The first cluster comprised the estimated contributions of protein, dietary fiber, ω-3 fatty acids (α-linolenic acid), total polyunsaturated fatty acids (PUFA), total phenolic compounds, DPPH and ABTS antioxidant activities, and ash, together with key dough rheological attributes, including water absorption, dough stability, and maximum elasticity. These variables exhibited predominantly strong positive correlations ($r = 0.91–1.00$), indicating a coordinated response of CSP-associated compositional enrichment and dough functionality across the tested substitution levels. A second cluster comprised the principal loaf and sensory attributes, including loaf volume, specific volume, crust lightness (L*), crumb lightness (L*), and overall acceptability. Strong positive associations were observed among several variables within this cluster ($r = 0.79–1.00$), whereas moderate to strong negative associations ($r = −0.74$ to $−0.95$) occurred between these attributes and most CSP-derived compositional and rheological variables. The clustering pattern therefore separated the nutritional–rheological response from the loaf appearance and sensory response, highlighting a measurable trade-off associated with increasing CSP incorporation. The CSP-derived variables also showed highly similar correlation patterns with the rheological attributes, particularly protein, dietary fiber, ω-3 fatty acids, total PUFA, phenolic compounds, and antioxidant activity. This similarity indicates that these variables followed a common treatment-associated gradient rather than demonstrating independent effects on dough functionality. Likewise, the close clustering of water absorption, dough stability, and maximum elasticity is consistent with the strong relationships identified in the rheological analysis and supports their interpretation as interconnected descriptors of dough functionality.

The hierarchical analysis therefore provides a complementary multivariate view of the relationships identified by the targeted regression analyses. Rather than establishing independent causal effects, the clustering demonstrates how CSP-associated nutritional characteristics, dough functionality, loaf development, color, and sensory response were organized across the substitution gradient. The resulting pattern supports the existence of a nutritional–technological versus appearance–sensory trade-off, in which increasing CSP incorporation was associated with enhanced compositional and rheological attributes but reduced crust and crumb lightness and changes in sensory preference.

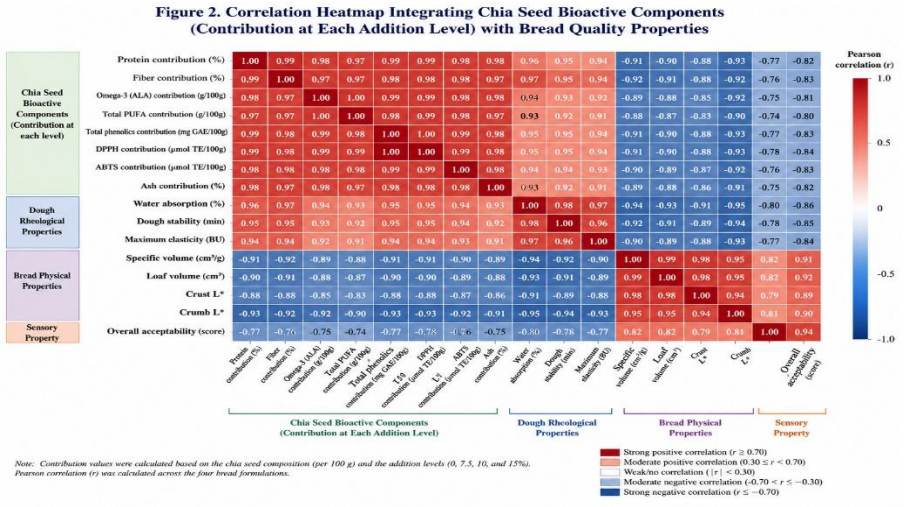


Figure 8. Integrated Pearson correlation heatmap showing the relationships among the estimated contributions of chia seed bioactive components and the rheological, physical, color, and sensory properties of bread supplemented with different levels of chia seed powder (CSP). Positive correlations are represented in red and negative correlations in blue, with color intensity proportional to the Pearson correlation coefficient (r).

