

## Research Article

## Open Access

# Effect of pH on the properties of protein isolates obtained from defatted groundnut cake

Nerella Susheela<sup>\*1</sup>, Aparna Kuna<sup>2</sup>, Afifa Jahan<sup>3</sup>, Lakshmi prasanna Kata<sup>4</sup><sup>1</sup>Department of Foods and Nutrition, College of Community Science, PJTSAU, Rajendranagar- 500 030, India<sup>2</sup>MFPI - Quality Control Laboratory, P.J.T.S. Agricultural University, Rajendranagar, Hyderabad – 500030, India<sup>3</sup>Department of Foods and Nutrition, College of Community Science, PJTSAU, Saifabad, Hyderabad-500004, India<sup>4</sup>MFPI - Quality Control Laboratory, P.J.T.S. Agricultural University, Rajendranagar, Hyderabad – 500030, India

## ABSTRACT

With the ever-growing demand for protein, that is produced sustainably and economically maintaining environmental equilibrium, new concepts of food production where waste is efficiently utilized and transformed into value-added products are drawing the attention of many researchers. Hence a study was designed to utilize defatted groundnut oilseed cake - a byproduct of oil industry to produce protein isolates. The research investigated the impact of pH on the properties of protein isolates derived from defatted groundnut cake. Cold-pressed defatted groundnut cake was used to generate groundnut protein isolates using alkaline extraction (pH 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0) and acid precipitation at pH 4.5. The protein content of defatted flour was 50.75±0.03% which significantly increased in all protein isolates, with highest protein content (88.76±0.50%) observed in isolates extracted at pH 7. The highest isolate recovery and protein yield were observed at pH 12 (61.22±0.01g/100g), (94.44±0.13%). The highest WHC and OAC concentrations were found in protein isolates extracted at pH 12 (1.44±0.0 g/g, 1.28±0.01 ml/g) respectively. The highest emulsion activity was reported at pH 8 (50.10±0.1%), but ES was higher in GPIs extracted at pH 7 and 9 (48.61±0.04% and 47.34±0.02%). Bulk density and tapped density were higher in defatted flours than in protein isolates. Tapped density decreased in all protein isolates, as pH increased. Protein isolates from defatted peanut flour had low foaming and gelation capacities. The protein isolates extracted at various pH levels, had potential functional properties which are suitable for various product formulations. Hence, depending on the desirable functional property needed, the extraction of protein isolates can be taken up to develop various protein rich products. The alkaline extraction of protein isolates from plant sources presents a range of challenges, including quality control, pH optimization, denaturation risks, co-extraction of undesired compounds, and management of various process complexities. Addressing these challenges through meticulous control, conducted extensive experimentation within the pH range and innovative techniques contributes significantly to the food industry by advancing sustainable protein sources, improving food quality, and enhancing nutritional value. This optimization of extraction processes not only positions plant-based protein isolates as valuable alternatives to animal-derived counterparts but also promotes resource efficiency and reduced environmental impact. Together, these efforts pave the way for the development of nutritious, environmentally sustainable, and economically viable protein-rich food products to meet the ever-growing global demand.

**Keywords:** Acid precipitation, Alkaline extraction, Cold pressed, Defatted groundnut oilseed cake, Environmental equilibrium, Functional properties, Isolate recovery, pH extraction, Product formulations, Protein isolates, Protein-rich products, Value-added products.

## INTRODUCTION

Peanuts, also known as groundnuts (*Arachis hypogaea*), are edible seeds of a legume that are primarily farmed for oil extraction, human consumption, and animal use. They account for around 60% of global groundnut production, with 80% used for oil extraction, 12% for seed, 2% for export, and the remainder for culinary reasons [1]. A large portion of groundnut meals after oil extraction yields a protein-rich, inexpensive, and

underutilized by-product known as defatted groundnut meal (DGM), which offers several health and dietary benefits [2]. DGM contains 47-55% high-quality protein with essential amino acids, a desirable volatile profile, a low level of antinutritional factors, and a steady supply, making it a potential ingredient in many food applications. Apart from being used as an ingredient in food formulations, the DGM can also be used for the production of peanut protein isolates (90% protein) and concentrates (70% protein), which can also be used for incorporation in various new product formulations and protein fortification [3].

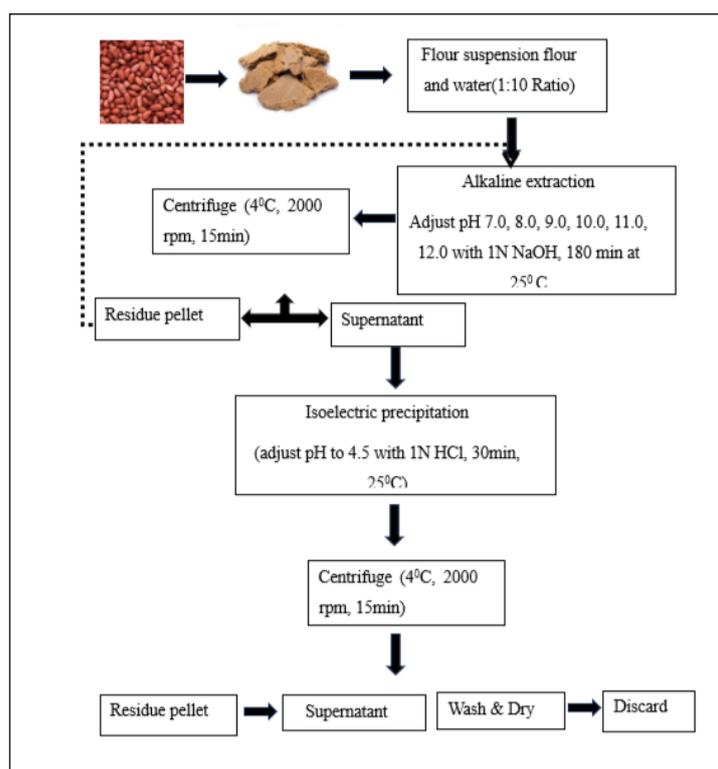
The world population in 2023 has exceeded 8 billion people and is expected to reach over 9 billion by 2050 [4]. Rapid population growth puts immense pressure on food production (approximately double the current food production), which is why special emphasis should be placed on sustainable food production, in particular, the production of foods rich in high-

\*Corresponding Author: K.M. Singh  
Email Address: m.krishna.singh@gmail.com

DOI: <https://doi.org/10.58321/AATCCReview.2023.11.03.446>  
© 2023 by the authors. The license of AATCC Review. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

quality protein to tackle food insecurity and malnutrition [5]. Projected demand for protein shows that the world demand for animal-derived protein will double by 2050, resulting in concerns for environmental sustainability. In part, this is because it is generally accepted that animal-based foods produce higher levels of greenhouse gases (GHG) than plant-based foods [6]. Due to these reasons, plant-based proteins are in great demand and the global market value for plant-based proteins is estimated to increase to about 40.3 billion dollars by 2025 [7].

