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Protein Quality Characterization of Defatted White Sesame Cake and Its Protein Isolate

Bakam Himabindhu [a++*], Aparna Kuna [b#], T. Sucharitha Devi [c†], M. Preethi [d‡], D. Srinivasa Chary [e^] and M. M. Azam [f##]

[a] Department of Foods and Nutrition, College of Community Science, PJTSAU, Rajendranagar-500 030, India.
[b] MFPI Quality Control Laboratory, P.J.T.S. Agricultural University, Rajendranagar, Hyderabad – 500030, India.
[c] Department of Foods and Nutrition, College of Community Science, PJTSAU, Saifabad, Hyderabad-500004, India.
[d] Extension Education Institute, PJTSAU, Rajendranagar, Hyderabad - 500 030, India.
[e] Department of Statistics and Mathematics, College of Agriculture, PJTSAU, Rajendranagar-500030, India.
[f] Agricultural Chemicals Unit, Crop Production Section, Indian Institute of Rice Research, Rajendranagar, Hyderabad – 500030, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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[++] PhD Scholar;
[#] Principal Scientist and Head (Foods and Nutrition);
[†] Professor and University Head (Foods and Nutrition);
[‡] Professor (H. Sc Extension);
[^] Associate Professor (Statistics and Maths);
[##] Principal Scientist (Organic Chemistry);
**Corresponding author: E-mail: himabindhu2121@gmail.com;*

ABSTRACT

Present work evaluated the protein quality of defatted white sesame flour and the protein isolates obtained from sesame cake through the alkaline extraction at a pH 9.5. The study was conducted at 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) during 2021-2023. Defatted white sesame cake was subjected to alkaline extraction at pH 9.5 and resultant isolates were evaluated for nutrient composition, scanning electron microscope imaging, amino acid composition and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The obtained data was subjected to one-way analysis of variance. The white sesame protein isolates had a protein content of 93.83%, isolate recovery of 37.00 g/100 g and protein yield of 71.77%. The non-essential amino acids (NEAAs) content of the defatted white sesame flour and white sesame protein isolates ranged between 68.06% to 70.60% of the total protein content, while 29.40% to 31.94% was essential amino acids (EAAs). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of defatted white sesame flour WSF and white sesame protein isolate (WSPI) exhibited protein bands within the molecular weight range of 20 to 62 kDa. The protein isolates derived from sesame seed cake has promising potential for integration into diverse food formulations, offering an avenue to combat protein deficiencies.

Keywords: Protein isolates; Defatted white sesame cake; Amino acid profiling; SDS-PAGE.

1. INTRODUCTION

A balanced healthy diet should include carbohydrates, fats and protein to provide nutrients required for growth, energy, maintenance and the repair of body tissues [1]. Protein being an essential nutrient, plays a crucial role in various metabolic and physiologic functions, including the regulation of appetite, body weight and body composition. It is a very important dietary macronutrient required for life [2]. Proteins also contribute to the regulation of blood pressure, glucose and lipid metabolism, bone health and the immune system [3]. The protein has to be obtained through the diet, which the human body is unable to produce naturally, necessitating its acquisition through the consumption of foods and beverages.

The consumption of dietary protein is crucial as it serves as the primary source of amino acids required for protein synthesis in humans. Proteins fulfil various important functions within the body, including (1) contributing to cell and extracellular structures; (2) catalysing enzyme reactions; (3) influencing gene expression; (4) hormone-mediated actions; (5) facilitating muscle contraction; (6) regulating osmotic regulation; (7) providing protection against oxidative stress, infection, and bleeding; (8) regulation of metabolism; and (9) storage and transport of nutrients (including long-chain fatty acids, iron, vitamin A and zinc) and oxygen. Consequently, dietary protein intake has a significant influence on the status of other nutrients in the body and adequate protein nutrition is crucial for optimal human growth and health [4].

Dietary protein come from various sources including meat, milk, egg, soy and various plants-based foods [5]. The increased demand for animal-based protein has negative environmental consequences such as greenhouse gas emissions, water consumption and more land use [6]. With the growing global population and increasing demand for protein amalgamated with environmental consciousness and scarcity of space for landfilling, wastes/byproduct utilization has become attractive alternate to disposal. Oilseeds could be used as a valuable source of plant-based proteins, as oilseeds are characterized not only by their rich composition in oils, but also by their high level of proteins. The increase in oilseed production globally to meet the edible oil requirements inevitably is resulting in an increased production of by-product (high protein fat-free meal) with a high potential for valorisation [7].