4. DISCUSSION

4.1. Nutritional and bioactive value of chia seed powder

The composition profile of CSP clearly defines its role as a multifunctional ingredient for bakery. Compared with wheat flour the higher protein, lipid, dietary fiber, ash and mineral contents of the bread suggest that partial flour replacement can significantly alter the nutritional density of the bread matrix. Further, the amino acid profile further confirms the nutritional potential of CSP with all the essential amino acids evaluated exceeding the reference requirements by the FAO/WHO and a good balance between the essential and non-essential amino acids [3, 16, 17]. CSP has a high percentage of unsaturated fatty acids, especially α-linolenic acid, which brings an extra nutritional aspect to CSP, and its phenolic profile and measurable DPPH and ABTS activities indicate the presence of antioxidant-related phytochemicals [18–20]. These characteristics are important because CSP does not contribute a single nutritional component; rather, it introduces a combination of protein, fiber, unsaturated lipids, minerals, and phenolic constituents into the wheat-based matrix. Thus the change seen in the composition of the fortified breads is due to the inherent nutritional properties of CSP and not an enrichment effect. This multifunctional composition is the basis for further modifications of dough hydration, rheology, loaf development and color. [21, 22].

4.2. CSP-driven changes in dough hydration and rheological functionality

The farinograph water absorption increased progressively with the addition of CSP, indicating the higher hydration demand of the wheat-CSP system. This result is consistent with the high fiber content of chia and the presence of hydrophilic components capable of retaining water. Importantly, the increase in water absorption occurred under a fixed bread-processing water level, demonstrating that the instrumental hydration response represents an intrinsic property of the flour–CSP blends rather than a consequence of increasing the water added during bread preparation [2, 23, 24]. Dough stability increased markedly with CSP incorporation, while the tolerance index decreased and dough weakening increased at higher substitution levels. This combination suggests that CSP strengthened resistance to mechanical mixing while simultaneously altering the behavior of the gluten-based network during prolonged mixing [25, 26]. The response is therefore better interpreted as a modification of dough functionality rather than a simple strengthening of gluten. The non-gluten ingredients provided by CSP may increase the competition for water and change the continuity of the wheat gluten network, resulting in dough which is initially mechanical resistant but more susceptible to softening with further mixing [27, 28]. The extensograph results provide additional evidence of this functional modification. The maximum resistance and energy increased progressively with CSP incorporation, while the extensibility showed a less consistent response. An increasing ratio of resistance to extensibility indicates a trend toward a stronger, less extensible dough structure. Such modifications indicate that CSP modified both hydration and mechanical behavior, demonstrating a direct technological consequence of its compositional incorporation [29, 30].

4.3. Translation of dough functionality into loaf development

Changes in rheology were reflected in loaf performance. Addition of CSP increased loaf volume and specific volume indicating that the modified dough system was able to maintain sufficient structural integrity to support expansion during baking. The strong quantitative relationships between dough stability and loaf volume, and between maximum resistance and both loaf volume and specific volume, provide additional evidence that the rheological response was functionally relevant to bread developmen [24, 31, 32]. The particularly high coefficients of determination for these relationships suggest that dough stability and maximum resistance were more closely aligned with loaf expansion than dough energy. In contrast, the positive association between dough energy and loaf volume did not reach the predefined significance level. This distinction is useful because it indicates that not all rheological descriptors contributed equally to loaf development. Rather, the ability of the dough to maintain structural integrity and resist deformation appeared to be more closely associated with the final loaf dimensions [33, 34]. The higher loaf weight under the fixed processing-water condition could be attributed to an increased water retention in the CSP-containing matrix. Thus, the physical response of the bread can be interpreted as the combined expression of altered hydration and mechanical functionality rather than as a simple consequence of flour replacement. [35, 36].

4.4. Nutritional enrichment versus color and sensory response

The incorporation of CSP significantly modified the nutritional profile of the bread in favor of increased protein, lipid, fiber and ash contents and reduced carbohydrate proportion.

These compositional improvements were accompanied by progressive decreases in crust and crumb lightness. The darker color may be due to the color characteristics of CSP itself and the higher levels of protein and phenolics that may facilitate browning during baking [34, 37]. Quantitative relationships are more informative than treatment comparisons alone in explaining the response. Protein content was negatively correlated with crust lightness, and fiber content was negatively correlated with crumb lightness. These associations imply that nutritional enrichment and color development co-occurred along the CSP substitution gradient. However, they should be interpreted as treatment-associated relationships rather than evidence that individual nutrients independently caused the observed color changes [38, 39]. The sensory response reveals an important trade-off. The positive association between crust lightness and the sensory crust-color score indicates that the darker appearance was reflected in panel perception. A similar direction was observed for crumb lightness, although the corresponding relationship did not reach statistical significance. With these changes, the breads presented good scores in the sensory evaluation at all substitution levels. Thus the reduction in lightness was not enough to lead to a loss of overall acceptability that could outweigh the nutritional and technological benefits of incorporating CSP [40, 41].