Plant proteins are becoming popular from a nutritional perspective, creating a sustainable food supply chain [8], and offering an inexpensive source of protein. These proteins offer health benefits, soil fertility, and protein content (20-40%). Emerging plant protein ingredients include soy, pea, lentil, and chickpea. Discarded oil seed cakes are a cost-effective protein source. These proteins have important properties in food processing and product formulation, requiring study of their properties and characteristics to develop effective methodologies. Protein-rich sources in food engineering use processing parameters involving pH and temperature adjustments for stability [9]. This study aims to investigate the effect of alkaline pH extraction on groundnut protein isolates, focusing on protein content, yield, functional properties, physico-chemical properties, and in-vitro protein digestibility. The results will guide a better understanding of protein yield and properties at various protein isolation process conditions.



**Figure 1. Schematic presentation of groundnut protein isolates preparation.**

**Protein isolates assay:** was conducted on the groundnut protein isolates using the [11] method. Protein content, isolate recovery, and protein yield were analyzed in the protein isolates and compared with control (defatted oilseed cake).

**Isolate Recovery:** Oilseed protein isolates recovery was assessed as the weight of protein isolates obtained after isoelectric precipitation per 100 g sample [12].

## MATERIALS AND METHODS

The study was conducted at the Department of Foods and Nutrition, Post Graduate and Research Center (PGRC), College of Community Science and MFPI - Quality Control Laboratory, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad (India).

**Procurement of raw material and chemicals:** Defatted groundnut oilseed cake was procured from the local market. All the chemicals and required consumables used in the present study were of AR grade and were procured from local vendors.

**Preparation of groundnut protein isolate using alkaline extraction:** Defatted groundnut cake was ground to fine powder using the grinder and was sieved using a mesh screen (52 BSS), followed by drying at 60°C for 8 hours in a hot air oven for flour stabilization. To prepare protein isolates, the resultant defatted groundnut flour was dispersed in distilled water using a solid-liquid ratio of 1:10 (w/v), and pH was adjusted at 7, 8, 9, 10, 11 & 12, using 1 N NaOH solution. Furthermore, the supernatant was separated by centrifugation at 2000 rpm for 15 minutes. The supernatant was then adjusted to pH 4.5 using 1 N HCl for protein precipitation, followed by re-centrifugation, neutralization, and drying [10]. Schematic presentation of groundnut protein isolates preparation is depicted in Figure 1.

**Isolate Yield:** Protein yield of resultant isolates was calculated by using the expression as described by [12].

$$\text{Yield(\%)} = \frac{\text{Weight (g) of protein isolates}}{\text{Weight (g) of defatted meal}} \times \frac{\text{Protein content of Protein isolates(\%)}}{\text{Protein content (\%) of defatted meal}} \times 100$$

**Nutrient composition analysis:** Nutrients analyzed in this study were moisture and ash (IS: 1155 – 1968 Reaffirmed 2010), crude fat, crude fiber, and crude protein [13].

**Water holding capacity (WHC) and Oil binding capacity (OBC):** WHC and OBC were determined by the method of [14]. Duplicate samples (1 g) and 5 ml deionized water (adjusted to pH 7.0), or 5 ml sunflower oil were stirred for 1 min in a graduated tube and allowed to stand for 30 min at 25°C. The mixtures were centrifuged at 3000g for 25 min respectively. The volume of free liquid was then measured and retained liquid was expressed as ml of water or oil absorbed per gram of sample.

**Emulsifying Activity (EA) and Emulsion stability (ES):** EA and ES were determined by the method described by [15]. About 0.7 g of sample was added to 10 ml of distilled water and mixed well before adding it 10 ml of refined groundnut oil. The mixture was blended in an electric blender for 5 min and centrifuged at 2000x for 5 min and for determining emulsifying stability, re-centrifugation was done, followed by heating at 80°C for 30 min and subsequent cooling to 15°C. The supernatant was then poured into 50 ml measuring cylinders and allowed to stay a few min until the emulsified layer became stable. EA and ES were expressed as the height of the emulsified layer divided by the height of total content in the tube and expressed as a percentage.

**Foaming capacity (FC):** A 2% aqueous dispersion of the sample was mixed thoroughly, whipped for 3 minutes at high speed, and transferred immediately to a 250 ml measuring cylinder and the foam volume was measured at different time intervals. It was

calculated as the increase in volume (ml) of the protein dispersion upon mixing and expressed as a percentage increase in volume.

**Colour:** The colour of the defatted sesame cake and its protein isolate was performed by using a spectrophotometer (Hunter lab Colorflex, Firmware versions 1.1, Reston, Virginia) with a measuring aperture of 36 mm [13]. A circular glass cuvette was used to measure the samples after calibrating the equipment with white, green, and black tiles. Samples were placed on the reading lens and tested. A mean of 3 readings of each sample, produced values of L\* (lightness), a\* (redness), and b\* (yellowness).

**Bulk density and tapped density:** A 100 ml measuring cylinder was taken and weighed ( $W_1$ ). Samples were filled into the measuring cylinder up to 100 ml mark. The weight of the sample filled into the measuring cylinder was noted ( $W_2$ ) and the process was repeated thrice. For tapped density, after weighing samples ( $W_1$ ) in a 100 ml measuring cylinder, it was gently tapped to eliminate the space between the sample and reweighed ( $W_2$ ) in triplicates and expressed in g/ml.

$$\text{Bulk Density and tapped density} = \frac{(W_2 - W_1) \text{ in g}}{\text{Volume (ml)}}$$

**Least gelation Concentration (LGC) [16]:** Defatted WSF and WSPI flour suspensions in 5 ml distilled water were prepared to obtain 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18% and 20% (w/v) suspension concentrations. The test tubes containing flour suspensions were heated in boiling water for 1h and then

cooled down under running tap water for an hour. The LGCs were determined as the concentration at which the sample from the inverted test tubes did not fall or slip.

**Statistical analysis of data:** The experiments were performed in triplicates. The results were statistically analysed by analysis of variance and expressed as mean  $\pm$  standard deviation. Means were compared using the least significant difference (L.S.D.) at 0.05 levels.