Sesame (*Sesamum indicum* L.), one of the oldest oilseed crops that has ever been grown by humans belongs to Pedaliaceae family and is majorly farmed is for its oil-rich seeds. The protein content of the seed is also high and the amino acid composition of the protein has good nutritional value. According to sesame's chemical composition, the seed is a significant source of oil (44-58%), protein (18-25%), carbohydrate (around 13.5%) and ash (about 5%) [8]. By-products generated from oil industries is defatted

sesame cake, whose protein content can reach 50% depending on the extraction method. Sesame seed cake is commonly used as a cattle feed or to make compost. It contains proteins with balance amino acid composition, dietary fiber and bioactive compounds. However, this leftover material has the potential to be processed into a finely ground flour suitable for culinary applications, thereby generating increased value for the food industry [9].

Along with increasing world population, the demand for protein rich products are also increasing. The agro-waste generated during food processing such as defatted oilseed cakes and protein isolates from them can be used as alternative protein sources to meet the increasing protein demand by the growing population. The valorization of defatted oilseed cakes by extraction, precipitation based on the isoelectric point of the proteins and isolating the proteins is a sustainable method of creating wealth from waste. Currently, the food industry has proactively embraced the incorporation of protein isolates into a wide array of food items to address protein needs [10]. The main objective of the present project was to study the protein quality of defatted white sesame seed cake and the proteins isolated from them. The protein isolates derived from sesame seed cake has promising potential for integration into diverse food formulations, offering an avenue to combat protein deficiencies in the general population.

2. MATERIALS AND METHODS

The study was conducted at 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).

2.1 Procurement of Seed Cake

Defatted white sesame seed cake was procured from Regional Agricultural Research Station, Jagtial and commercial outlets in Hyderabad, Telangana State, India. The defatted sesame seed cakes were a result of pressing dehulled sesame seeds in a cold expeller.

2.2 Nutrient Composition Analysis

Nutrient composition of defatted white sesame and its protein isolates were analysed following the methods of [11] for moisture and ash, [12] for estimation of crude fat, crude fiber and crude protein.

2.3 Preparation of Defatted White Sesame Flour

To stabilize the flour, defatted white sesame cake was ground to fine powder in a blender (WCG75, Torrington, CT) at medium speed for 3 min, followed by drying at 60^0C for 8 hours in a hot air oven and sieved using a sieve (52 BSS) to get even textured powder.

2.4 Preparation of White Sesame Protein Isolate Using Alkaline Extraction

White sesame protein isolation was carried at different pH (9.0, 9.5, 10.0). The resulting protein content varied, with 77.01% obtained at pH 9.0, 93.83% at pH 9.5, and 88.0% at pH 10.0. Notably, the highest protein content was achieved when isolating the protein at pH 9.5. Therefore, pH 9.5 was chosen as the optimal condition for further analysis and processing of the protein.

The protein from defatted white sesame meal was obtained by alkaline extraction at room temperature by adjusting the pH according to the method of [13]. Defatted white sesame flour was mixed with water in the ratio of 1:10 (w/v) and the pH of the solution was adjusted to 9.5 with 1 N NaOH. The mixture was homogenised by using a mechanical shaker for two hours at room temperature (25-28°C) and subsequently centrifuged at 3000 rpm for 30 min, to obtain supernatant and residue precipitate. The alkaline extraction procedure was repeated for residue precipitate to get protein yield. Supernatant was collected and subjected for isoelectric precipitation by adjusting to pH 4.5 with 1N HCl for 30 min at room temperature (25-28°C) followed by centrifugation. The precipitated protein extract was washed repeatedly with distilled water to free it from the acid tinge. Later it was neutralized to pH 7.0 using sodium salts. Finally, the proteins were dried in hot air oven. The dry protein isolate powder was stored in the refrigerator until experimental use.

2.5 Protein Isolate Assay

Protein isolate assay was analysed according to the method of [10] method. Oilseed protein isolates recovery was assessed as weight of protein isolates obtained after isoelectric precipitation per 100 g sample. The protein yield of protein isolates obtained by wet processing method was estimated by using the following formulas.

