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Nutritional and functional characterization of defatted white sesame seed cake and protein isolates

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ABSTRACT

With the ever growing population globally, there is a consistent demand for protein, that is produced in a sustainable and economic way to maintain the environmental equilibrium. New concepts of food production are pivotal, where waste is efficiently utilized and transformed into value added products. Hence a study was designed to utilize oilseed cake - a byproduct of oil industry to produce protein isolates. This work evaluated the nutrient composition, anti-nutrients, physico-chemical characteristics, functional properties, and fatty acid profile of defatted white sesame cake and protein isolates produced from sesame seed cake, which were obtained through alkaline extraction under optimal conditions. The nutrient composition revealed that protein content was high in protein isolates (93.83±0.34%) than in defatted flour (48.37±0.34%). Bulk density and tapped density were higher in defatted flours than in protein isolates. The fatty acid profile of the defatted flour and protein isolate was composed of saturated fatty acids (SFA) ranging between 16.10 to 19.47%, 37.25 to 43.89% monounsaturated fatty acids (MUFA) and 43.28 to 43.09% polyunsaturated fatty acids (PUFA). The major MUFA and PUFA were oleic acid (37.04 to 40.58%) and linoleic acid (41.69 to 41.93%). The results showed that defatted white sesame flour and protein isolates are components suitable to be included in a healthy diet.

Keywords: *Protein isolates, Defatted white sesame flours, SFA, PUFA, MUFA, Bulk density, Tapped desnity, Nutrient composition.*

INTRODUCTION

Good nutrition is an elementary human right. To have a healthy population that can promote development, the relationship between food, nutrition and health should be strengthened. In developing nations, one of the ways of attaining this is through the exploitation of locally available resources, to satisfy the increasing population needs [8]. Consumers today seek out sufficient nutrient intake as well as other bioactive substances with positive health effects. They demand for foods that are both naturally sourced, low in cost, and functionally improved in terms of nutrition [37].

Interest in plant-based proteins has risen globally because of the fact that the production and consumption of foods derived from animal sources has health and environmental concerns. This presents an opportunity to explore the oilseed meal value addition, as oilseed cakes are a good source of plant-based protein. Oilseeds are nutrient-dense crops high in fat, protein, minerals, phytochemicals, and vitamins [32].

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Sesame (*Sesamum indicum L.*) seed belongs to the genus sesamum of the family Pedaliaceae and is also referred to as sesamum, gingelly, benniseed, sim-sim and til [1]. Sesamum is also referred to as "seed of immortality" as it is one of the oldest cultivated short-duration crops, was one of the first crops used to make oil, and one of the earliest condiments that grows all year long. The crop is now produced in a variety of climates, from temperate areas to the semi-arid tropics and subtropics. It is cultivated for its seeds, which contain 38-54% oil of exceptionally high quality and 18-25% protein, making it a significant cash crop for small and marginal producers in several developing nations [17].

It is eaten straight up as a sweetmeat and a refreshment. The seed has been called the "queen of the oil seed crop" due to its high oil yield and its quality. The oil is primarily used for cooking, with a small amount also being used in the pharmaceutical, cosmetic and perfumery industries [23]. The USA, Australia, Vietnam, Japan and the UK are the major importers of this oil, but China and India are the top consumers [13].

Defatted sesame oilseed cake, a by-product of the edible oil industry, is superior in terms of quantity and quality of its protein (about 35–50%). However, it is mostly used as feedstock for fertilizer or for animal feed, in spite of its excellent protein content, bioactive metabolites and fatty acids content. Scientific evidence indicates that the defatted sesame seed cake is underutilized as a protein source for human consumption.

Defatted sesame cake and its protein isolates can offer the viable nutritious composition for usage as a primary ingredient in various food formulations to improve the protein content of the product and also for their functional properties as flavor foams, emulsions, and gels [24]. Applying proper isolation technology which enhances the yield and characteristics of the final protein isolates are important and hence the current study was undertaken to understand the nutritional and functional properties of defatted white sesame and its protein isolates using an alkaline extraction method.

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

Preparation of defatted white sesame flour: Defatted white sesame cake was ground to a fine powder using a 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.

Preparation of white sesame protein isolates using alkaline extraction: The protein from defatted white sesame meal was obtained by alkaline extraction at room temperature by adjusting the pH according to the method of [20]. Schematic presentation of white sesame protein isolates preparation is depicted in Figure 1.

