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Protein extraction from sesame meal and its quality measurements

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Abstract

Sesame is an important source of plant protein and edible vegetable oil. Due to the quantity and quality of its protein (about 35-50%), sesame oilseed cakes (press cakes), a leftover from the oil industries, are a potential protein source. This work evaluated simultaneous protein extraction and modification from sesame oilseed cake. The extraction yield of sesame protein isolate (SPI) was found to be 26.04%. The isoelectric pH of the SPI was observed to be 4.5. The highest solubility and dispersibility results were at pH 11, while pH 3 had the lowest. The present study discovered that the more solubility, the more dispersibility, which are the essential requirements for other functional attributes. The protein content of SPI was found to be 88.05%. Protein dispersions made at pH 4.5 showed the highest levels of turbidity. In summary, this research offers a way to extract SPI and its potential use in the future.

Keywords: Protein extraction, animal proteins, functional potentials, the scarcity of protein

1. Introduction

The scarcity of protein among humans and the quick increase in the cost of some dietary ingredients derived from animals have boosted interest in plant protein as a substitute for dairy and meat products. The food sector sparked the study of specific oilseeds' functional potentials (Teh *et al.*, 2014) [18]. It is essential to find plant proteins with appropriate levels of sulfur-containing amino acids and comprehend how extraction procedures affect their isolation. Sesame seeds have between 20 and 27% protein and 40 to 50% oil (Fasuan *et al.*, 2018) [2]. 70% of the world's sesame seeds are produced in Asia, with China, India, and Myanmar as the top three producers worldwide (Sharaby & Butovchenko, 2019) [15]. Sesame oilseed meals (press cake), after the oil extraction, are often thrown away or used as a feedstock for fertiliser and animal feed due to the quantity and quality of their protein (Onsaard, 2012; Sibte-abbas *et al.*, 2020) [12, 16]. There have been initiatives to explore it as a human protein source. There are fewer plant sources of cysteine and methionine than there are for animal proteins. Sesame stands out from other oil seeds in that it contains many essential amino acids like cysteine, methionine, and tryptophan that are scarce in plant proteins (Escamilla-Silva *et al.*, 2003). Sesame protein isolate can be utilised to enhance the nutritional quality of foods.

Alkali extraction of plant proteins often involves pH modulation, which entails extraction with a mildly basic pH, precipitation at its isoelectric pH, and re-solubilisation at neutral pH (Gandhi & Srivastava, 2007) [3]. Although this method regularly recovers about half of the proteins, the remaining half is insoluble in the leftover solids (Rahman & Lamsal, 2021) [14]. Functional features of meals complement their sensory qualities and significantly impact how they behave physically during preparation, processing, and storage. The unique characteristics of protein isolates, known as their functional properties, can be changed by a set of variables, including pH, ionic strength, temperature, physical, chemical, and enzymatic processes (Kumar *et al.*, 2021) [7]. Many food enterprises increase the desired functional qualities to make them better and suit their needs. However, in this research, extraction of protein isolate by alkaline/isoelectric precipitation from sesame by-product is carried out, and its selected Physico-chemical and functional properties have been studied.

2. Materials and Methods

2.1 Materials

Sesame seed cake was collected from the local oil mills in Thanjavur. Chemicals were purchased from Sigma-Aldrich, such as sodium hydroxide, sodium chloride, n-hexane, and hydrochloric acid (Bengaluru, India). To prepare samples and to clean glassware, double distilled water was employed.

2.2 Methods

2.2.1 Defatting of sesame meal

Soxhlet extraction was used to remove the leftover oil in sesame oilseed cakes. Sesame oilseed cake was ground into powder using an attrition mill and a hammer mill before being sieved through a 600 µm sieve to create a homogenous sample. The obtained sesame cake flour was subsequently dissolved in hexane at a solid-liquid ratio of 1:5 (w/v) for 2 hours at room temperature (32 °C) (Teh *et al.*, 2014) [18]. Flour that had been partially defatted was subsequently processed in a Soxhlet extraction apparatus for 3 hours at ambient temperature with hexane (boiling point: 70 °C). The resulting defatted sesame flour was dried in a fume cupboard to remove traces of the organic solvent. It was again comminuted, vacuum dried, and kept at 4 °C before protein extraction and further characterisation.

