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Evaluation of Functional Properties of Protein Isolates from Groundnut and Sesame Oilseed Meal

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Abstract

The rising global population has increased demand for sustainable protein sources, thereby enhancing the utilisation of oilseed meal, an oil industry by-product, to produce value-added protein isolates. Hence, the study was designed to investigate the functional properties of groundnut and sesame meal protein extracted through alkaline solubilization. The functional attributes of the protein were assessed for percent yield, bulk density, tap density, oil holding capacity (OHC), water holding capacity (WHC), emulsifying activity index (EAI), emulsion stability (ES), foaming capacity (FC), foaming stability (FS), least gelation concentration (LGC), and color. The findings showed that both protein isolates had good functional attributes with different features ideal for application in varied food products. Groundnut meal protein isolate showed greater bulk density (0.34 g/mL), tapped density (0.67 g/mL), water holding capacity (2.67 g/g), emulsifying activity index (33.77 m^2/g), and foaming capacity (58%). On the other hand, sesame meal protein isolate exhibited greater oil holding capacity (2.40 g/g), emulsion stability (25.76), and foaming stability (63.33%). Groundnut meal protein isolate showed a lower least gelation concentration (12%), indicating superior gel-forming ability compared with sesame meal protein isolate. The findings indicate that protein isolates derived from groundnut meal and sesame seed meal exhibit promising functional properties, supporting their use as sustainable food ingredients. Their favourable water and oil holding capacity, emulsification, foaming, and gelation characteristics make them suitable for improving texture, stability, and overall quality in a variety of food products.

Keywords: Oilseed Meal, Protein isolates, Functional Properties

1. Introduction

The rapidly growing population has intensified the demand for protein that can be produced sustainably and economically while maintaining environmental balance. New concepts in food production are pivotal, particularly the efficient use of waste to produce value-added products. Among agro-industrial by-products, oilseed cake, a byproduct of the oil industry, has been utilized [1] for its high protein content and nutritional value [2].

Oilseeds are important sources of lipids and are primarily processed for their edible oils, yielding a substantial protein-rich meal [3]. Groundnut (*Arachis hypogaea*) and sesame seeds (*Sesamum indicum*) are among the valuable oilseed crops farmed worldwide for oil extraction. After oil removal, a large portion of oilseed crops leaves defatted meals that retain high levels of protein and other nutrients. Defatted groundnut flour contains approximately 50-55% high-quality protein, whereas sesame oilseed meal contains about 35-50% protein along with bioactive metabolites and fatty acids [1,2,3].

Attention to plant-based proteins has increased globally, creating scope to explore value addition to oilseed meal, which is a good source of plant-based protein. Despite their rich nutritional content, these oilseed meals remain underutilized. Defatted sesame oilseed cake is widely used as animal fodder or as a fertilizer feedstock, despite its excellent protein, bioactive metabolite, and fatty acid content [1]. Similarly, peanut meal has traditionally been used as feed in the poultry and livestock sector, and it is not much explored in food processing industry. Therefore, it is of great significance to explore efficient approaches to maximizing the economic value of these underutilized, protein-rich by-products [4].

Protein concentrates and isolates derived from sesame and groundnut meals can be used in a variety of cuisines to flavour foams, emulsions, and gels [2,5]. In food product development, the functional properties of proteins are highly essential, i.e., amino acid sequence, secondary/ternary structures, surface potential and charge distributions, hydrophilic and hydrophobic characteristics, aggregation behaviors, etc. which are all adjustable for because they determine their performance in applications such as emulsification, foaming, water and oil binding, and gelation. These functionalities are determined by their different heterogeneous structures as well as the resultant physicochemical properties appropriate for food applications [6]. In this context, the objective of this study is to assess the functional characteristics of protein isolates from groundnut and sesame meal.

2. Materials and Methods

Procurement of raw materials

White sesame seed meal was procured from Samarth Lakda Ghani (Wood pressed Oil), Vadodara, while Groundnut meal was procured from Gayatri Industry, Undra, Kheda, Gujarat.

Preparation of Sample

White sesame seed meal and groundnut meal were ground to a fine powder using a mixture and sieved using a mesh screen (120 BSS).

Protein Extraction Method

Protein extraction from selected oilseed meal was performed using a modified method by Moghadam et al. (2020) [7]. A 5% (W/V) sample of oilseed flour was combined with distilled water and adjusted to pH 9 using 1N NaOH. After one hour of shaking, the mixture was centrifuged at 2000 rpm for 15 minutes. The collected extract was adjusted to pH 4.5 with 1N HCl to precipitate the protein. The protein was then separated by centrifugation at 2000 rpm for 15 minutes, washed with distilled water, and dried.