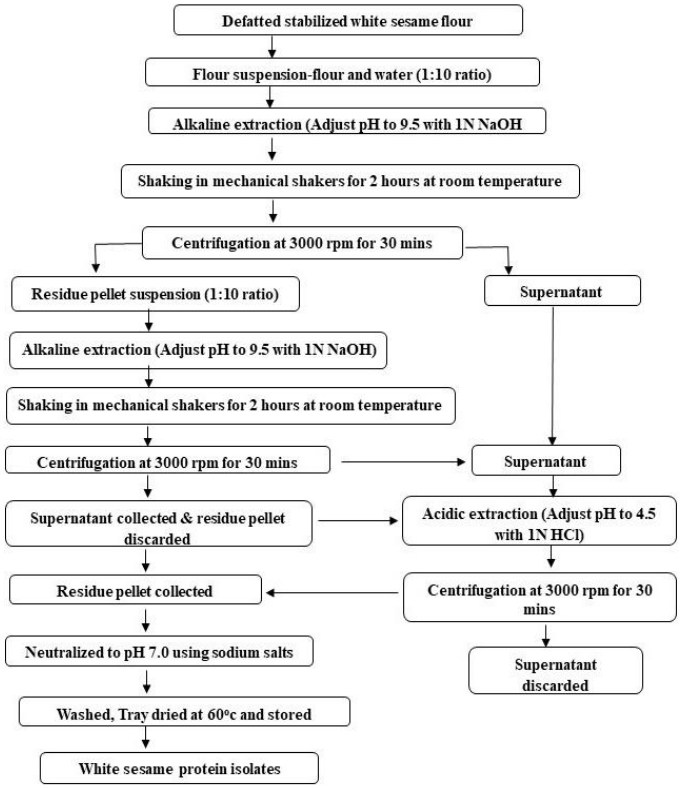

Figure 1. Schematic presentation of white sesame protein isolates preparation

Nutrient and antinutrient composition analysis: Nutrients analyzed in this study were moisture and ash [19], crude fat [4], crude fiber [5], and crude protein [7]. Antinutrients like phytates, saponins, oxalates, and tannins were estimated by following the methods described by [9], [39], [26] and [3] respectively.

Water holding capacity (WHC) and Oil binding capacity (OBC): WHC and OBC were determined by the method of [38]. 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 3000*g* 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 of [36]. About 0.7 g of sample was added to 10 ml of distilled water and mixed well before adding to it 10 ml of refined groundnut oil. The mixture was blended in an electric blender for 5 min and centrifuged at 2000 × g 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 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 : Colour of the defatted sesame cake and its protein isolate was performed by using a spectro-colorimeter (Hunter lab Colorflex, Firmware versions 1.1, Reston, Virginia) with a measuring aperture of 36 mm [6]. 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 the 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 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.

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

Least gelation Concentration (LGC) [27]: 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.

Estimation of fatty acids and trans fatty acids: Fatty acids were analysed using Gas Chromatograph (7890B of Agilent Technologies) equipped with flame ionization detector and Agilent-DB-FFAP column (nitro terephthalic-acid-modified polyethylene glycol (PEG) of high polarity for the analysis of volatile fatty acids) as described by [16]. Using 0.5 M methanolic KOH, the extracted fat was trans-esterified to form fatty acid methyl esters (FAME). The column's temperature was kept at its starting point of 100°C for 5 minutes before being raised to 240°C at a rate of 4°C/min. 1.0 ml/min of nitrogen was employed as the column flow rate as the carrier gas. The detector's temperature was kept at 280°C. 47885-U Supelco® 37 Component FAME Mix, 10 mg/mL in methylene chloride, was utilized as the reference standard. Sample fatty acid composition was compared to a reference fatty acid composition and percentages were calculated by normalization of peak areas.

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

RESULTS AND DISCUSSION

Nutrient analyses, such as moisture, ash, crude fat, crude protein and crude fiber of defatted white sesame flour (WSF) and WSPI (white sesame protein isolates) were shown in Figure 2. The comparison between WSF and WSPI revealed significant differences ($p<0.05$) in all nutritional parameters. WSF had higher moisture, ash, fat and crude fiber content than WSPI. The moisture and protein content in WSF were comparable with the results of [15]. As WSF and WSPI have low moisture, they can be used various in food formulations, as low moisture content is indicative of long shelf life and keeping quality. High ash content in defatted WSF (9.03±0.08%) typically indicates a high concentration of several minerals and vitamins, hence WSF may be a good source of minerals. Excessive fat consumption has been linked to some cardiovascular problems, including cancer, atherosclerosis and aging. As defatted WSF and WSPI have low-fat content, they can be consumed as an alternative to animal protein with high-fat content.

Protein content in defatted WSF was 48.37±0.34%, which significantly improved in the extracted protein isolates (93.83±0.34%). This shows that WSPI is a very rich source of protein and therefore can be used as an ingredient in low-protein flours and various food formulations. Also WSPI has a great potential as a therapeutic ingredient in battling the protein-calorie malnutrition due to its high quality and quantity of protein. WSPI has low fibre levels when compared with WSF, which might be related to the isolation process and its conditions of processing. The crude fiber content of current findings are in agreement with the results of [36] of defatted WSF.