2.2.2 Isolation of Protein

50.0 g defatted sesame cake was dissolved in deionised water at a 1:10 (w/v) ratio at 40 °C for 1.5 hours. According to (Onsaard, 2012) [12] the protein in defatted sesame flour was produced via alkaline extraction/isoelectric pH precipitation at room temperature with a pH range of 8.0 to 11.0. The solution was centrifuged at 4500 rpm for 20 minutes after being stirred at 1200rpm for 1 hour at 30 °C to free the remaining insoluble carbohydrate residue. After collecting the supernatant, the pH was adjusted to 4.5 by adding 1N HCL to cause the precipitation of proteins. It had a faint cream colour. It was centrifuged at 5000 rpm for 15 minutes to recover the proteins. It was repeatedly rinsed with distilled water to remove traces of acidic tinge. The pH was then adjusted to 7.0 using sodium salts. The proteins were finally freeze-dried at -40 °C and 0.6096 mm H₂O. Sesame protein isolates were given the acronym SPI. The protein extraction yield (%) was obtained based on the method followed by X. Zhang *et al.*, 2020 [22].

$$\text{Protein Yield (\%)} = \frac{\text{Weight (g) of SPI X Protein content (\% of SPI X 100)}}{\text{Weight (g) of DSF X Protein content (\% of DSF)}} \quad (1)$$

Where,

SPI – Sesame protein isolate, DSF- Defatted sesame flour

2.2.3 Determination of isoelectric point of SPI

Alkaline soluble extracts of SPI were made by centrifuging them at 4500 rpm for 20 min, then titrating them with 1N HCL to different pH values starting from 3.5 to 7.0. The resulting precipitates underwent 20-minute centrifugation at 4500 rpm. Plotting the per cent of precipitated protein over several pH levels led to the isoelectric point calculation for SPI (Malik & Saini, 2018) [8].

2.3 Chemical characteristics of protein isolates

The protein, moisture, fat, ash and crude fibre contents of SPI were ascertained using the standard procedures of AOAC (2006) with numbers 925.1, 981.10, 923.03, 920.85, and 962.09, respectively. The carbohydrate content was calculated

by deducting the total contents of protein, moisture, fat, ash, and crude fibre from 100%.

2.3.1 Measurement of Moisture Content

The hot air oven method was used to determine the moisture content. The empty dish was dried at 105 °C before being left in the desiccator to cool. Next, the dish was weighed while being empty. Three grams of material were placed on the plate. The dish and the sample were dried for 3 hours at 105 °C in the oven. The desiccator was used to cool the dish with a lightly closed lid after being dried. The dried sample and the plate's weight were then reweighed.

$$\text{Moisture (\%)} = \frac{(W_2 - W_1) - W_3 - W_1}{(W_2 - W_1)} \times 100 \quad (2)$$

Where W_1 is the weight (g) of the empty dish, W_2 is the weight (g) of the sample and dish, and W_3 is the weight (g) of the dish and dried sample

2.3.2 Estimation of Fat Content

The amount of fat was calculated using the Soxhlet apparatus unit. A sample weighing around 2g was put into a cotton wool-lined, spotless thimble. Then the thimble was placed in the extractor for over 8 hours, and fat was extracted using hexane. At least 15 siphons should be produced every hour at the chosen temperature. Evaporation has dried up the remaining solvent. At 105 °C, the flask was completely dried before being cooled and weighed.

$$\text{Fat(\%)} = \frac{W_1 - W_2}{W_3} \times 100 \quad (3)$$

W_1 = weight of the fat extraction flask with fat (g), W_2 = weight of the extraction flask (g), and W_3 = weight of the sample

2.3.3 Measurement of Fiber Content

2g of the sample was mixed with 200ml of Sulphuric acid, which was then boiled for 30 minutes before being filtered. The residue was gently rinsed three times in hot water to remove traces of acid. After being heated to boiling for 30 minutes, 100ml and 50ml of NaOH were added. The sample was vacuum-dried, moved to an oven overnight at 105°C, and then weighed. Following 3 hours of ashing at 550°C in a muffle furnace until a light grey ash had formed, the residue was weighed, and the calculation below was used to compute the total crude fiber%.

$$\text{Crude fiber (\%)} = \frac{W_1 - W_2}{W_3} \times 100 \quad (4)$$

Where W_1 denotes the sample's initial weight, W_2 its weight following ignition, and W_3 is the sample's original weight

2.3.4 Calculation of Ash Content

Ash content was calculated using the ash drying method. After weighing an empty crucible, add 2 g of sample into it, and the crucible was heated in a furnace at 550°C till the sample turned grey. When the furnace had heated the crucible to the desired temperature, it was put in a desiccator to cool.

$$\text{Ash (\%)} = \frac{W_1 - W_2}{S} \times 100 \quad (5)$$

Where W_1 is the weight of the crucible with the sample, W_2 is

the weight of the crucible without the sample, and S represents the sample weight