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

2.6 Scanning Electron Microscope (SEM)

SEM was used to examine the micro-structural changes of defatted white sesame flour (WSF) and white sesame protein isolates (WSPI) as described by [14]. Surface morphology of the samples was observed and acquired using a scanning electron microscope (S-3700N SEM, HITACHI, Japan) with magnification from 100x to 1000x. For microstructure study, the samples were mounted over the stubs with double-sided conductivity tape and applied a thin layer of gold over the samples using an automated sputter coater for about 3 min. The samples were scanned in a scanning electron microscope at various magnifications, with an accelerating potential of 15 kV for imaging.

2.7 Estimation of Amino-acids

Amino acid profiles in samples were determined using HPLC (Agilent 1260 Infinity HPLC system; Santa Clara, CA, equipped with diode array and multiple wavelength detector) after samples were hydrolysed with 6 N HCl as reported by [15]. Method validation was performed to ensure the precision and accuracy of the results as per the standard procedure [12]. Hydrolysed peptide samples were derivatized with OPA (o–phthalaldehyde for primary amino acids) and FMOC (9–fluorenylmethyl chloroformate for secondary amino acids). The same samples were analyzed for amino acid composition using a Zorbax Eclipse–AAA column (250 mm, 9 4.6 mm, L 9 ID, particle size 5 µm) [Agilent Technologies, Santa Clara, CA]. Amino acid composition was expressed as percent amino acid of the total protein content of each sample.

2.8 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was run according to the method of [16] in order to ascertain the molecular weight distribution of defatted white sesame flour and its protein isolates using a 5% stacking gel and a 12% separating gel. To make the protein concentration 10 mg/ml of the samples were dissolved in water and mixed with 1 ml of lysis buffer, vortexed for 10 mins (1 min on ice, 1 min on vortex). Centrifuged the sample at 10,000 rpm for 15 mins at 4°C and the supernatant was collected and loaded. A protein marker with a molecular weight of 10-250 kDa was used as a standard.

2.9 Statistical Analysis

The experiments were carried out in triplicates. The results were statistically analysed by analysis of variance and expressed as means ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Nutrient Composition

The nutrient composition of WSF indicates that the defatted sesame flour had moisture content of 6.10±0.55%, ash content of 9.03±0.08%, fat content of 12.76±0.45%, crude fiber content of 7.64±0.08% and protein content of 48.37±0.34%. The defatted sesame cake is a rich source of protein, minerals and crude fiber as indicated by the results. The nutrient composition of WSPI indicates that the WSPI had moisture content of 5.95±0.56%, ash content of 1.27±0.00%, fat content of 4.42±0.17%, crude fiber content of 0.34±0.00%, and protein content of 93.83±0.34%. It is evidenced that, the process of protein isolation lead to a significant ($p < 0.05$) reduction in the moisture, ash, fat and crude fiber content, while there a significant ($p < 0.05$) improvement in the protein content of the WSPI. The current findings are in agreement with the results of [10] for defatted sesame flour. Poor fiber content in protein isolates has also been reported by [17], who attributed low fiber content in protein isolates, to the processing method in which other nutrients have been removed.

3.2 Protein Isolate Assay

The protein isolate assay of white sesame protein isolates (WSPI) are presented in the Fig. 1. The protein content in WSPI was 93.83%, which was obtained at a pH 9.5 from defatted white sesame flour. The isolate recovery and protein yield in WSPI was 37.00 g/100g and 71.77%. Current findings for recovery of oilseed

protein isolates are in conformity with the outcomes of [10], who reported protein content (90.14 ± 2.37%), isolate recovery (36.86 ± 1.22 g/100g), protein yield (79.03 ± 2.18%) in sesame protein isolates. As demonstrated by [18], the various pH conditions are most likely to be responsible for variations in protein isolate yield, while plant material and process conditions have a significant impact on the protein recovery rate.

In nutshell, the high-yield extraction of protein isolates from defatted white sesame flour (WSF) holds a crucial importance for the development of protein-based product innovation. This potential incorporation opens doors for novel food products, serving as a viable alternative. Furthermore, protein isolates sourced from defatted seed cakes play a pivotal role in alleviating the protein requirements of a wider population.

The protein isolation process is important as it helps in extracting the proteins with high protein content from raw materials and also helps in converting the industrial byproducts into useful products and protein quality evaluation helps in understanding the usage of protein isolates in different food formulations.