**Results and discussion:** The values for crude protein, protein yield and protein isolate recovery are given in Table 1 and Fig.2. It was observed that protein content increased significantly in all GPIs compared to DGF, with the highest protein content observed at pH 7.0 (88.76 $\pm$ 0.50%). [17] reported the highest solubility of pea protein was observed at pH 9.0, possibly due to the salt-soluble nature of pea proteins. Protein isolate yield also increased with increasing pH values, attributed to the neutralization of side amine groups of basic amino acids. This increased the total negative charge and solubility of protein [18]. The optimal pH for protein extraction is assumed to be pH 7.0. Protein isolate recovery increased significantly with increasing pH values from 36.18 $\pm$ 0.15g/100g to 61.22 $\pm$ 0.01g/100g. The recovery of oilseed protein isolates conformed with the outcomes of rapeseed protein extracted at pH 12 and precipitated at pH 4–4.5. The highest isolate recovery was observed at pH 12 with a maximum of 61.22 $\pm$ 0.01g/100g. The protein recovery yield was correlated with both extraction and precipitation pH as reported by [19]. Alkaline extraction was effective for increasing the yield of tartary buckwheat protein isolates as reported by [20].

**Table 1. Protein Assay of Groundnut protein isolates**

Sample tested	Protein g%	Protein Yield %	Isolate Recovery g/100g
DGF	50.75 $\pm$ 0.03 <sup>a</sup>		
GPI 7	88.76 $\pm$ 0.50 <sup>g</sup>	63.57 $\pm$ 0.40 <sup>a</sup>	36.18 $\pm$ 0.15 <sup>a</sup>
GPI 8	85.21 $\pm$ 0.68 <sup>f</sup>	65.56 $\pm$ 0.18 <sup>b</sup>	38.34 $\pm$ 1.03 <sup>b</sup>
GPI 9	83.04 $\pm$ 0.94 <sup>e</sup>	75.52 $\pm$ 0.16 <sup>c</sup>	46.09 $\pm$ 0.95 <sup>c</sup>
GPI 10	81.87 $\pm$ 0.10 <sup>d</sup>	86.70 $\pm$ 0.45 <sup>d</sup>	53.46 $\pm$ 0.01 <sup>d</sup>
GPI 11	79.37 $\pm$ 0.13 <sup>c</sup>	89.36 $\pm$ 0.46 <sup>e</sup>	56.80 $\pm$ 0.52 <sup>e</sup>
GPI 12	78.33 $\pm$ 0.23 <sup>b</sup>	94.44 $\pm$ 0.13 <sup>f</sup>	61.22 $\pm$ 0.01 <sup>f</sup>
Grand Mean	78.18	79.19	48.68
SE of Mean	2.609	2.863	2.260
C.D	0.617	0.594	2.019
CV%	0.444	0.422	2.332

Note: Values are expressed as Mean  $\pm$  SD. Level of significance (p <0.05). DGF- Defatted Groundnut flour, GPI7- Groundnut protein isolate extracted at pH 7.0, GPI8- Groundnut protein isolate extracted at pH 8.0, GPI9- Groundnut protein isolate extracted at pH 9.0, GPI10- Groundnut protein isolate extracted at pH 10.0, GPI11- Groundnut protein isolate extracted at pH 11.0, GPI12- Groundnut protein isolate extracted at pH 12.0. Values with similar superscripts within columns are statistically similar at 0.05% level.

**Table 2. Nutrient composition of defatted flour and protein isolates**

Sample tested	Moisture (g%)	Ash (g%)	Fat (g%)	Protein (g%)	Crude fibre (g%)
DGF	6.46 $\pm$ 0.02 <sup>d</sup>	6.02 $\pm$ 0.01 <sup>e</sup>	10.2 $\pm$ 0.05 <sup>g</sup>	50.75 $\pm$ 0.03 <sup>a</sup>	5.42 $\pm$ 0.01 <sup>e</sup>
GPI 7	5.72 $\pm$ 0.02 <sup>c</sup>	1.23 $\pm$ 0.03 <sup>a</sup>	9.93 $\pm$ 0.01 <sup>f</sup>	88.76 $\pm$ 0.50 <sup>g</sup>	0.85 $\pm$ 0.02 <sup>b</sup>
GPI 8	5.44 $\pm$ 0.12 <sup>b</sup>	1.21 $\pm$ 0.02 <sup>a</sup>	9.61 $\pm$ 0.00 <sup>e</sup>	85.21 $\pm$ 0.68 <sup>f</sup>	0.93 $\pm$ 0.01 <sup>cd</sup>
GPI 9	5.23 $\pm$ 0.02 <sup>a</sup>	1.82 $\pm$ 0.02 <sup>d</sup>	8.75 $\pm$ 0.15 <sup>d</sup>	83.04 $\pm$ 0.94 <sup>e</sup>	0.90 $\pm$ 0.02 <sup>c</sup>
GPI 10	5.65 $\pm$ 0.02 <sup>c</sup>	1.33 $\pm$ 0.02 <sup>b</sup>	7.81 $\pm$ 0.01 <sup>c</sup>	81.87 $\pm$ 0.10 <sup>d</sup>	0.22 $\pm$ 0.02 <sup>a</sup>

<b>GPI 11</b>	6.85±0.02 <sup>e</sup>	1.73±0.03 <sup>c</sup>	7.21±0.01 <sup>b</sup>	79.37±0.13 <sup>c</sup>	0.83±0.02 <sup>b</sup>
<b>GPI 12</b>	7.85±0.03 <sup>f</sup>	1.82±0.03 <sup>d</sup>	6.35±0.04 <sup>a</sup>	78.33±0.23 <sup>b</sup>	0.94±0.03 <sup>d</sup>
<b>Grand Mean</b>	6.173	2.169	8.555	78.18	1.448
<b>SE of Mean</b>	0.194	0.356	0.305	2.609	0.367
<b>C.D</b>	0.092	0.047	0.105	0.617	0.036
<b>CV%</b>	0.844	1.231	0.690	0.444	1.410

Note: Values are expressed as Mean ± SD. Level of significance (p <0.05). DGF- Defatted Groundnut flour, GPI7- Groundnut protein isolate extracted at pH 7.0, GPI8- Groundnut protein isolate extracted at pH 8.0, GPI9- Groundnut protein isolate extracted at pH 9.0, GPI10- Groundnut protein isolate extracted at pH 10.0, GPI11- Groundnut protein isolate extracted at pH 11.0, GPI12- Groundnut protein isolate extracted at pH 12.0. Values with similar superscripts within columns are statistically similar at 0.05% level.