Extraction Yield

The protein extraction yield for alkaline extraction was derived by the method of Miranda et al. (2022) [8].

$$\% \text{ Protein Yield} = \frac{Supernatant\ Protein}{Sample\ flour\ protein} \times 100$$

Water Holding Capacity

Water Holding Capacity (WHC) was determined using the method of Sofi et al. (2013) [9]. 0.5g sample was mixed with 6 ml of distilled water and soaked for 20 mins. The slurry was then centrifuged at 4000 rpm for 15 minutes, and the supernatant was removed. The residue weight was recorded, and WHC was assessed by the method (g/g).

Oil Holding Capacity

Oil Holding Capacity (OHC) was measured according to the procedure of Sofi et al. (2013) [9]. 0.5 g sample was soaked with 10 ml of oil for 24 hours. Then, the content was centrifuged 4000 rpm for 10 minutes, and the supernatant was discarded. The weight of the residue was measured and OHC was calculated from the equation (g/g).

Bulk Density

Bulk Density (BD) was calculated using the procedure described by Sofi et al. (2013) [9]. A sample was carefully added to a 10 mL measuring cylinder. After filling to the 10 mL mark, the bottom of each cylinder was tapped lightly on a laboratory platform multiple times until the sample level stopped dropping. The bulk density was then evaluated as the weight per unit volume of the sample (g/mL).

Tapped Density

The Tapped Density was calculated using the method described by Anandharamakrishnan and Ishwarya et al. (2015) [10]. Tapping the cylinder 300 times, followed by measurement of compacted volume (V_T), the weight of the sample with the cylinder (m_2), and the weight of the empty cylinder (m_1), The tapped density was then evaluated by the method of weight per unit volume of sample (g/mL).

Emulsifying Activity Index

The Emulsifying Activity Index (EAI) method was proposed by Lopez et al. (2003) with minor modifications [11]. 10 ml of oil was dispersed into 30 mL of protein suspensions (0.5 g protein/100 mL) using a homogenizer at 5000 rpm for 5 minutes. The emulsions obtained were immediately diluted with a solution of 0.1 g/100 mL sodium dodecyl sulfate at pH 7, containing 0.1 mol/L sodium chloride. The turbidity T (= 2.303 A/l, where A is the absorptivity of the emulsion and l is the path length of the cuvette) of the dilutions was immediately measured at 500 nm using a spectrophotometer. Emulsifying activity index (EAI) was described as:

$$\text{EAI} = \frac{2T}{\emptyset C}$$

where Ø is the volume fraction of the oil phase (here Ø = 0.25), and C is the protein concentration in the aqueous phase. The EAI, expressed in (m^2/g), was related to the interfacial area stabilized per unit weight of protein.

Emulsion Stability

Emulsion Stability (ES) of the protein isolates was investigated by following the method described by Shrestha et al. (2023) [12]. ES was measured by a turbidimetric method. 12 mL of protein solution (0.25% w/v) in phosphate-buffered saline (0.1 M, 0.15 M NaCl, pH 7) was stirred at 300 rpm for 24 hours at room temperature (20 ± 5 °C) to ensure complete protein hydration. Oil (4 mL) was added to the protein dispersion, and the mixture was homogenized using a homogenizer at 6000 rpm for 1 min to form a stable mixture, then at 9500 rpm for 5 min to form a homogeneous emulsion. Take an emulsion (50 µL) from the bottom of the tube and add it to 5 mL of 0.1 % w/v sodium dodecyl sulfate (SDS) at 0 min and 10 min after homogenization. The absorbance of the diluted emulsion was measured at 500 nm using a spectrophotometer, and ES was calculated as follows.

$$\text{Emulsion stability} = \frac{A_0 \times \Delta t}{\Delta A}$$

where A_0 = Absorbance at 0 min, Δt = time interval of 10 min, and ΔA = the difference in absorbance between 0 and 10 min.