3.3 Scanning Electron Microscope (SEM)

SEM was used to examine the micro-structural changes of protein. Figs. 2 and 3 shows the scanning electron micrographs of WSF and WSPI. WSPI has more compact structure and more smaller particle size than WSF. Both WSF and WSPI have smooth and non-porous structure. [19] suggested that the resemblance in particle morphology and size can be attributed to the tray drying conditions. During tray drying, the water surrounding the material rapidly evaporates, leaving the dried items with a distinctive shape. In the case of tray drying, the droplet encounters hot dry air and the droplet shrinks due to the high flux of moisture leaving the droplet. The shape is because of the complex interplay between the moisture diffusivity of protein isolates and the changes in the surface tension at the gas-liquid interface. Polymeric macromolecules (i.e., proteins and carbohydrates) have extremely strong concentration-dependent moisture diffusivity, the magnitude of which decreases very quickly as the solid content in the droplet/ particle increases. The functional properties of defatted WSF and WSPI were closely associated with their morphology. The difference in the surface morphology of defatted WSF and WSPI can cause difference in the functional properties.

3.4 Amino Acid Profile

The amino acid profile of defatted WSF and WSPI are depicted in the Fig. 4. The total protein content in defatted WSF was 48.37%, which significantly increased to 93.83% in WSPI. As per the results obtained, the NEAAs constituted 70.60% in WSF and 68.06% in WSPI of the total proteins, while the essential amino acids (EAAs) content was higher in WSPI (31.94%) than WSF (29.4%). The branched chain amino acid (BCAAs) included leucine, isoleucine and valine, which are three of the nine EAAs. The BCAAs content in WSF and WSPI was 14.72% and 17.51% of the total protein content. It was observed that the total NEAAs content decreased, while the EAAs and BCAAs content increased in WSPI when compared to the defatted WSF, which might be due to the isolation process and thus can be considered as a good source of protein.

Highest NEAAs of the total protein content present in the defatted WSF and WSPI were Glutamate (18.20%, 18.86%), Aspartate (8.04%, 9.61%), Arginine (11.28 %, 11.24%), Serine (7.75%, 6.67%) and Glycine (6.86%, 8.21%) followed by Alanine (6.98%, 9.72%), Cystine (6.01%,0.60%), Tyrosine (2.66, 2.79%) and Proline (2.16%, 1.02%) respectively. The EAAs present in higher quantities in defatted WSF and WSPI were Leucine (7.75%, 7.45%), Phenylalanine (3.94%, 4.10%), followed by Valine (3.93%, 5.83%), Lysine (2.88%, 3.12%), Threonine (4.71%, 3.82%) and Isoleucine (3.04%, 4.23%) respectively. Histidine and Methionine were present in small quantities. The amino acid content in WSPI (Glutamate, Serine, Threonine, Valine, Isoleucine, Leucine and Lysine) were similar with the sesame protein isolates amino acid composition reported by [20]. Glutamate is also an important neurotransmitter and also serves as a precursor for bioactive molecules, including glutathione [21].

The high levels of EAAs are particularly pertinent as they cannot be synthesised by the human body and must be acquired from food, which are the building blocks for protein synthesis and they regulate human health. Moreover, protein functionality and bioavailability are governed by the amino acid composition and the amino acid sequence. Additionally, owing to their better

amino acid profile, WSF and WSPI have the ability to impart better functional attributes to the food.

As per the results obtained, it was observed that defatted WSF and WSPI have >25% EAAs, along with good BCAAs content, making them suitable as a competent ingredient that can be used to formulate protein rich products and can be used as supplements for low protein flours.

WSF and WSPI had higher arginine content. The biological significance of arginine intake stems from the fact that it is required for the nitric oxide synthase enzymes to produce nitric oxide in cells. It functions as a neurotransmitter in the central nervous system and may have favourable effects on the immune system, cerebral vascular system and have a positive impact in learning and memory processes [22]. Athletes can also use arginine as a supplement. Numerous researches revealed that its ingestion enhanced bodily composition and athletic performance while lowering cardiovascular risk factors, which may be related to elevated serum levels of nitric oxide [23]. Hence WSF and WSPI can be used as a good source of arginine, in various sports beverage formulations.

