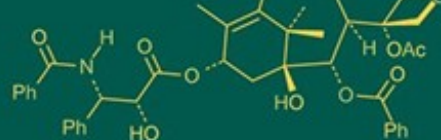
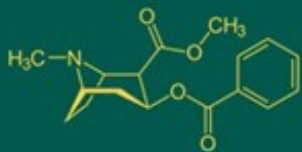


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Physicofunctional properties and amino acid composition of chia seeds (*Salvia Hispanica* L.): potential for functional food applications

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ABSTRACT

Chia (*Salvia hispanica* L.) is a nutrient-dense seed with considerable potential as a functional food ingredient. This study characterized the physical properties, functional properties, and amino acid composition of chia seeds from Guntur, Andhra Pradesh, India. Physical properties included thousand-kernel weight, thousand-kernel volume, bulk density, hydration capacity and index, and swelling capacity and index. Functional properties of chia flour comprised water and oil absorption capacities, emulsion activity and stability, and foaming capacity and stability. Amino acids were quantified by high-performance liquid chromatography following acid hydrolysis. The seeds had a thousand-kernel weight of 1.46 ± 0.01 g, a thousand-kernel volume of 1.80 ± 0.01 ml, and a bulk density of 0.75 ± 0.01 g ml⁻¹. Hydration capacity, hydration index, swelling capacity, and swelling index were 81.20 ± 0.06 mg seed⁻¹, 55.57 ± 0.03 , $95.10 \pm 0.09\%$, and 52.83 ± 0.02 , respectively. *Chia* flour showed a water absorption capacity of $166.0 \pm 1.20\%$, an oil absorption capacity of $63.00 \pm 0.80\%$, emulsion activity of $54.23 \pm 0.18\%$, emulsion stability of $82.98 \pm 0.23\%$, foaming capacity of $90.00 \pm 0.60\%$, and foaming stability of $2.79 \pm 0.05\%$. Eight essential amino acids were quantified, with leucine the most abundant (1.61 ± 0.04 g 100 g⁻¹ dry matter), and glutamic acid predominated among the non-essential amino acids (4.25 ± 0.07 g 100 g⁻¹ dry matter). These findings indicate that chia seeds from Guntur, Andhra Pradesh, India, have favorable hydration, water-binding, and emulsifying characteristics, along with a nutritionally relevant amino acid profile, supporting their use in protein- and fiber-enriched food formulations.

Keywords: Chia seed, Physical properties, Functional properties, Amino acid profile, Functional foods, Food formulation

Introduction

Nutrition is a major determinant of health, and interest is growing in foods that supply essential nutrients along with additional physiological or technological benefits. Shifting dietary patterns, greater consumption of processed food, and sedentary lifestyles have increased demand for nutrient-dense and functional food ingredients. Functional foods are generally defined as foods or food components that confer benefits beyond basic nutritional requirements, and plant derived seeds have received particular attention as sources of protein, dietary fiber, lipids, and bioactive compounds.

Chia (*Salvia hispanica* L.) is an ancient oilseed crop native to Mexico and Guatemala that was historically consumed by the Mayan and Aztec civilizations. In contemporary food systems, it has attracted scientific and commercial interest for its nutrient density and its content of protein, dietary fiber, polyunsaturated fatty acids, minerals, and antioxidant-associated constituents (Peerzada *et al.* 2023) [1], (Ferreira *et al.* (2023) [1, 2]. The lipid fraction is notable for its α -linolenic acid content, and the seed also supplies minerals such as calcium, iron, magnesium, and phosphorus (Kulczyński *et al.*, 2019) [3], (Almoselhy *et al.*, 2025) [4], [3, 4].