Foaming Capacity and Stability

The protein isolates were analysed for foaming capacity (FC) and foaming stability (FS) using the method represented by Shrestha et al. (2023) with minor modifications [12]. 50 mL of protein solution (1% w/v) was mixed with phosphate buffer saline (0.1 M, 0.15 M NaCl, pH 7) and then blended at 300 rpm for 24 h at room temperature to form complete protein hydration. The protein solution was homogenised using a homogeniser at 6000 rpm for 1 min to form a constant mixture, then at 9500 rpm for 5 min. The homogenised mixture was shifted into a graduated measuring cylinder. Foaming capacity and stability were determined using formulas (1) and (2), respectively.

$$\text{Foaming capacity (\%)} = \frac{V_0}{V} \times 100 \qquad (1)$$

$$\text{Foaming stability (\%)} = \frac{V_{120}}{V_0} \times 100 \qquad (2)$$

where, V = total volume before stirring, V_0 = foam volume at time 0 min, and V_{120} = foam volume at time 120 min.

Least Gelation Concentration

The method was proposed by Mengozzi (2024) with slight modifications [13]. Aqueous solutions were prepared by adding protein at 10, 12, 14, 16, 18, and 20% (w/w) to distilled water. The suspensions were magnetically stirred for 3 h at room temperature, and after 2 h and 30 min, the pH was adjusted to 5.0 by adding 1 M HCl or 1 M NaOH. To influence gelation, protein suspensions were transferred to sugar tubes

and placed in a water bath at 95 °C for 1 h, then left to cool for 10 min. The heat-treated samples were then stored at 4 °C for 24 h before analysis. The Least Gelation Concentration (LGC) was determined as the sample with the lowest concentration that did not flow when the tubes were inverted.

Colour analysis (Hunter Lab)

The colour of the protein was determined using a colour flex EZ 45º/ 0° spectro-colorimeter (Hunter Lab) after standardisation of the instruments using Hunter Lab Colour standards & 'L*' (lightness), ' a* ' (redness to greenness) and 'b*' (yellowness to blueness) values were measured by placing the sample in a sample cup.

Statistical analysis:

The obtained data was analysed using SPSS (Version 22.0) for Mean, standard deviation (S.D.), and t test.

3. Result and Discussion

Yield of protein

The percent protein yield of sesame seed meal protein was higher yield (75.24 %) compared to groundnut meal protein (71.55 %) as shown in Figure 1. The findings reported by Abbas et al. (2020) and Susheela et al. (2022) indicated higher protein isolate yields of 79.03% for groundnut and 75.52% for the same protein, respectively [14, 5]. The higher protein yield observed may be attributed to the extraction condition employed. As reported by Susheela et al. (2022), protein recovery yield is influenced by both extraction and precipitation pH. Moreover, alkaline extraction has been shown to enhance protein isolate yield in various plant protein sources [5].

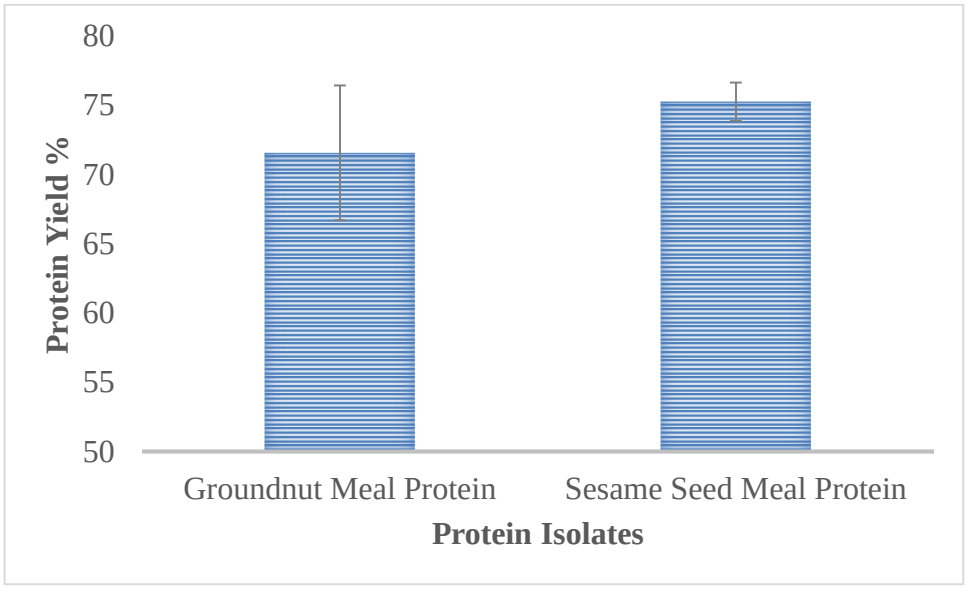

Figure 1: Protein Yield (%) of Oilseed Meal Protein Isolates

Water Holding Capacity and Oil Holding Capacity

The water-holding capacities (WHC) of proteins indicate amount of water held per unit mass, while the oil-holding capacities (OHC) indicate amount of oil held per unit mass. These characteristics are vital for some food products, such as the syneresis of plant-based yoghurts and the cookability and juiciness of plant-derived meats [15].