DGF had higher moisture, ash, fat, and crude fiber content than GPI. The moisture content of DGF was 6.46±0.02%, while GPI had a higher moisture content at pH 11 and 12 (Fig.3). [21] found that low moisture content in food items can enhance shelf life and quality, as spoilage microorganisms do not thrive on low-moisture items. The ash content of DGF was 6.02±0.01%, which decreased in all protein isolates. The ash content ranged between 1.21±0.02 to 1.82±0.03% among GPIs, with the least content in GPI extracted at pH 8 and the highest in GPI extracted at pH 12. [10] found that repeated washing can reduce ash content in traditional protein extraction processes. The fat content in DGF was 10.2±0.05%, which significantly reduced among GPIs. An inverse association between protein isolation pH and fat content was observed (as the pH increased, the fat content among the GPIs decreased). Defatted flour had higher fat content than protein isolates, possibly due to inadequate oil expeller pressing [22]. Crude fiber content was significantly higher in DGF (5.42±0.01%), which drastically reduced to less than 1% in all protein isolates extracted at various pH levels. The study aligns with previous research by [22] and [11], who found low crude fiber content in defatted groundnut flour and defatted sesame flour protein isolates.

**Table 3. Functional properties of defatted flour and protein isolates**

<b>Sample tested</b>	<b>Water Holding Capacity g/g</b>	<b>Oil Binding Capacity ml/g</b>	<b>Emulsion activity%</b>	<b>Emulsion stability%</b>	<b>Foaming Capacity%</b>
<b>DGF</b>	1.73±0.04 <sup>g</sup>	1.05±0.02 <sup>ab</sup>	45.8±0.13 <sup>c</sup>	47.17±0.12 <sup>c</sup>	5.067±0.11
<b>GPI 7</b>	0.57±0.00 <sup>a</sup>	1.01±0.01 <sup>a</sup>	45.8±0.07 <sup>c</sup>	48.61±0.04 <sup>d</sup>	--
<b>GPI 8</b>	0.68±0.00 <sup>b</sup>	1.04±0.04 <sup>ab</sup>	50.10±0.1 <sup>d</sup>	44.43±0.01 <sup>a</sup>	--
<b>GPI 9</b>	0.99±0.02 <sup>c</sup>	1.06±0.03 <sup>ab</sup>	45.92±0.02 <sup>c</sup>	47.34±0.02 <sup>d</sup>	--
<b>GPI 10</b>	1.14±0.01 <sup>d</sup>	1.09±0.04 <sup>b</sup>	48.63±0.01 <sup>d</sup>	44.50±0.06 <sup>a</sup>	--
<b>GPI 11</b>	1.19±0.01 <sup>e</sup>	1.17±0.02 <sup>c</sup>	44.74±0.01 <sup>b</sup>	47.23±0.01 <sup>c</sup>	--
<b>GPI 12</b>	1.44±0.01 <sup>f</sup>	1.28±0.01 <sup>d</sup>	44.43±0.01 <sup>a</sup>	45.93±0.01 <sup>b</sup>	--
<b>Grand Mean</b>	1.109	1.101	46.50	46.45	0.723
<b>SE of Mean</b>	0.084	0.020	0.431	0.324	0.396
<b>C.D</b>	0.041	0.054	0.127	0.091	0.077
<b>CV%</b>	2.084	2.776	0.154	0.111	6.030

Note: Values are expressed as Mean ± SD. Level of significance (p <0.05). DGF- Defatted Groundnut flour, GPI7- Groundnut protein isolate extracted at pH 7.0, GPI8- Groundnut protein isolate extracted at pH 8.0, GPI9- Groundnut protein isolate extracted at pH 9.0, GPI10- Groundnut protein isolate extracted at pH 10.0, GPI11- Groundnut protein isolate extracted at pH 11.0, GPI12- Groundnut protein isolate extracted at pH 12.0. Values with similar superscripts within columns are statistically similar at 0.05% level.

Water holding capacity (WHC) is the ability of proteins to hold water against gravity [23]. DGF had higher WHC than GPI (1.73±0.04 g/g) as presented in Table.3. Protein isolates showed a significant reduction in WHC, with the lowest at pH 7 and highest at pH 12 (Fig.4). Carbohydrates contribute to high WHC in legume flours, but decreased carbohydrates may result in lower WHC. [24] reported that high-solubility proteins have low WHC. [25] reported that higher denaturation isolates have greater WHC due to unfolding polypeptide chains and have less WHC, suggesting that membrane processing doesn't disrupt structures enough to increase WHC.

Oil absorption capacity (OAC) is crucial in food processing, as the OAC enhances mouthfeel and flavor retention. Studies show a significant difference in OAC between DGF (1.05±0.02 ml/g) and GPIs at different pH levels. GPIs extracted at 7 and 8 pH had slightly lower OAC as compared with other GPIs extracted from 9 to 12 pH (Fig.5). Higher OAC values promote flavor retention, palatability, and extended shelf life in food products [15].

Emulsion Adsorption Index (EAI) is the protein's ability to rapidly adsorb to the water/lipid interface during emulsion formation.

The highest emulsion activity was observed at pH 8 (50.10±0.1%), while the lowest was at pH 12 (Fig.6). Emulsion stability (ESI) refers to a protein isolate's ability to create resistance against emulsion breakdown. GPI showed significantly higher ES at pH 7 and 9 i.e., 48.61±0.04%, 47.34±0.02% (Fig.7). Physicochemical factors like pH, temperature, and ionic strength can influence protein emulsion properties [26].

Foaming capacity (FC) is crucial for proteins' functional characteristics, indicating their ability to retain air in bubbles [27] DGF had a foaming capacity of 5.067±0.11%. Groundnut protein isolates (GPIs) did not exhibit foaming properties, making them unsuitable for foam-requiring food systems like cake and ice creams. FC is affected by penetration, transportation, and rearrangements of molecules under the air-water surface. High temperature denatures protein structure, affecting foaming capacity and stability [28]. Therefore, peanut protein isolates may not be suitable for certain food systems.