Beyond its nutritional composition, chia has distinctive physicochemical and functional characteristics. On hydration, the seed forms a mucilaginous layer that can contribute to water retention, viscosity, and structural stability in food systems. These properties have promoted the incorporation of chia and chia-derived fractions into bakery products, beverages, dairy-type products, snacks, and gluten-free formulations (Coorey *et al.*, 2014) [5], (Muñoz *et al.*, 2013) [5, 6]. Functional performance depends on the seed mucilage, proteins, and other matrix components, so water and oil absorption, emulsification, and foaming behavior are key parameters for evaluating suitability in food formulation. Amino acid characterization complements these measurements by describing the nutritional quality of the seed protein and its potential contribution to protein-enriched foods.

Accordingly, this study characterized selected physical and functional properties and the amino acid profile of chia seeds from Guntur, Andhra Pradesh, India, to evaluate their potential as an ingredient in nutritionally enhanced food products.

Objective

1. To evaluate the physicofunctional properties and amino acid composition of chia seeds (*Salvia hispanica* L.)

2. Materials and Methods

2.1 Procurement and preparation of samples

Chia (*Salvia hispanica* L.) seeds were procured as a single lot from a local market in Guntur, Andhra Pradesh, India. The seeds were manually cleaned to remove dust, broken seeds, and other extraneous material before analysis. A single lot was used to reduce batch-to-batch variation.

2.2 Preparation of chia seed flour

The cleaned chia seeds were dried at room temperature and ground using a laboratory grinder. The ground seeds were sieved to obtain a fine and uniform flour. The chia seeds were defatted prior to milling, and the resulting defatted chia seed flour was stored in airtight containers until further analysis.

Collection of chia seeds ↓ Sorting and cleaning ↓ Room-temperature drying ↓ Grinding ↓ Sieving ↓ Airtight packaging and storage

Figure 1. Flow chart for the preparation of chia seed flour

2.3 Determination of physical properties

Thousand kernel weight was determined according to (Williams *et al.*, 1983)^[7]. One hundred intact seeds were counted and weighed using an electronic balance, and the measured mass was multiplied by ten to obtain the thousand-kernel weight. Thousand-kernel volume was determined by the water-displacement method using a graduated cylinder containing 25 ml of distilled water, and the volume displaced by 1000 seeds was recorded.

Bulk density was determined according to (Okaka *et al.*, 1977)^[8]. A 50 g seed sample was transferred to a 100 ml graduated cylinder and gently tapped 20–30 times until a constant volume was obtained. Hydration capacity and hydration index were determined according to (Bishnoi *et al.*, 1993)^[9]. A 0.10 g seed sample was soaked overnight in 10 ml of distilled water at ambient temperature, after which the hydrated seeds were drained and the relevant measurements were calculated. Swelling capacity and swelling index were determined using the same soaking procedure, based on the increase in seed volume following hydration.

2.4 Determination of functional properties

Water absorption capacity (WAC) and oil absorption capacity (OAC) were determined using the method of (Sosulski *et al.*, 1976)^[10]. One gram of chia seed flour was separately mixed with distilled water or soybean oil, allowed to stand for 30 min at room temperature, and centrifuged at 2000 rpm for 10 min. Absorption capacity was calculated from the change in sample mass following centrifugation.

Emulsion activity (EA) and emulsion stability (ES) were evaluated according to (Yasumatsu *et al.*, 1972)^[11]. An emulsion was prepared by homogenizing 1 g of chia seed flour with 10 ml of distilled water and 10 ml of peanut oil, followed by centrifugation at 2000 × g for 5 min. The height of the emulsified layer was measured for calculation of the corresponding parameters.

Foaming capacity (FC) and foaming stability (FS) were determined according to (Narayana *et al.*, 1982)^[12]. One gram of chia seed flour was dispersed in 50 ml of distilled water and whipped for 5 min. Foam volume was recorded immediately after whipping and again after 1 h of storage, and the corresponding values were used to calculate foaming capacity and stability.

2.5 Amino acid analysis

Amino acid composition was determined following (Landry *et al.*, 1992)^[13]. Samples were hydrolysed with 6 N HCl at 110 °C for 22 h in sealed glass ampoules under a nitrogen atmosphere. The hydrolysates were diluted, filtered, and evaporated to dryness, followed by reconstitution in sodium citrate buffer (pH 2.2). Samples were passed through a 0.45 µm syringe filter and analysed by high-performance liquid chromatography (HPLC) using a Nova-Pak column and fluorescence detection.