4.5. Integrated structure–function interpretation

The data support the following structure–function sequence: CSP composition; hydration response; dough functionality; loaf development; color response; sensory perception. The correlation and hierarchical clustering analyses supported this integrated pattern by separating a group dominated by nutritional/CSP-derived characteristics and rheological variables from a group comprising loaf, color, and sensory attributes. However, because these analyses were performed across four CSP substitution levels, the correlations primarily describe coordinated changes along the substitution gradient rather than independent causal relationships. This distinction is important when interpreting the strong correlations observed among several variables. The value of the multivariate analysis was not to attribute causality to individual components, but to demonstrate that nutritional enrichment, dough functionality, loaf development and sensory response were systematically coupled. The targeted regression analyzes also revealed dough stability and maximum resistance as particularly informative rheological descriptors of loaf development. Instrumental lightness allowed a quantitative link to color-related sensory perception. The Pearson correlation analysis provided a comprehensive view of the relationships among the nutritional composition of chia seed powder, dough rheology, bread characteristics and sensory performance. The quality attributes were not separated but were part of a total system where changes in the composition impacted the processing behavior and thus the quality of the final product [23, 24]. The positive correlations of protein, dietary fiber and mineral fortification with the rheological parameters implied the close correlation of the compositional changes with the improved hydration and mixing properties with CSP addition. This improves water uptake and dough stability and also improves loaf volume and specific loaf volume. The results showed that the technological benefits of CSP are

related to its ability to modify the dough functionality through the improvement of water retention and structural stability [37, 42].

The correlation matrix also showed that the darker crust and crumb observed at higher substitution levels were strongly associated with the compositional characteristics of CSP, particularly its higher protein and phenolic contents. Although these color changes were accompanied by slight reductions in some sensory scores, their influence on panel acceptance remained limited. The weak negative relationships between color attributes and overall acceptability suggest that visual changes were less important to panelists than improvements in bread quality and nutritional value [32, 40, 43]. The multivariate analysis therefore confirms that nutritional enhancement, rheological performance, and loaf quality are closely interconnected. The favorable correlation pattern observed at the higher substitution levels indicates that chia seed powder functions as a multifunctional ingredient, simultaneously improving nutritional composition, dough behavior, and bread quality while maintaining satisfactory panel acceptance. These relationships provide additional evidence supporting the use of CSP at substitution levels up to 15% in nutritionally enriched Balady bread.

5. Conclusion

Chia seed powder (CSP) was identified as a promising multifunctional ingredient for wheat bread with simultaneous nutritional and technological benefits. Moreover, its addition improved the nutritional values of bread in terms of protein, lipid, dietary fiber, ash, mineral, and phenolic compounds but reduced the relative proportion of carbohydrates. CSP also modified dough hydration and rheological properties, mainly by increasing water absorption, dough stability and maximum resistance and energy. The rheological changes were correlated quantitatively with loaf development. Loaf volume was strongly related to dough stability and maximum resistance, while maximum resistance was also strongly associated with specific volume. These relationships indicate that the functional contribution of CSP extended beyond nutritional enrichment to the mechanical behavior of the dough and the physical development of the baked loaf.

CSP incorporation also produced a progressive darkening of the crust and crumb. Although this change was reflected in lower color-related sensory scores, the bread remained well accepted across the tested substitution levels. Pearson correlation and hierarchical clustering further revealed coordinated relationships among nutritional enrichment, dough functionality, loaf characteristics, color, and sensory response, supporting a nutritional–technological versus appearance–sensory trade-off. the highest substitution level tested (15% CSP) balance under the processing conditions investigated yielded significant nutritional enrichment, desirable rheological and loaf properties, but acceptable sensory quality. Therefore, the present study supports the potential of CSP as a multi-functional ingredient to produce nutritionally fortified Balady bread and the applicability of quantitative structure-function analysis to understand the interlinked responses of functional bakery formulations.

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Institutional Review Board Statement: The study was reviewed and approved by the Research Ethics Committee at King Faisal University, Saudi Arabia (Ref. No. KFU-REC-2026-SEP–ETHICS4481; approval date: 14 September 2026). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the applicable institutional ethical guidelines.

Consent to Participate: Written informed consent was obtained from all participants before sensory evaluation. Participation was voluntary, and participants were informed that they could withdraw at any time without providing a reason.

Clinical trial number: not applicable.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: During the preparation of this manuscript, the authors used QuillBot to improve the English language, grammar, and readability of the text, and FigureLabs to assist in the preparation and refinement of scientific figures, Graphical analyses and figure preparation were performed using standard scientific data-visualization software, including FigureLabs. These AI-assisted tools were used solely to enhance the presentation of the manuscript and did not contribute to the study design, data collection, data analysis, interpretation of results, or scientific conclusions. All outputs generated with the assistance of these tools were carefully reviewed, verified, and edited by the authors. The authors take full responsibility for the accuracy, originality, and integrity of the final manuscript.