2.3.5 Estimation of crude protein

The protein content was calculated using the Kjeldahl method, and the total estimated nitrogen was multiplied by 6.25 to get the total protein%. The procedure has three steps: digestion, distillation, and titration. 0.5 g of the sample, 1 g of copper Sulphate, 5 g of sodium Sulphate, and 10ml of concentrated Sulphuric acid were used in the digestion, which was done in a fume hood and heated for 3 hours before cooling. The distillation unit contains 40% NaOH and 4% boric acid, and the cooled tube was filled with 30ml of distilled water. A conical flask was also kept to collect the freed nitrogen from the condensate. After adding 4 drops of methyl red indicator, titration was performed against 0.1N HCL.

$$\text{Protein(\%)} = \frac{1.4 \times V \times 0.1}{W} \times 6.25 \quad (6)$$

V - Titration value (ml), W- Weight of sample (g)

2.4 Measurement of quality characteristics of sesame protein isolate

2.4.1 Water Activity (a_w)

Using a water activity meter, the freeze-dried SPI's water activity was assessed (Aqua Lab, USA). One milligram of the sample is placed in a plastic container and kept in the water activity meter to get the values.

2.4.2 Turbidity of SPI

Turbidity was assessed by Xu *et al.*, 2011 [20]. A 50mM phosphate buffer solution (pH 7.0) was made, which contained 2 mg/mL of protein. The dispersions' absorbance at 600 nm was measured on a UV-Vis spectrophotometer (UV1800, Shimadzu, Tokyo, Japan. Analysis was done for triplicates.

2.4.3 Protein solubility

Using a slightly modified version of Vargas *et al.*, 2021, the solubility of the sesame protein isolates was assessed. A 20ml of freeze-dried protein was dissolved to give it a 1 mg/ml protein concentration. The solution's pH was changed and then centrifuged at 4 °C for 20 minutes at 4500rpm. The

supernatant was collected, and the protein content was estimated using the Lowry method with a standard Bovine Serum Albumin (BSA) curve. The protein solubility is given by Equation (7).

$$\text{Solubility\%} = \frac{\text{Protein content in supernatant}}{\text{Protein content in the sample}} \times 100 \quad (7)$$

2.4.4 Protein dispersibility

The modified method of Kulkarni *et al.*, 1991 was adopted to measure the dispersibility of SPI. In measuring cylinders, 3 g of SPI was mixed with distilled water of 30ml to achieve the desired pH (3–11), utilising either 0.5N HCL or 0.5 N NaOH. After giving the mixtures a thorough swirl, they were given 2 hours to settle. Following the measurement of the settling particle volumes, the below formula was used to determine dispersibility:

$$\text{Dispersibility(\%)} = \frac{\text{Total volume} - \text{settled volume}}{\text{Total volume}} \times 100 \quad (8)$$

2.4.5 Statistical Analysis

The analysis's findings were demonstrated as a mean and standard deviation (SD) in triplicates. The data for the treatments and varieties were examined independently using a one-way analysis of variance (ANOVA). To find any significant variations in the mean values, Duncan's multiple range tests (DMRT) ($p < 0.05$) were carried out using IBM SPSS software.

3. Results and discussion

3.1 Extraction yield

Sesame protein was extracted by following the alkaline/isoelectric pH method, and protein yield was observed to be 26.04%. The effect of process parameters i.e., solid to liquid (S/L) ratio, pH, temperature, and time remarkably influenced resultant protein yield.

3.2 Isoelectric pH of sesame protein isolates

The yield of precipitates at various pHs was shown in (Fig. 3.1). At a pH of 4.5, the most significant yield of 90% was attained. The findings showed that SPI's isoelectric pH was 4.5, which is similar to other plant protein sources, including wheat protein ($p_i = 4.22$), rice protein ($p_i = 4.46$) and legume protein ($p_i = 4.5$) (Mir *et al.*, 2019) [10].