2.6 Statistical analysis

All analyses of physical, functional, and amino acid parameters were performed in triplicate unless otherwise stated. Results are presented as mean ± standard deviation (SD). The coefficient of variation (CV, %) was calculated as (SD/mean) × 100 to assess analytical precision and repeatability.

3. Results and Discussion

3.1 Physical properties of chia seeds

Table 1 presents the physical properties of chia seeds: thousand-kernel weight, thousand-kernel volume, bulk density, hydration capacity, hydration index, swelling capacity, and swelling index. We selected these parameters because they collectively govern seed behavior during cleaning, grading, storage, transport, soaking, and downstream processing, and because they influence chia's functional performance in food formulations, particularly its well-known gelling and water-binding behavior. Each value represents the mean of three independent determinations. The low coefficients of variation for all seven parameters (0.04–1.33%) indicate uniformity within the seed lot and good reproducibility of the analytical procedures.

The thousand-kernel weight (TKW) and thousand-kernel volume of chia seeds were 1.46 g and 1.80 mL, respectively, and the bulk density was 0.75 g mL⁻¹. These values are broadly comparable with those reported by (Ixtaina *et al.*, 2008)^[14], who observed a thousand-seed mass of 1.323 g for dark chia seeds and 1.301 g for white chia seeds, along with a bulk density ranging from 0.667 to 0.722 g cm⁻³ for *Argentinian chia* seed accessions. The marginally higher TKW obtained in the present investigation may be attributed to varietal and agro-climatic differences, a trend also noted by (Porrás-Loaiza *et al.*, 2014)^[15], who reported that the physical characteristics of chia seeds vary appreciably among Mexican production regions (Chiapas, Oaxaca, Michoacán, and Puebla) as well as in comparison with Argentinian seed stocks. Similarly, (Guiotto *et al.*, 2013)^[16] reported thousand-seed mass values ranging from 1.170 to 1.352 g, depending on seed moisture content, further supporting the moisture- and origin-dependent variability of this parameter.

Bulk density is an important physical property influencing storage efficiency, transportation, and packaging requirements. The observed value (0.75 ± 0.01 g mL⁻¹) is consistent with the findings of (Ayerza *et al.*, 2004)^[17], (Muñoz *et al.*, 2013)^[6], who reported bulk density values ranging from 0.70 to 0.78 g mL⁻¹ for chia seeds. This relatively high bulk density supports economical storage and transportation while influencing the textural properties of processed food products. Hydration capacity was 81.20 ± 0.06 mg seed⁻¹ and the hydration index was 55.57 ± 0.03. The pronounced hydration behavior is consistent with the mucilaginous polysaccharide fraction associated with the seed coat, which hydrates rapidly and forms a gelatinous layer. Previous studies have similarly linked chia mucilage with strong water-holding and hydration characteristics (Muñoz *et al.*, 2013)^[6]. This behavior is technologically relevant because water retention can influence dough handling, texture, viscosity, and moisture retention in formulated foods.

The swelling capacity and swelling index of chia seeds were 95.10 ± 0.09% and 52.83 ± 0.02, respectively, demonstrating their high water-absorption and swelling potential. The pronounced swelling can be attributed to the hydration of mucilage polysaccharides, which facilitates water retention and the formation of a hydrated, viscous ma

trix. Consequently, chia's high swelling capacity is directly related to its ability to increase viscosity, improve moisture retention, and contribute to gel formation, supporting its functionality as a natural hydrocolloid in food formulations. Similar water-binding and swelling properties have been reported previously (Segura-Campos *et al.*, 2014) [18], (Dick *et al.*, 2015) [18, 19]. These characteristics indicate chia's potential for applications involving thickening, stabilization, texture modification, and moisture management.