References

1. Adamczyk G, Ivanišová E, Kaszuba J, et al (2021) Quality assessment of wheat bread incorporating chia seeds. Foods 10:2376

2. Fernandes SS, Egea MB, Salas-Mellado M de las M, Segura-Campos MR (2023) Chia oil and mucilage nanoemulsion: potential strategy to protect a functional ingredient. International Journal of Molecular Sciences 24:7384

3. Sayed-Ahmad B, Talou T, Straumite E, et al (2018) Evaluation of nutritional and technological attributes of whole wheat based bread fortified with chia flour. Foods 7:135

4. AOAC (1990) Association of official analytical chemists. Official methods of analysis of AOAC International

5. Block RJ, Durrum EL, Zweig G (2013) A manual of paper chromatography and paper electrophoresis. Elsevier

6. FAO WH (1991) Protein Quality Evaluation: Report of the Joint FAO/WHO Expert Consultation, Bethesda, Md., USA 4-8 December 1989. Food & Agriculture Org.

7. Alsmeyer RH, Cunningham AE, Happich ML (1974) Equations predict PER from amino acid analysis.

8. El-Shirbeeny A, Farag SA, Nassef AE (1996) Physicochemical studies for preparing quick-cooking rice by using gamma irradiation

9. Labuda J, Kacerovský O, Kováč M, Štěrba A (1982) Výživa a kŕmenie hospodárskych zvierat; Ján Labuda, Oto Kacerovský, Michal Kováč, Antonín Štěrba, Magdaléna Brázdilíková. Príroda

10. Slinkard K, Singleton VL (1977) Total Phenol Analysis: Automation and Comparison with Manual Methods. Am J Enol Vitic 28:49–55. https://doi.org/10.5344/ajev.1977.28.1.49

11. Goupy P, Hugues M, Boivin P, Amiot MJ (1999) Antioxidant composition and activity of barley (Hordeum vulgare) and malt extracts and of isolated phenolic compounds. J Sci Food Agric 79:1625–1634. https://doi.org/10.1002/(SICI)1097-0010(199909)79:12%3C1625::AID-JSFA411%3E3.0.CO;2-8

12. Brand-Williams W, Cuvelier M-E, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology 28:25–30

13. Re R, Pellegrini N, Proteggente A, et al (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine 26:1231–1237

14. Romankiewicz D, Hassoon WH, Cacak-Pietrzak G, et al (2017) The Effect of Chia Seeds (*Salvia hispanica* L.) Addition on Quality and Nutritional Value of Wheat Bread. Journal of Food Quality 2017:1–7. https://doi.org/10.1155/2017/7352631

15. AOAC international (2000) Official methods of analysis of AOAC International. AOAC International

16. Biswas S, Islam F, Imran A, et al (2023) Phytochemical profile, nutritional composition, and therapeutic potentials of chia seeds: A concise review. Cogent Food & Agriculture 9:2220516. https://doi.org/10.1080/23311932.2023.2220516

17. Mesías M, Gómez P, Olombrada E, et al (2023) Risk/benefit evaluation of chia seeds as a new ingredient in cereal-based foods. International journal of environmental research and public health 20:5114

18. Agarwal A, Rizwana, Tripathi AD, et al (2023) Nutritional and functional new perspectives and potential health benefits of quinoa and chia seeds. Antioxidants 12:1413

19. Rabail R, Sultan MT, Khalid AR, et al (2022) Clinical, nutritional, and functional evaluation of chia seed-fortified muffins. Molecules 27:5907

20. Zare T, Fournier-Level A, Ebert B, Roessner U (2024) Chia (Salvia hispanica L.), a functional 'superfood': new insights into its botanical, genetic and nutraceutical characteristics. Annals of Botany 134:725–746

21. Matondo JD, Abihudi SA (2026) Chia seeds: a nutrient-dense functional food for health and nutrition. Cogent Food & Agriculture 12:2626606. https://doi.org/10.1080/23311932.2026.2626606

22. Tak Y, Kaur M, Kumar R, et al (2022) Repurposing chia seed oil: A versatile novel functional food. Journal of Food Science 87:2798–2819. https://doi.org/10.1111/1750-3841.16211