WHC of groundnut meal and sesame seed meal protein is 2.67 g/g and 2.26 g/g, respectively, as presented in Table 1. A significant difference ($P \leq 0.05$) in WHC was observed between groundnut meal and sesame meal protein isolate. Comparatively higher WHC values of 2.86 g/g and 3.64 g/g were reported for groundnut protein concentrate and isolates, respectively, by Jain et al. (2015) [3]. At the same time, a lower WHC of 2.12 ml/g for sesame oilcake protein is reported by Sibt-e-Abbas et al. (2020) [14]. The OHC for groundnut meal and sesame meal protein was (2.22 g/g) and (2.40 g/g), respectively, in this study. No significant difference ($p > 0.05$) was observed in OHC between protein isolates. Groundnut (3.48 g/g) and sesame (3.11 ml/g) proteins have higher OHC reported by Jain et al. (2015) and Sibt-e-Abbas et al. (2020) [3,14], respectively. The differences in protein composition, level of interaction with water and oil, and structural properties may clarify the varied findings on protein's effects on oil at different ratios in terms of physical retention [2].

Table 1: Functional Properties of Oilseed Meal Protein Isolates

Name of Protein	**Water Holding Capacity (g/g)**	**Oil Holding Capacity (g/g)**	**Bulk Density (g/mL)**	**Tapped Density (g/mL)**
Groundnut meal protein	2.67 ± 0.19	2.22 ± 0.14	0.34 ± 0.20	0.67 ± 0.025
Sesame seed meal protein	2.26 ± 0.07	2.40 ± 0.31	0.31 ± 0.00	0.64 ± 0.01
t - value	3.45*	-0.90^{NS}	2.13^{NS}	2.13^{NS}

Values are mean ± standard deviation of three observations; * indicates a significant difference at $P \leq 0.05$; NS indicates no significant difference.

Bulk Density and Tapped Density

The bulk density (BD) is a major parameter for food powders during storage, processing, packaging, and handling, and it depends on powder size, shape, surface properties, and particle size. Smooth, uniform powder has a higher bulk density, indicating smaller air gaps between powder particles, thereby reducing lipid oxidation and improving storage stability [16]. Tapped density (TD) is an important property that reflects the packaging arrangement and compaction behaviour of particles. It influences hydration characteristics during food processing [17].

The BD of groundnut meal protein was higher (0.34 g/mL) than that of sesame seed meal (0.31 g/mL), as presented in Table 2. No significant difference ($P > 0.05$) in BD and TD was observed between the protein isolates from groundnut and sesame meal. Comparatively higher BD values for groundnut oilcake protein

(0.49 g/mL) extracted at pH 9.0 and sesame seed oilcake protein (0.53 g/cm^3) extracted by the conventional method were reported by Susheela et al. (2022) and Mathews et al. (2023), respectively [5, 2]. The tapped density was (0.67 g/mL) for groundnut meal protein and (0.64 g/mL) for sesame meal protein, as shown in Table 1. Relatively low TD values of 0.64 g/mL and 0.55 g/cm^3 were observed for groundnut protein isolate and sesame protein isolate, respectively [2,5]. These differences are attributed to particle size, protein conformation, the number of contact sites, and interparticle attractive forces [5]. The porous structure developed during processing may also affect density characteristics, thereby affecting their suitability for complementary and infant food formulations. This property is valuable from economic and functional perspectives in food packaging, as it can improve packaging performance and lower packaging costs [5].

Emulsifying Activity Index (EAI) and Emulsion Stability (ES)

Protein emulsifying properties are commonly evaluated using the Emulsifying Activity Index (EAI) and Emulsion Stability (ES). EAI indicates the interfacial area stabilized per unit weight of protein, determined by turbidity measurements, whereas ESI assesses emulsion stability by tracking the decrease in turbidity over time [18]. Emulsifying properties are an important functional property of proteins that improve the formation and stabilization of oil-water emulsions. It enhances the stability and quality of food products by preventing coalescence, creaming, sedimentation, or flocculation [6].