The low CV values observed across the measured physical properties indicate good repeatability of the measurements under the experimental conditions. However, interpret CV values alongside the number of replicates and the precision of the analytical methods used. Overall, the physical properties are consistent with previous reports and highlight the pronounced hydration and swelling capacity of chia seeds, primarily associated with their mucilage polysaccharides. These properties underpin chia's technological functionality, particularly in improving texture, moisture retention, and structural stability, and support its incorporation into functional foods, bakery products, beverages, and protein-enriched formulations.

Table 1. Physical properties of chia seeds

Physical property	Observation	CV (%)
Thousand-kernel weight (g)	1.46 ± 0.01	0.68
Thousand-kernel volume (ml)	1.80 ± 0.01	0.56
Bulk density (g ml ⁻¹)	0.75 ± 0.01	1.33
Hydration capacity (mg seed ⁻¹)	81.20 ± 0.06	0.07
Hydration index	55.57 ± 0.03	0.05
Swelling capacity (%)	95.10 ± 0.09	0.09
Swelling index	52.83 ± 0.02	0.04

Values are mean ± SD (*n* = 3). CV, coefficient of variation.

3.2 Functional properties of chia seed flour

Chia seed flour exhibited a WAC of 166.0 ± 1.20% and an OAC of 63.00 ± 0.80% (Table 2), indicating a greater apparent affinity for water than for oil under the assay conditions. This high WAC is consistent with the hydrophilic nature of chia mucilage and its soluble polysaccharide fraction. Higher values have been reported for fiber-rich or defatted chia fractions, where hydrophilic components are

concentrated (Vázquez-Ovando *et al.* 2009) [20], (Capitani *et al.*, 2012) [20, 21]. The lower value observed for whole flour is compatible with the presence of oil, protein, and other seed constituents that dilute the concentration of highly hydrophilic components.

The OAC of 63.00 ± 0.80% indicates measurable oil-binding ability. In plant flours, oil retention can reflect interactions with hydrophobic protein regions and physical entrapment within the flour matrix. Differences among chia preparations may therefore result from defatting, milling, protein concentration, and processing history.

Emulsion activity and emulsion stability were 54.23 ± 0.18% and 82.98 ± 0.23%, respectively, indicating that chia flour can form and stabilize oil-in-water emulsions. Chia proteins provide amphiphilic interfacial activity, whereas mucilage can increase the viscosity of the continuous phase and thereby influence droplet mobility and coalescence ((Sandoval - Oliveros and Paredes Lopez) *et al.* 2013) [22]. The measured emulsion activity was close to that reported for a chia fiber-rich fraction by (Vázquez-Ovando *et al.*, 2009) [20], although differences in flour composition and extraction procedures can affect absolute values.

Foaming capacity was high (90.00 ± 0.60%), whereas foaming stability was low (2.79 ± 0.05%), indicating that the flour can incorporate air readily but does not maintain the resulting foam effectively over time. Protein adsorption at the air–water interface can facilitate initial foam formation, while inadequate interfacial film strength, drainage, and bubble coalescence can contribute to subsequent collapse. The results suggest that applications requiring prolonged foam stability may require formulations with additional proteins or hydrocolloids.

Table 2. Functional properties of chia seed flour

Functional property	Observation	CV (%)
Water absorption capacity (%)	166.0 ± 1.20	0.72
Oil absorption capacity (%)	63.00 ± 0.80	1.27
Emulsion activity (%)	54.23 ± 0.18	0.33
Emulsion stability (%)	82.98 ± 0.23	0.28
Foaming capacity (%)	90.00 ± 0.60	0.67
Foaming stability (%)	2.79 ± 0.05	1.79

Values are mean ± SD (*n* = 3). CV, coefficient of variation.