#### Physical properties of defatted groundnut flour and protein isolates

The study reveals that color is a crucial criterion of sensory quality, affecting the acceptability of food products. It is measured using L\*, a\* and b\* values. The L\* values of DGF and protein isolates ranged from 36.47±0.00 to 60.51±0.01, with DGF being lighter than protein isolates. As pH increased, the L\* values in protein isolates decreased, indicating increased darkness. This decrease could be due to Maillard-type browning reactions during drying. The dark color might be attributed to the combination of pigments and polyphenols dissolved in the extraction solution with protein isolates. The a\* values of DGF and protein isolates ranged from 6.64±0.02 to 10.71±0.01, with no significant difference between GPI at pH 11 and pH 12 (Fig.8). As pH increased, the a\* values in protein isolates significantly increased, with the highest a\* value observed in GPI isolated at pH 12 (60.51±0.01). The defatted flour and protein isolates have positive b\* values, indicating yellowness. The highest b\* value among GPIs was observed at pH 12 (24.82±0.02), indicating more yellowness. These findings are supported by previous studies conducted by [29].

**Table 4. Bulk Density and Tapped Density of defatted flour and protein isolates**

Sample Tested	Bulk Density g/ml	Tapped Density g/ml
DGF	0.50±0.02 <sup>d</sup>	0.71±0.01 <sup>e</sup>
GPI 7	0.43±0.01 <sup>c</sup>	0.65±0.01 <sup>d</sup>
GPI 8	0.39±0.04 <sup>b</sup>	0.65±0.00 <sup>d</sup>
GPI 9	0.49±0.00 <sup>d</sup>	0.64±0.00 <sup>cd</sup>
GPI 10	0.48±0.05 <sup>d</sup>	0.63±0.00 <sup>c</sup>
GPI 11	0.34±0.01 <sup>a</sup>	0.61±0.01 <sup>a</sup>
GPI 12	0.37±0 <sup>b</sup>	0.61±0.01 <sup>b</sup>
Grand Mean	0.429	0.644
SE of Mean	0.013	0.007
C.D	0.025	0.016
CV%	3.291	1.406

Note: Values are expressed as Mean ± SD. Level of significance (p < 0.05). DGF- Defatted Groundnut flour, GPI7- Groundnut protein isolate extracted at pH 7.0, GPI8- Groundnut protein isolate extracted at pH 8.0, GPI9- Groundnut protein isolate extracted at pH 9.0, GPI10- Groundnut protein isolate extracted at pH 10.0, GPI11- Groundnut protein isolate extracted at pH 11.0, GPI12- Groundnut protein isolate extracted at pH 12.0. Values with similar superscripts within columns are statistically similar at 0.05% level.

Bulk density is crucial for material handling, food industry applications, and packaging requirements [30]. Protein isolates have a significantly reduced bulk density compared to DGF, with a range of 0.34 to 0.49 g/ml (Table 4), (Fig.9). [31] reported that the variance is attributed to protein structure, particle size, number of contact point and attractive inter-particle forces. [32] reported that protein isolates are high in protein, resulting in low bulk density due to minimal carbohydrate content. The porous texture from defatting processes can enhance the bulk density, making them suitable for complementary foods and child food formulations. [33] reported that high bulk density limits caloric and nutrient intake per feed, potentially causing growth faltering. Groundnut protein isolates are suitable for producing complementary foods and food formulations for children as seen from the above results. Tapped density significantly decreased in protein isolates compared to DGF, with lower density at pH 11 and 12. This decrease is attributed to rapid moisture removal at higher drying temperatures. Tapped density is also related to contact site dimensions and particle attraction. This is an economical and functional aspect of food packaging, as it lowers packing costs [34].

**Table 5. Least gelation Concentration of DGF and Protein isolates**

Flour concentration (% w/v)	DGF	GPI 7	GPI 8	GPI 9	GPI 10	GPI 11	GPI 12
2%	—	—	—	—	—	—	—
4%	±	—	—	—	—	—	—
6%	±	—	—	—	—	—	—

8%	±	-	-	-	-	-	-
10%	±	-	-	-	-	-	-
12%	±	±	±	±	±	±	±
14%	+	±	±	±	±	±	±
16%	+	±	±	±	±	±	±
18%	+	±	±	±	±	±	±
20%	+	±	±	±	±	±	±

Note: Gelation levels: (-) no, (±) partial, (+) complete gel; DGF- Defatted Groundnut flour, GPI7- Groundnut protein isolate extracted at pH 7.0, GPI8- Groundnut protein isolate extracted at pH 8.0, GPI9- Groundnut protein isolate extracted at pH 9.0, GPI10- Groundnut protein isolate extracted at pH 10.0, GPI11- Groundnut protein isolate extracted at pH 11.0, GPI12- Groundnut protein isolate extracted at pH 12.0. Values with similar superscripts within columns are statistically similar at 0.05% level.

The least gelation concentration (LGC) for DGF showed no gel at 2% flour concentration, while slight gel was observed at 4%, 6%, 8%, 10%, and 12%, and a strong gel at 14% to 20% (w/v), as given in Table.5. The lower the LGC, the greater the gelling ability of the protein constituent [35]. The GPIs demonstrated no gel formation from 2% to 10% flour concentration, regardless of pH. However, they showed a slight gelling capacity from 12% flour concentration, which may be advantageous for making curd or supplementing other gel-forming ingredients in food product formulations [36]. Many meals, including vegetables and other goods, rely on protein gelation for preparation and acceptance.