3.5 SDS PAGE

SDS-PAGE is used to compare the subunit profile of proteins. The SDS-PAGE electrophorogram of defatted WSF and WSPI along with standard are represented in Fig. 5. The electrophoretic pattern of defatted WSF and WSPI showed a large number of bands with a wide range of molecular sizes ranging from 20 to 62 kDa. In WSF and WSPI intense bands were visible at 20 kDa, 29 to 31 kDa [10], Observed that sesame protein isolates exhibited a range of polypeptide bands with molecular weights between 15 and 45 kDa on SDS-page, which vary with the findings of our study. It was observed that most of the poly-peptides contained in flour are also present in the protein isolates, as evidenced by the electrophoretic pattern of defatted flours and their protein isolates. The electrophoretic patterns of defatted WSF and WSPI were similar. The comparable subunit profiles of defatted flours and protein isolates show that isolation processing (alkaline extraction) preserved the protein subunit composition in its native state.

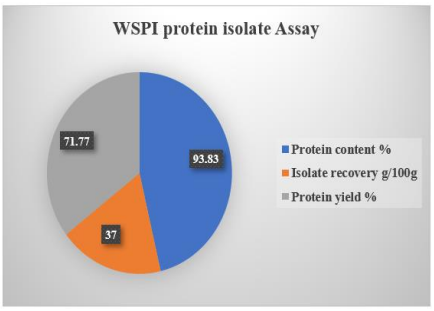

Fig. 1. Protein isolate assay

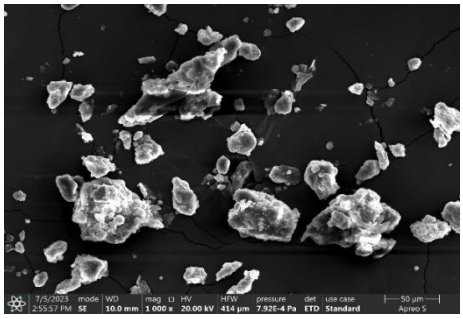

Fig. 2. SEM images of WSF

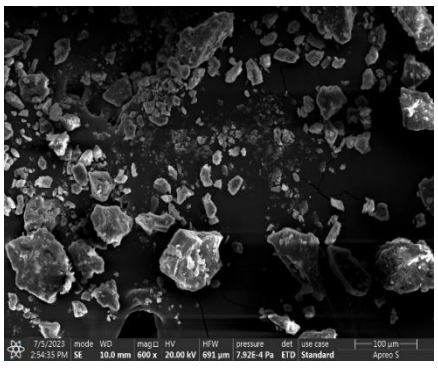

Fig. 3. SEM images of WSPI

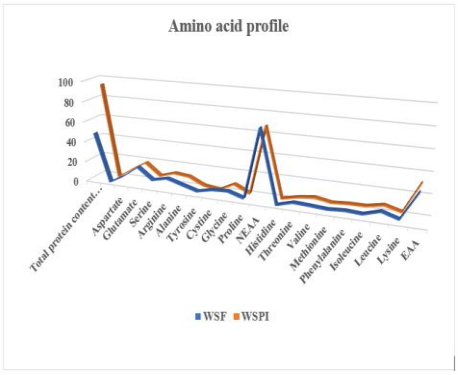

Fig. 4. Amino acid profile of defatted WSF and WSPI

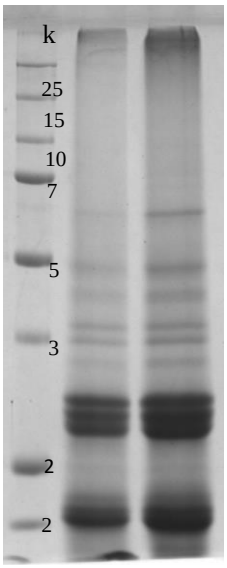

Fig. 5. SDS PAGE patterns of WSF and WSPI

4. CONCLUSION

In this study, the protein quality revealed that both defatted flours and protein isolates had good amount of protein and amino acid profile. The protein content and essential amino acid content was higher in WSPI than WSF. The amino acid profile of WSPI and WSF revealed good amount of essential amino acids and branched chain amino acids content, indicating the superiority of the protein. Therefore, WSF and WSPI can be considered as a potential source of protein in food formulations, especially for developing countries where protein deficiency is a major health challenge.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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