Groundnut meal protein (33.77 m^2/g) showed a significantly higher EAI ($P< 0.01$) than sesame meal seed protein (19.86 m^2/g), as presented in Table 2. The higher EAI of groundnut meal protein than that of sesame seed meal protein may indicate that the protein contains both polar and nonpolar amino acids, thereby increasing interactions between water and oil molecules. The protein from groundnut meal can act as an emulsifier since it is capable of being associated with both water and oil components of the food system and also contains hydrophobic and hydrophilic properties [1]. Higher EAI values have been observed in peanut meal (42.5 m^2/g) and sesame protein isolate (27.89 m^2/g) by Zhoa et al. (2023) and Mathews et al. (2023), respectively [4,2]. The Emulsion Stability of groundnut and sesame seed meal protein was 18.85 min and 25.76 min, respectively, which indicates that sesame seed meal protein has a greater ability to form and maintain stable emulsions. A significant difference ($P < 0.01$) in emulsion stability was observed between groundnut and sesame protein meal isolates. Similar findings were reported by Zhao et al. (2023) with an ES of 18.5 min for groundnut protein [4]. Protein emulsification is affected by solubility and surface hydrophobicity [19]. The higher emulsifying activity of proteins may be associated with their flexible size and smaller size, which enhance compact packing at the oil-water interface and the formation of stable interfacial networks around oil droplets, thereby enhancing emulsion stability [20].

Table 2: Emulsifying Properties of Oilseed Meal Protein Isolates

Name of Protein	**Emulsifying Activity Index (m^2 /g)**	**Emulsion Stability (min)**
Groundnut meal protein	33.77 ± 0.56	18.85 ± 1.41
Sesame seed meal protein	19.86 ± 0.95	25.76 ± 2.39

t - Value	-21.76**	-4.30*

Values are mean ± standard deviation of three observations, **indicates a significant difference at P<0.01, * means significant difference at P ≤ 0.05

Foaming Capacity and Stability

The foaming properties, including Foam Capacity (FC) and Foam Stability (FS), are the main features used for aeration and whipping in the food sector [21].

Groundnut meal protein (58.00%) has a higher FC than sesame seed meal protein (33.33%), as shown in Figure 2. However, a statistically significant difference ($p < 0.0001$) was observed. Zhao et al. (2023) and Yuzer et al. (2023) reported that defatted peanut meal protein and defatted sesame seed protein had foamability values of 60% and 36.21%, respectively [4, 22]. FS of sesame seed meal protein and groundnut meal protein is 44.8% and 39.66%, respectively as shown in Table 4. No significant difference was observed. Comparatively, higher FS values for defatted peanut meal protein (50.2%) and sesame oilcake protein isolate (46.98%) were reported by Zhoa et al. (2015) and Abbas et al. (2020), respectively [4, 14]. Similarly, Abbas et al. (2015) reported higher FS for defatted peanut protein (DPF) from the local varieties Golden and BARI-2011, at 52.45% and 54.75%, respectively [23].

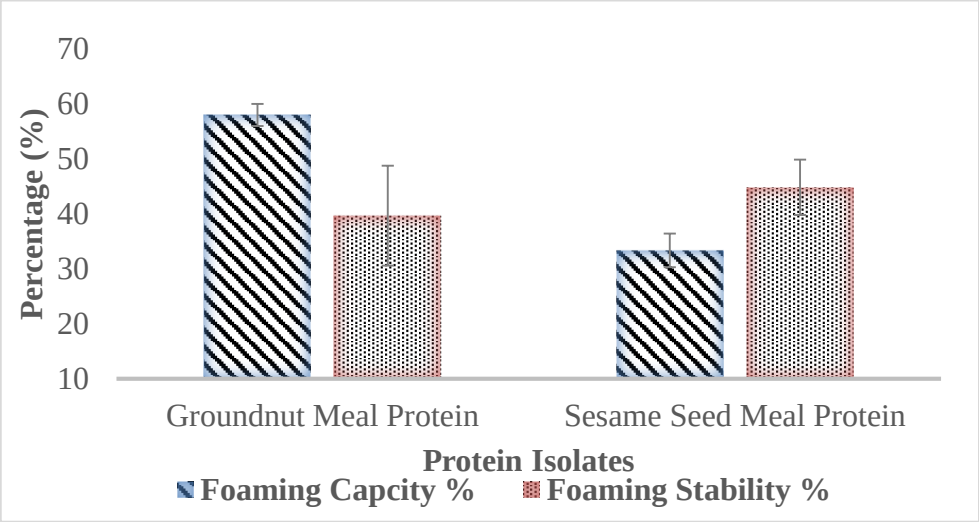

Figure 2: Foaming Properties of Oilseed Meal Protein Isolates

Least Gelation Concentration

The least gelation concentration (LGC) is a widely used property for measuring the gelling ability of proteins [24] and is the most common method, determining the protein concentration at which the protein solution forms a gel that does not slide from a test tube when inverted [15].