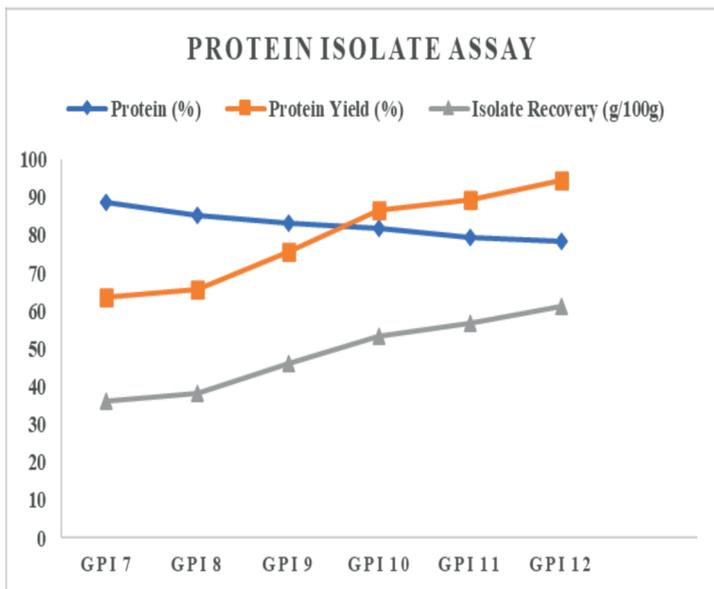


Figure 2. Protein assay of groundnut protein isolates

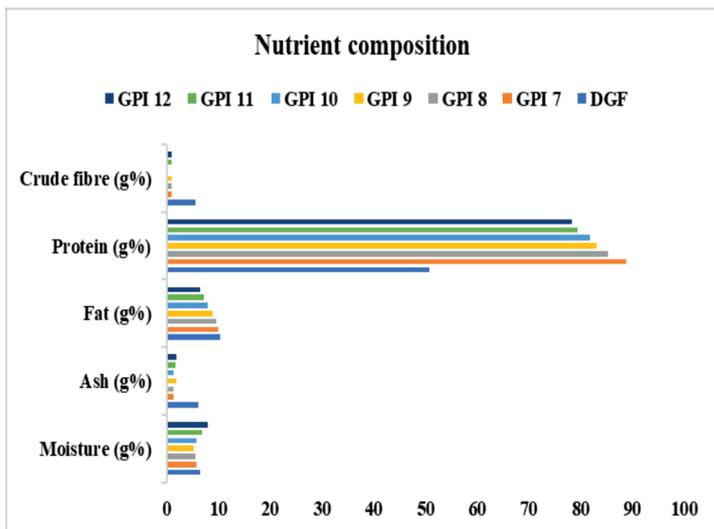


Figure 3. Nutrient composition of defatted groundnut flour and protein isolates

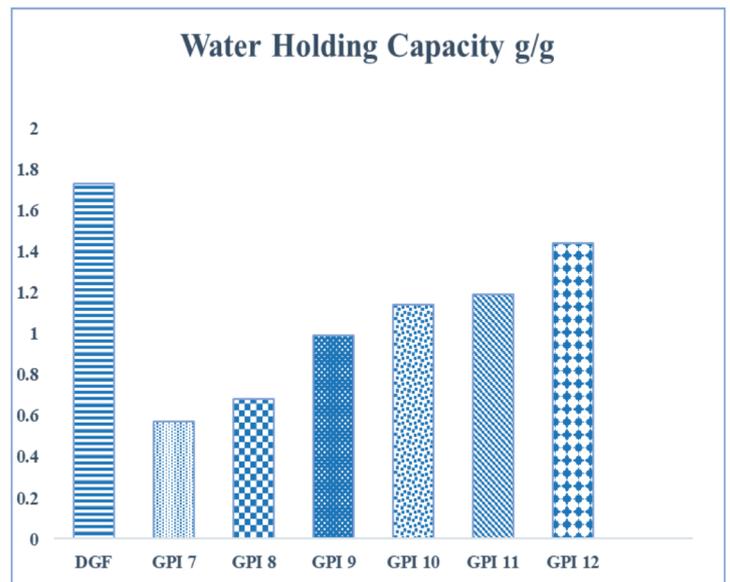


Figure 4. Water Holding Capacity of defatted groundnut flour and protein isolates