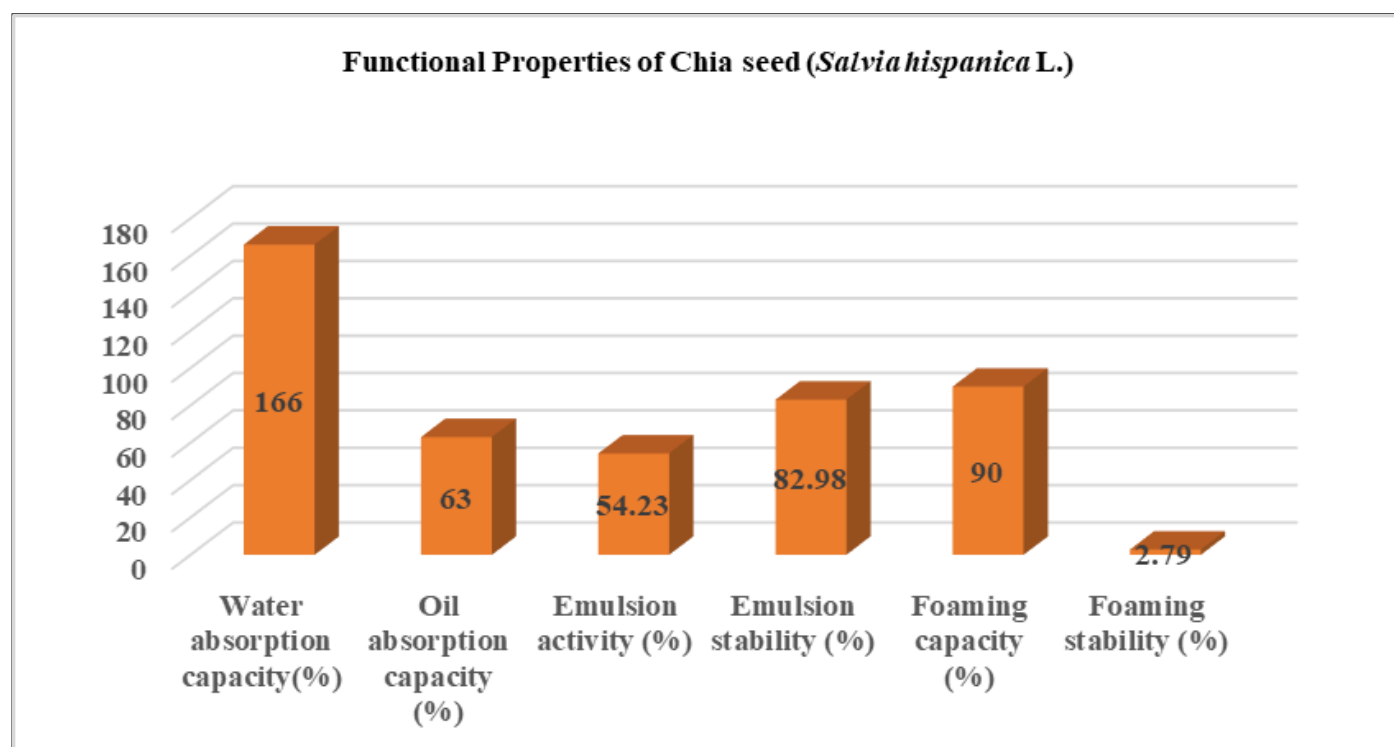


Figure 2. Functional properties of chia seed

3.3 Amino acid composition of chia seeds

Table 3 presents the amino acid composition of the analyzed chia seed sample. We quantified eight essential amino acids and eleven non-essential amino acids. Among the essential amino acids, leucine was the most abundant (1.61 ± 0.04 g 100 g⁻¹ dry matter), followed by valine (1.33 ± 0.005 g 100 g⁻¹), phenylalanine (1.25 ± 0.04 g 100 g⁻¹), lysine (1.09 ± 0.03 g 100 g⁻¹), threonine (0.96 ± 0.01 g 100 g⁻¹), histidine (0.89 ± 0.05 g 100 g⁻¹), isoleucine (0.88 ± 0.04 g 100 g⁻¹), and methionine (0.79 ± 0.02 g 100 g⁻¹). Tryptophan is destroyed during acid hydrolysis and was therefore not determined.