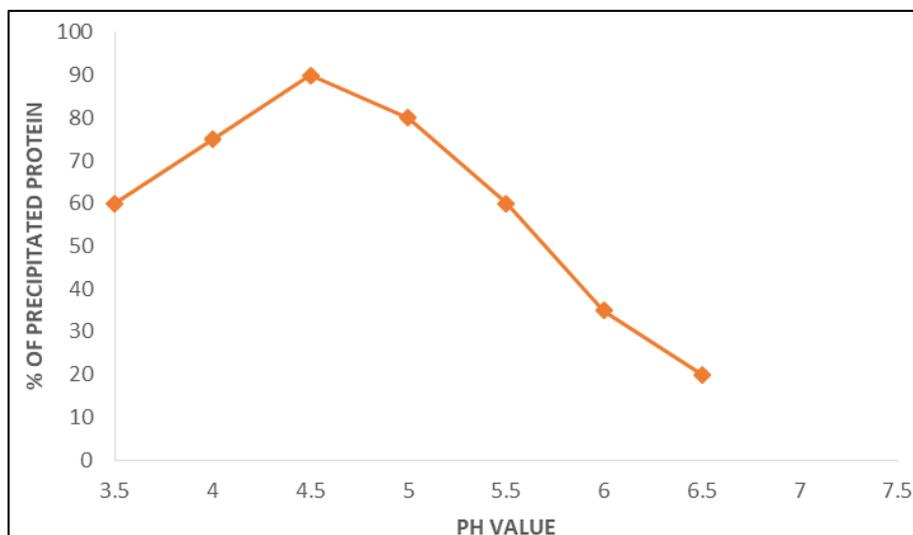


Fig 1: % of Precipitated protein at different pH levels

3.3 Physico-chemical and functional characteristics of SPI

3.3.1 Proximate Analysis of DSF and SPI

The powdered SPI had a composition of 88.05% protein, 0.25% fat, 0.89% moisture, 2.4% ash, 0.75% fibre and 6.65% carbohydrate (Table 1). Defatted sesame flour processing raised the protein content from 41.57% to 88.05%, as expected. Lipid content reduced from 8.64% to 0.25%. Results show the superiority of the defatting process. SPI composition may alter due to drying conditions and extraction procedures. These findings thus demonstrate the potential of the sesame seed by-product as a protein source for human nutrition in food formulation. Consistent conditions were used for all treatments to eliminate probable errors.

Table 1: Proximate composition of defatted sesame meal (DSF) and sesame protein isolate (SPI)

Constituents	DSF	SPI
Moisture	4.19±0.03 ^c	0.89±0.15 ^d
Fat	8.64±0.12 ^c	0.25±0.05 ^d
Protein	41.57±0.64 ^a	88.05±0.17 ^a
Fibre	3.46±0.63 ^c	0.75±0.10 ^d
Ash	6.15±0.88 ^d	2.4±0.01 ^c
Carbohydrate	38.18±0.52 ^b	6.65±0.17 ^b

Means that do not share a letter are significantly different

3.3.2 Water activity

The most crucial aspect of food items is water activity, which affects the storage stability of powdered foods and is closely related to their chemical, physical, and microbiological stability (Soottantawat *et al.*, 2004) [17]. It is the product's equilibrium relative humidity divided by 100. The water activity of SPI was observed to be 0.38±0.01. It can therefore be stored for longer with no risk of microbial contamination. Protein isolates' water activity can benefit from heat treatment.

3.3.3 Effect of pH on Turbidity

The aggregation of protein molecules by applying pH shift was studied further by measuring turbidity. According to Pouzot *et al.*, 2004 [13], the size of the scattering particle

present directly relates to the turbidity of the solution. Compared to solutions made from protein isolates prepared at pH 3.5 and 5.5, those made at pH 4.5 showed the greatest turbidity. This could be explained by the protein molecules' low surface charges, which enable them to combine at pH 4.5, resulting in aggregate formation with increased molecular weight. Protein molecules carrying charge on the surface caused electrostatic repulsion and inhibited intermolecular protein aggregation, which may explain why protein isolates at pH 5.5 and 3.5 had lower turbidity values. This might be caused by protein molecules unfolding in response to their eventual aggregation (Malik & Saini, 2018) [8].

3.3.4 Effect of pH on protein solubility

Solubility has gained recognition as a salient functional aspect of proteins, and it considerably impacts the product's colour, texture, and sensory qualities (Idowu *et al.*, 2021) [4]. It directly impacts how proteins are used in food compositions and are closely related to their ability to retain water and other physical, chemical, and functional characteristics like emulsification and gelling. The solubility of SPI was 28.34±0.33% at pH 3, and it increased to 74.34±0.49% at pH 7. The Hydrophilic-Hydrophobic balance of the proteins and the thermodynamics of its interaction with the solvent are the factors responsible for the solubility of SPI.