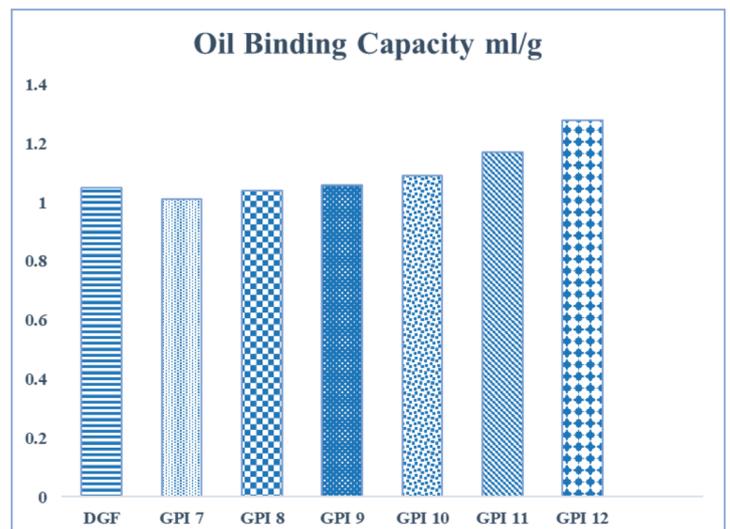


Figure 5. Oil Binding Capacity of defatted groundnut flour and protein isolates

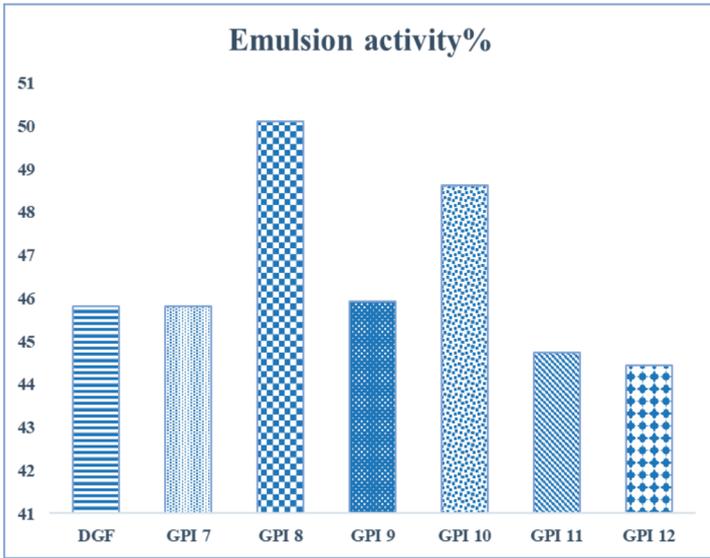


Figure 6. Emulsion activity of defatted groundnut flour and protein isolates

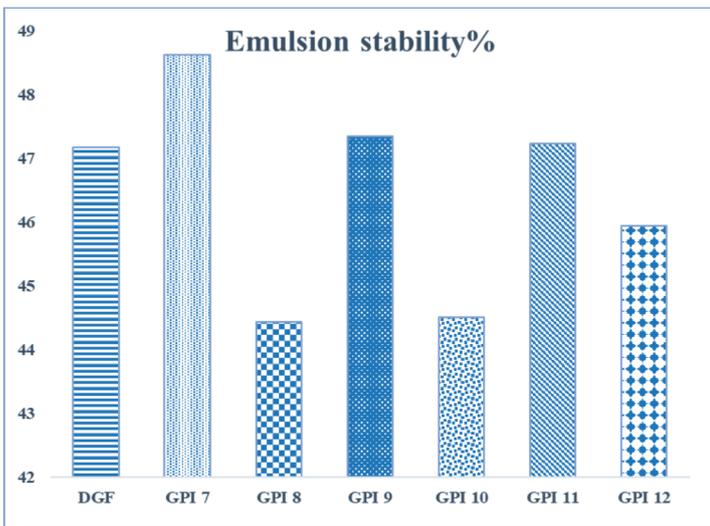


Figure 7. Emulsion stability of defatted groundnut flour and protein isolates

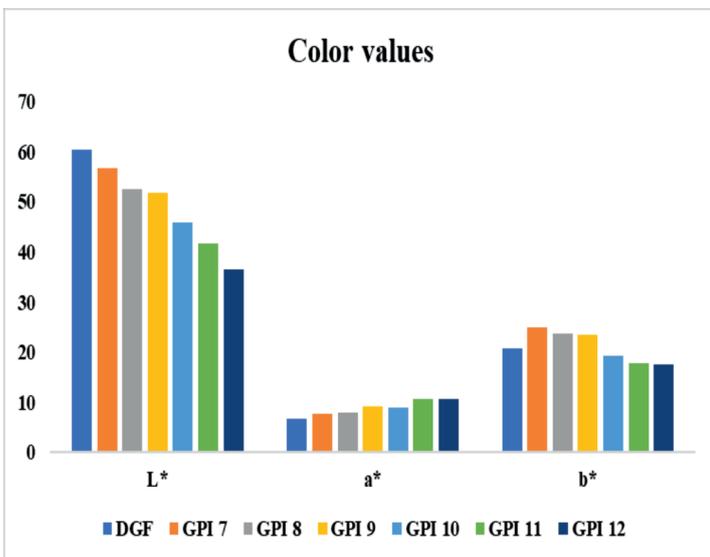


Figure 8. Color values of defatted groundnut flour and protein isolates

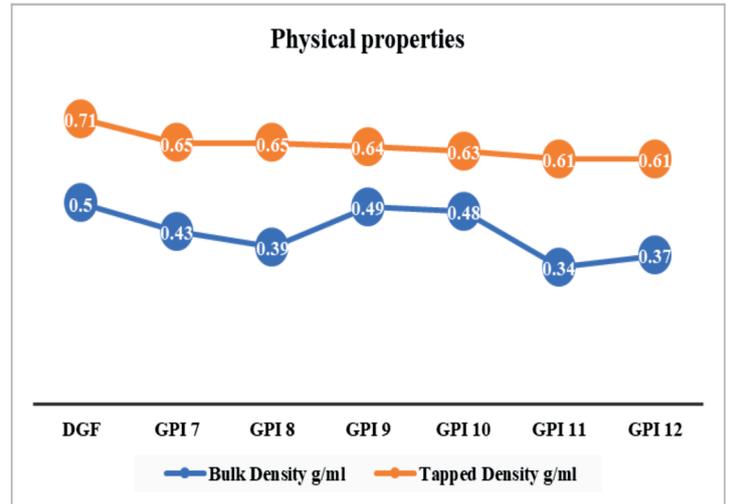


Figure 9. Physical properties of defatted groundnut flour and protein isolates

### Conclusion

GPIs were obtained by alkaline precipitation at their isoelectric point (pH 4.5), and they had a high protein content extracted at pH 7 (88.76%). This demonstrated that the aqueous extraction approach for obtaining GPIs was practical for industrial applications, implying that protein isolates from peanuts can be very useful for the formulation of protein-rich beverage and bakery food products and are important food formulation characteristics. GPIs showed superior functional qualities such as EAI, ESI, and WAC. Although the foaming and gelation capacity of the groundnut protein isolates is quite low, the high protein solubility implies that they have intriguing food applications. Overall, protein isolates extracted from groundnut seed cakes, an inexpensive by-product of groundnut oil industries, can provide an economic advantage to the groundnut oil industries by providing food processors with an affordable source of plant proteins with unique flavor and functional characteristics.

**Future scope of the study:** Further research aimed at expanding the usage of protein isolates from defatted groundnut cake includes the development of low-cost, value-added food products aimed at improving the nutritional status of diverse age groups can be validated.

**Acknowledgment:** The authors thank Professor Jayashankar Telangana State Agricultural University, Rajendranagar for providing constant encouragement, infrastructure, and support.

**Funding:** The research was conducted as a part of M.Sc Student Research work, supported by Professor Jayashankar Telangana State Agricultural University, Hyderabad, India.

**Conflict of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**Credit authorship contribution statement:** Nerella Susheela: Methodology, Investigation, Formal analysis, Writing -original draft. Aparna Kuna: Conceptualization, Validation, Writing -review and editing, Funding acquisition, Supervision. Afifa Jahan: Methodology, Investigation, Supervision. Lakshmi prasanna Kata: Project administration, Writing -review and editing.

draft. Aparna Kuna: Conceptualization, Validation, Writing - review and editing, Funding acquisition, Supervision. Afifa Jahan: Methodology, Investigation, Supervision. Lakshmiprasanna Kata: Project administration, Writing - review and editing.