The gelation behaviour of protein suspensions was evaluated at pH 5. Groundnut meal protein exhibited gel formation at 12 % (w/w), whereas sesame seed meal protein exhibited gelation at 18 % (w/w), as shown in Table 3. Similar findings for groundnut protein isolate were reported by Susheela et al. (2022), in which gel formation occurred at a protein concentration of 12% [5]. Himabindhu et al. (2023) noted similar gel formation in white sesame protein isolate at concentrations of 18% and 20%. The differences

in the LGC of white sesame meal protein isolate may be due to the ratios of proteins, carbohydrates, and lipids, as well as their interactions, which affect functional properties [1].

Table 3: Least Gelation Concentration of Oilseed Meal Protein Isolates

Name of protein	**10 %**	**12 %**	**14 %**	**16 %**	**18 %**	**20 %**
Groundnut meal protein isolate	✗	✓	✓	✓	✓	✓
Sesame seed meal protein isolate	✗	✗	✗	✗	✓	✓

Color Analysis

Colour is a key feature influencing the appearance of finished products [1]. Similarly, Susheela et al. (2022) stated that colour plays an essential role in the sensory quality of food, impacting its acceptability. It is typically measured using L*, a*, and b* values [5].

There was significant difference ($p < 0.01$) in the 'L*', 'a*' and 'b*' values in the meal protein isolates as presented in Table 4. The 'L*' value of the sesame seed meal protein (69.63) was significantly higher than the groundnut protein meal (64.61). This suggests that there is better lightness in the protein isolate, which might make it more acceptable visually in foods. The 'a*' values are indicative of the red-green spectrum were 8.26 and 8.14 for sesame seed and groundnut meal protein, respectively. Sesame seed meal protein exhibited a higher b* value (33.09) than that of groundnut meal protein (23.62). Similar 'L*' value (65), lower 'b*' value ranging from 5 to 8, and similar 'b*' value ranging from 20 to 25 were observed for groundnut protein isolate extracted at pH 9.0 by Susheela et al. (2022) [5]. Himabindhu et al. (2020) reported lower values of 'L*' (55.05), 'a*' (5.67), and 'b*' (18.75) for white sesame protein isolates [1]. As pH increased, the 'L*' values of the protein isolates decreased, indicating greater darkness. This decrease could be due to Maillard-type browning outcome during drying. The dark colour might be due to the mixture of pigments and polyphenols dissolved in the protein isolate extraction solution, as reported by Susheela et al. (2022) [5].

Table 4: Color Analysis of Oilseed Meal Protein Isolates

Name of protein	**L***	**a***	**b***
Groundnut meal protein	64.61 ± 0.16	8.14 ± 0.01	23.62 ± 0.01
Sesame seed meal protein	69.63 ± 0.40	8.26 ± 0.00	33.09 ± 0.01
t-value	-208.70**	-20.78**	-856.60**

Values are mean ± standard deviation of three observations, **indicates a significant difference at P<0.01

4. Conclusion

The present study concludes that protein isolates extracted from groundnut and sesame seed meal possess desirable functional properties and distinct application potential. The groundnut meal protein isolates showed greater water holding capacity, bulk density, tapped density, emulsifying activity index, foaming capacity, and lesser least gelation concentration, which suggests their suitability in foods that need better water retention and efficient emulsification. Because of its low LGC value, it is clear that the groundnut protein isolate has the potential to develop into a gel structure even at low levels of proteins, thus making it an appropriate addition to food products. In contrast, sesame seed meal protein isolate showed greater protein yield, oil-holding capacity, emulsion-stabilised formulations, and aerated products with greater stability during processing and storage. Overall, the results demonstrate that protein isolates obtained from groundnut and sesame oilseed meals possess complementary functional properties. Their combined potential highlights the value of these underutilised by-products as sustainable plant-based protein ingredients for the development of functional and value-added food products.

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