The protein's solubility grew noticeably with rising pH levels until it reached its maximum solubility (79.46±0.67%) at pH 11. SPI displayed similar solubility trends, with the isoelectric point region (pH 4-5) having the lowest solubility. Because of their electrostatic repulsion, ionic hydration, net charge, and fewer hydrophobic residues, SPI showed an increased solubility (pi) above and below isoelectric pH (Yang *et al.*, 2021) [21]. The study's findings demonstrated that protein solubility is pH-dependent. Protein solubility is impacted by denaturation because the surface's Hydrophilicity/Hydrophobicity ratio changes (Moure *et al.*, 2006) [11]. The degree of protein denaturation caused by chemical or heat treatment and the efficiency of protein isolates in the food sector may be helpfully indicated by the solubility (Kaur & Singh, 2007) [5].

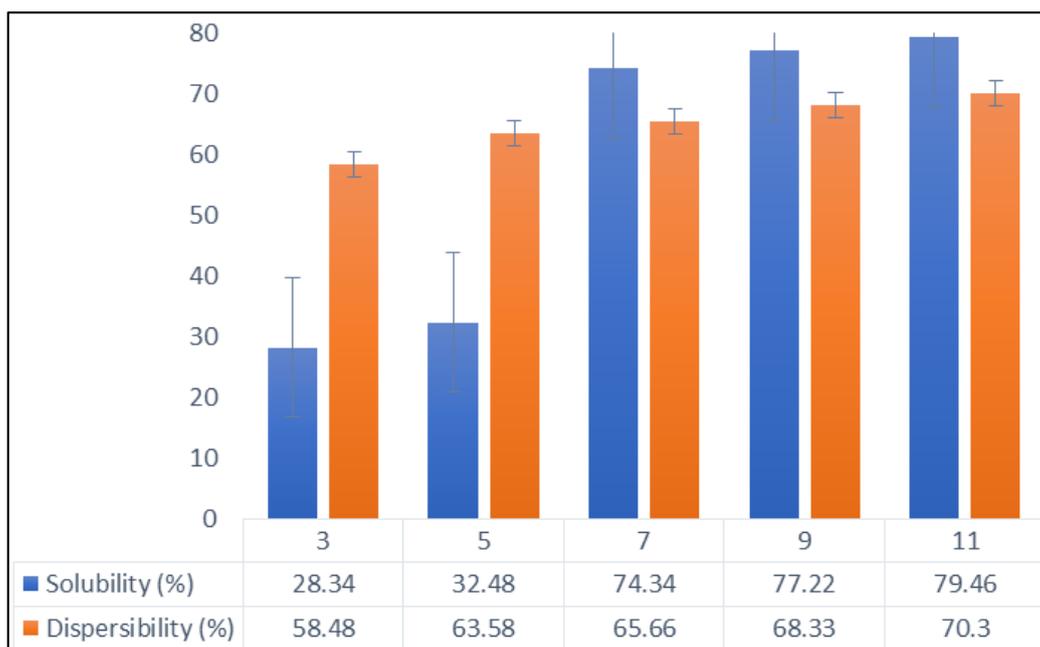


Fig 2: Solubility and dispersibility of SPI at various pH

3.3.5 Effect of pH on protein dispersibility

Dispersibility, which refers to a protein's capacity for reconstitution, is primarily influenced by the solvent's temperature, ionic make-up, degree of agitation, and pH. A higher dispersibility value improves the ability for emulsifying and foaming and other functional qualities. As the pH increases, the dispersibility of isolates of sesame protein dramatically increases ($p < 0.05$). According to the study's findings, pH 11 had the highest dispersibility values, at $70.30 \pm 0.67\%$, while pH 3 had the lowest values, at $58.48 \pm 0.36\%$. Additionally, it was shown that dispersibility varied significantly ($p < 0.05$) according to pH. Since solubility is the primary criterion for other functional qualities, the present study found that the greater solubility, the more dispersibility. Similar outcomes were noted by (Malik *et al.*, 2017)^[9] on sunflower seed and kernel protein isolates.

4. Conclusion

Sesame protein isolate (SPI) can be made by adjusting four variables: pH, time, temperature, and the solid to liquid ratio. Use of sesame oilcake for extraction of protein isolate given better protein yield. The proximate composition of DSF and SPI showed an enormous difference in protein content. SPI became completely neutral at its isoelectric pH and showed the least solubility and dispersibility. SPI was found extremely soluble at alkaline and acidic pH levels. Protein dispersions made at 4.5 pH showed maximum turbidity. Due to the recovery of high-quality proteins, reduced adverse environmental impact, and preserved finite natural resources, the agro-industrial by-products offer alternatives to the animal-based protein source. Emerging technologies can enhance the extraction of sesame protein isolate as pretreatments. The findings of this study have demonstrated the potential for using sesame protein isolate, a by-product of oil extraction, as a substitute plant-based protein with high nutritional content and a crucial role in food formulation.

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