23. Guiotto EN, Tomás MC, Haros CM (2020) Development of highly nutritional breads with by-products of chia (Salvia hispanica L.) seeds. Foods 9:819

24. Nassef SL, El-Hadidy GS, Abdelsattar AS (2022) Impact of defatted chia seeds flour addition on chemical, rheological, and sensorial properties of toast bread. Egyptian Journal of Agricultural Sciences 73:55–66

25. Gao H, Wang Z, Dai Y, et al (2023) Effects of chia seed gum on the physicochemical properties of frozen dough and the quality of dumplings. International journal of biological macromolecules 253:127280

26. Matita IC, Soedirga LC, Andriani I (2024) Utilization of chia seeds powder in wet noodle substituted with modified cassava flour. Caraka Tani J Sustain Agric 39:140–153

27. Renoldi N, Melchior S, Calligaris S, Peressini D (2023) Application of high-pressure homogenization to steer the technological functionalities of chia fibre-protein concentrate. Food Hydrocolloids 139:108505

28. Wojciechowicz-Budzisz A (2024) Impact of Chia Flour on the Nutritional and Rheological Properties of Wheat Flour Blends and Dough. Nauki Inżynierskie i Technologie 43–52

29. Verbeke C, Debonne E, Versele S, et al (2024) Technological evaluation of fiber effects in wheat-based dough and bread. Foods 13:2582

30. Wang J, Wang J, Al-Dalali S, Dai R (2026) The effect of soluble dietary fiber from chia seeds on dough characteristics and steamed bread quality. ITALIAN JOURNAL OF FOOD SCIENCE 38:69–83

31. Miranda-Ramos K, Millán-Linares MC, Haros CM (2020) Effect of chia as breadmaking ingredient on nutritional quality, mineral availability, and glycemic index of bread. Foods 9:663

32. Sarkar A, Miah MS, Masum M, et al (2025) Fortification of wheat biscuits with chia seed fiber and flower waste: Nutritional, antinutritional, functional, and storage properties. Applied Food Research 5:100864

33. Anwar D, Eid H, Rashad S, Soliman SA (2024) Nutritional, physical and microbiological properties of gluten free bread with chia seed flour as alternative thickening agent. Food Technology Research Journal 4:92–106

34. Kowalski S, Mikulec A, Pustkowiak H (2020) Sensory assessment and physicochemical properties of wheat bread supplemented with chia seeds. Polish Journal of Food and Nutrition Sciences 70:387–397

35. Hassan H, Elgharably A, Ashoush IS, et al (2023) Rheological, physical properties and sensory evaluation of biscuits enriched with Chia seed powder. Egyptian Journal of Nutrition 38:32–50

36. Mihaylova D, Popova A, Goranova Z, et al (2023) Characterization of Muffins Reformulated with Chia and Lyophilized Peach Powder in Terms of Some Technological and Sensory Aspects. Food Technol Biotechnol (Online) 61:273–282. https://doi.org/10.17113/ftb.61.03.23.7843

37. Zyada MAA, Elgharably A, Yousef EE, Saleh MAM (2025) Influence of Partial Substitution of Wheat Flour with Chia Seeds Powder on Dough Rheology and Quality Attributes of Pan Bread. Egyptian Journal of Chemistry 68:291–309

38. da Costa Borges V, Fernandes SS, da Rosa Zavareze E, et al (2021) Production of gluten free bread with flour and chia seeds (Salvia hispânica L). Food Bioscience 43:101294

39. Sanna M, Sanna S, Serra M, et al (2026) From composition to acceptance: linking nutritional, structural and sensory attributes in clean-label breads. Foods 15:831

40. Bartkiene E, Rimsa A, Zokaityte E, et al (2023) Changes in the physicochemical properties of chia (Salvia hispanica L.) seeds during solid-state and submerged fermentation and their influence on wheat bread quality and sensory profile. Foods 12:2093

41. Rahardjo M, Sihombing M (2025) Formulation of gluten-free bread using psyllium husk and chia seed seen from physical and sensory characteristics. AGROMIX 16:35â€“43-35â€“43. https://doi.org/10.35891/agx.v16i1.5636

42. Farbo MG, Fadda C, Marceddu S, et al (2020) Improving the quality of dough obtained with old durum wheat using hydrocolloids. Food Hydrocolloids 101:105467. https://doi.org/10.1016/j.foodhyd.2019.105467

43. Al-Ali RM, Al-Ibresam OT, Al-Hilifi SA, et al (2024) Chemical, rheological and sensory characteristics of wheat bread enriched with chia (Salvia hispanica L.) seed gum. Future Foods 10:100471