## References

- Arya, S. S., Salve, A. R., & Chauhan, S. (2016). Peanuts as functional food: a review. *Journal of food science and technology*, 53(1), 31-41.
- Uddin, M. S., Islam, M. A., Rahman, M. M., Uddin, M. B., & Mazumder, A. R. (2018). Isolation of protein from defatted peanut meal and characterize their nutritional profile. *Chemistry Research Journal*, 3(2), 187-196.
- Boukid, F. (2022). Peanut protein—an underutilised by-product with great potential: a review. *International Journal of Food Science & Technology*, 57(9), 5585-5591.
- Tuhumury, H. C. D. (2021, October). Edible insects: Alternative protein for sustainable food and nutritional security. In *IOP Conference Series: Earth and Environmental Science* (Vol. 883, No. 1, p. 012029). IOP Publishing.
- Premalatha, M., Abbasi, T., Abbasi, T., & Abbasi, S. A. (2011). Energy-efficient food production to reduce global warming and ecodegradation: The use of edible insects. *Renewable and sustainable energy reviews*, 15(9), 4357-4360.
- Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M., & Tiwari, B. (2017). Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods*, 6(7), 53.
- Sá, A. G. A., Moreno, Y. M. F., & Carciofi, B. A. M. (2020). Plant proteins as high-quality nutritional source for human diet. *Trends in Food Science & Technology*, 97, 170-184.
- Diedericks, C. F., Stolten, V., Jideani, V. A., Venema, P., & van der Linden, E. (2021). Effect of pH and mixing ratios on the synergistic enhancement of Bambara groundnut-whey protein gels. *Food Hydrocolloids*, 117, 106702.
- Ngui, S. P., Nyobe, C. E., Bassogog, C. B. B., Tang, E. N., Minka, S. R., & Mune, M. A. M. (2021). Influence of pH and temperature on the physicochemical and functional properties of Bambara bean protein isolate. *Heliyon*, 7(8).
- Jain, A., Prakash, M., & Radha, C. (2015). Extraction and evaluation of functional properties of groundnut protein concentrate. *Journal of food science and technology*, 52, 6655-6662.
- Sibt-e-Abbas, M., Butt, M. S., Khan, M. R., Tauseef, M., Sultan, M. S. S., & Shahid, M. (2020) Nutritional and functional characterization of defatted oilseed protein isolates. *Pakistan Journal of Agricultural Sciences*, 57(1), 219-228.
- Wang, M., N.S. Hettiarachchy, M. Qi, W. Burks and T. Siebenmorgen. 1999. Preparation and functional properties of rice bran protein isolate. *J. Agric. Food Chem.* 47:411-416.
- AOAC. 2016. Generic combustion method; (Leco F-528 Nitrogen Analyzer) - Official Methods of Analysis of the Association of Analytical Chemists. 20th edition (Volume-1 and 2). North Frederick Avenue, Maryland, U.S.A. AOAC 992.23.20877-2417.
- Tomotake, H. Shimaoka, I and Kayashita, J. 2002. Physicochemical and functional properties of buckwheat protein product. *Journal of Agriculture Food Chemistry*. 50: 2125-2129.
- Siddiq, M., R. Ravi, J.B. Harte and K.D. Dolan. 2010. Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris* L.) flours. *LWT- Food Science Technology*. 43: 232-237.
- Moongngarm, A., Moontree, T., Deedpinrum, P and Padtong, K. 2014. Functional properties of brown rice flour as affected by germination. *APCBEE procedia*. 8: 41-46.
- Tanger, C., Müller, M., Andlinger, D., & Kulozik, U. (2022). Influence of pH and ionic strength on the thermal gelation behaviour of pea protein. *Food Hydrocolloids*, 123, 106903.
- Gao, Z., Shen, P., Lan, Y., Cui, L., Ohm, J. B., Chen, B., & Rao, J. (2020). Effect of alkaline extraction pH on structure properties, solubility, and beany flavor of yellow pea protein isolate. *Food Research International*, 131, 109045.
- Manamperi, W. A., Wiesenborn, D. P., Chang, S. K., & Pryor, S. W. (2011). Effects of protein separation conditions on the functional and thermal properties of canola protein isolates. *Journal of Food Science*, 76(3), E266-E273.
- Wu, L., Li, J., Wu, W., Wang, L., Qin, F., & Xie, W. (2021). Effect of extraction pH on functional properties, structural properties, and in vitro gastrointestinal digestion of tartary buckwheat protein isolates. *Journal of Cereal Science*, 101, 103314.
- Ranganayaki, S., Vidhya, R., & Jaganmohan, R. (2012). Isolation and proximate determination of protein using defatted sesame seed oil cake. *International Journal of Nutrition and Metabolism*, 4(10), 141-145.
- Kalpna Devi, B., Vidhya, R., & Jaganmohan, R. (2013). Determination and isolation of protein from different fractions of defatted groundnut oil cake. *African Journal of Plant Science*, 7(8), 394-400.
- Shevkani, K., Kaur, A., Kumar, S., & Singh, N. (2015). Cowpea protein isolates: functional properties and application in gluten-free rice muffins. *LWT-Food Science and Technology*, 63(2), 927-933.
- Liu, C. M., Peng, Q., Zhong, J. Z., Liu, W., Zhong, Y. J., & Wang, F. (2018). Molecular and functional properties of protein fractions and isolate from cashew nut (*Anacardium occidentale* L.). *Molecules*, 23(2), 393.
- Arrese, E. L., Sorgentini, D. A., Wagner, J. R. and Anon, M. C. (1991) Electrophoretic, solubility and functional properties of commercial soy protein isolates. *J. Agric. Food Chem.* 39, 1029-1032.

26. Lam, R. S., & Nickerson, M. T. (2013). Food proteins: A review on their emulsifying properties using a structure–function approach. *Food chemistry*, 141(2), 975-984.
27. Boye, J. I., Aksay, S., Roufik, S., Ribéreau, S., Mondor, M., Farnworth, E., & Rajamohamed, S. H. (2010). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International*, 43(2), 537-546.
28. Jayaprakash, G., Bains, A., Chawla, P., Fogarasi, M., & Fogarasi, S. (2022). A narrative review on rice proteins: current scenario and food industrial application. *Polymers*, 14(15), 3003.
29. Surasani, V. K. R., Singh, A., Gupta, A., & Sharma, S. (2019). Functionality and cooking characteristics of pasta supplemented with protein isolate from pangas processing waste. *Lwt*, 111, 443-448.
30. Ocloo, F. C. K., Bansa, D., Boatin, R., Adom, T., & Agbemavor, W. S. (2010). Physico-chemical, functional and pasting characteristics of flour produced from Jackfruits (*Artocarpus heterophyllus*) seeds. *Agriculture and biology journal of North America*, 1(5), 903-908.
31. Kumarakuru, K., Reddy, C. K., & Haripriya, S. (2018). Physicochemical, morphological and functional properties of protein isolates obtained from four fish species. *Journal of food science and technology*, 55, 4928-4936.
32. Kanu, P. J., Kerui, Z., Ming, Z. H., Haifeng, Q., Kanu, J. B., & Kexue, Z. (2007). Sesame protein 11: Functional properties of sesame (*Sesamum indicum* L.) protein isolate as influenced by pH, temperature, time and ratio of flour to water during its production. *Asian Journal of Biochemistry*, 2(5), 289-301.
33. Olawuni, I.A., C.Osobie, C., Ebiringa, D., Am, C., ikwa, J.Ibeabuchi, C., & E.Uzoukwu, A. (2014). Effect of pH and temperature on selected functional properties of flour samples and protein isolate of cowpea (*Vigna unguiculata*) seeds.
34. Mathews, A., Tangirala, A. S., Kumar, S., Anandharaj, A., & Rawson, A. (2023). Extraction and Modification of Protein from Sesame Oil Cake by the Application of Emerging Technologies. *Food Chemistry Advances*, 100326.
35. Peyrano, F., Speroni, F., & Avanza, M. V. (2016). Physicochemical and functional properties of cowpea protein isolates treated with temperature or high hydrostatic pressure. *Innovative Food Science & Emerging Technologies*, 33, 38-46.
36. Aremo, M. O., & Olaofe, O. (2007). Functional properties of some Nigerian varieties of legume seed flours and flour concentration effect on foaming and gelation properties.