The overall ranking of essential amino acids broadly matches patterns reported for chia seeds from other growing regions. (Katunzi-Kilewela *et al.*, 2022)^[23] reported leucine, valine, and phenylalanine among the predominant essential amino acids, with methionine occurring at a comparatively lower concentration. Variation among studies can reflect genotype, environmental conditions, seed maturity, and analytical methodology.

Among the quantified non-essential amino acids, glutamic acid showed the highest concentration (4.25 ± 0.07 g 100 g⁻¹ dry matter), followed by arginine (3.70 ± 0.057 g 100 g⁻¹) and aspartic acid (2.06

± 0.01 g 100 g⁻¹). Serine, proline, glycine, and alanine were present at intermediate concentrations. The predominance of glutamic acid and arginine is consistent with published characterizations of chia protein (Kulczyński *et al.*, 2019)^[3], (Sandoval-Oliveros *et al.*, 2013)^[3, 22].

Cystine was present at 0.45 ± 0.03 g 100 g⁻¹ dry matter. Glutamine was reported as 0.00 ± 0.001 g 100 g⁻¹. This result should not be interpreted as definitive evidence for complete biological absence because conventional acid hydrolysis converts glutamine to glutamic acid. Hydroxyproline was detected at 0.94 ± 0.006 g 100 g⁻¹ dry matter; because this is an unusual constituent to emphasize in a seed-protein profile, confirmation using an independent analytical approach would strengthen its identification.

Overall, the amino acid data show a broad range of essential amino acids and a predominance of glutamic acid and arginine among the quantified non-essential amino acids. These characteristics support further investigation of chia as a plant-derived protein ingredient. However, conclusions regarding protein quality should ideally be complemented by total protein content, amino acid scoring against an established reference pattern and, where appropriate, digestibility measurements.

Table 3. Amino acid profile of chia seeds

Amino acid	Content (g 100 g ⁻¹ dry matter)
Essential amino acids	
Methionine	0.79 ± 0.02
Histidine	0.89 ± 0.05
Isoleucine	0.88 ± 0.04
Leucine	1.61 ± 0.04
Lysine	1.09 ± 0.03
Phenylalanine	1.25 ± 0.04
Threonine	0.96 ± 0.01
Valine	1.33 ± 0.005
Non-essential amino acids	
Aspartic acid	2.06 ± 0.01
Glutamic acid	4.25 ± 0.07
Serine	1.27 ± 0.01
Proline	1.28 ± 0.01
Glycine	1.05 ± 0.05
Alanine	1.11 ± 0.02
Cystine	0.45 ± 0.03
Arginine	3.70 ± 0.057
Tyrosine	0.61 ± 0.047
Glutamine	0.00 ± 0.001
Hydroxyproline	0.94 ± 0.006

Values are mean $\pm$ SD ($n = 3$) on a dry-matter basis.

4. Conclusion

This study showed that chia seeds from Guntur have favorable physical and functional characteristics for food formulation. The seeds showed substantial hydration and swelling behavior. Chia seed flour exhibited high water absorption capacity, measurable oil absorption capacity, appreciable emulsion activity and stability, and high initial foaming capacity, although foam stability was low. The amino acid profile comprised eight quantified essential amino acids, with leucine the most abundant, while glutamic acid and arginine were prominent among the quantified non-essential amino acids. Collectively, these findings support the potential of chia seed flour as a multifunctional ingredient for developing protein- and fiber-enriched foods. Further work should evaluate the effects of processing (roasting, milling, de-fatting), incorporation level, storage, and food matrix on nutritional

quality, functionality, sensory characteristics, and product stability. It should include total protein, dietary fiber, lipid, and mineral composition, amino acid scoring against a reference pattern, and independent confirmation of the hydroxyproline and glutamine findings.

5. Recommendations

Chia seed flour may be explored as a functional ingredient in developing protein-rich, fiber-rich and nutritionally enhanced bakery, snack and beverage products. Further studies may evaluate the effects of processing, storage and different incorporation levels on the nutritional, functional, sensory and shelf-life characteristics of chia-based products.

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