Anti-nutrients

Table 1. Anti-nutrients estimation of defatted flours and protein isolates

	Phytic acid mg/100G	Tannic acid mg TAE/100g	Saponins %	Oxalates mg/100gm
WSF	**200.2±0.01^{a}**	**0.33±0.01^{a}**	**12.64±0.10^{a}**	12.40±0.05^{b}
WSPI	158.1±1.63^{b}	0.32±0.01^{a}	10.22±0.04^{b}	**13.40±0.04^{a}**
Grand Mean	179.19	0.32	11.43	12.90
SE of Mean	9.41	0.04	0.54	0.22
C.D	2.61	0.02	0.18	0.10
CV%	0.64	3.93	0.70	0.37

Note: Values are expressed as Mean ± SD. WSF- White sesame flour, WSPI- White sesame protein isolates. Values with similar superscripts within columns are statistically similar at 0.05% level.

Antinutrients are naturally occurring compounds that interfere with the absorption of nutrients in the gastrointestinal tract, thereby affecting their bioavailability. As per the results obtained, there was a significant difference ($p<0.05$) observed among all the antinutrients expected in tannin content of WSF and WSPI (Table 1). According to [2], phytates and oxalates interfere with certain mineral components like calcium, iron, zinc and magnesium by producing insoluble complexes. High tannin diets are generally attributed for poor palatability. Oxalic acid's bitter taste decreases palatability and may also cause kidney lesions [21].

According to [18] phytates in canola and rapeseed protein isolates was found to be nearly eliminated by ultrafiltration. In our study a significant reduction ($p<0.05$) was observed in the phytate content in the WSPI as compared to their defatted WSF, which could be attributed to the isolation process. As tannins content is low in WSF and WSPI, these may not have an effect on the palatability of diet. According to [10], the application of steam injection processing during protein isolation led to a reduction of over 90% in antinutritional factors such as phytates, tannins and saponins levels. Sesame meal can also be treated with hydrogen peroxide at pH 9.5 to remove the oxalic acid [17]. Through the implementation of the above pretreatment methods, it is possible to further decrease the levels of antinutrients, thereby enhancing the safety of the food for human consumption.

Table 2. Functional properties of WSF and WSPI

	Water holding capacity (g water/g of sample)	Oil binding capacity (ml oil/ g sample)	Emulsification activity (%)	Emulsification stability (%)	Foaming capacity (%)
WSF	2.69±0.06[a]	1.33±0.23[a]	24.60±0.29[b]	74.70±0.24[a]	12.03±0.65[a]
WSPI	1.46±0.03[b]	0.86±0.26[b]	43.47±0.36[a]	30.47±0.38[b]	9.72±1.96[a]
Grand Mean	2.07	1.10	34.03	52.58	10.87
SE of Mean	0.27	0.15	4.22	9.89	0.83
C.D	0.14	0.69	0.92	0.90	4.06
CV%	3.08	27.77	1.20	0.75	16.48

Note: Values are expressed as Mean ± SD. WSF- White sesame flour, WSPI- White sesame protein isolates. Values with similar superscripts within columns are statistically similar at 0.05% level.

The results (Table 2) indicated that there was a significant difference ($p < 0.05$) among all functional properties in WSF and WSPI except for foaming capacity, which might be due to the isolation process. As per the results obtained, there was a reduction in the water and oil absorption of the protein isolates, when compared to the defatted WSF. Low water and fat absorption could be due to changes in the pH that affect the protein ionization and magnitude of the net charge on protein molecules influencing attractive and repulsive forces within the protein and thereby associating with water or oil [28]. WHC and OBC are important functional properties in foods because, they are the contributing factors of the flavour, mouthfeel, and texture of the foods [12]. As defatted WSF (2.69±0.06 g/g) and WSPI (1.46±0.03 g/g) have good WHC, they could be used in food formulations such as bakery, meat and pastry products. Current findings for WHC are in conformity with the outcomes of [31] in SPI. The oil binding capacity of WSF (1.33±0.23 ml / g) and WSPI (0.86±0.26 ml /g) was poor indicating poor hydrophobic interactions in them. Similar OBC values were reported by [28] and [22] in Mucuna bean protein isolates (1.13 ml/g) and pigeon pea protein isolates (1.67 ml/g) respectively at pH 8.5. The findings of our study are in contrast with the outcomes of [29], who stated EA as 27.43% in sesame protein isolates. It's interesting to note that the WSPI has higher emulsifying activity (EA) than the defatted WSF; this could indicate that the protein isolate contains both polar and nonpolar amino acids, increasing the interaction between water and oil molecules. WSPI can serve as an emulsifier as they may interact with both water and oil in food systems and possess both hydrophobic and hydrophilic qualities. Emulsion stability can be defined as the ability to resist changes in its physicochemical properties over time. The findings are in conformity with the outcomes of [29], who stated ES as 30.50% in sesame protein isolates. The emulsifying stability exhibited by defatted WSF and WSPI can serve as valuable stabilizers in food formulations. Foaming capacity is important in maintaining consistency, texture and appearance of food that involves leavening and aeration properties. The WSF has high foam capacity might be due to an increase in foam hydration and stable molecular layer development at the water and air interface. As WSF and WSPI had good foaming capacity they can be used in formulations of cakes, ice creams and deserts.

Physical Properties: The physical properties analyzed included colour, bulk density, tapped density and least gelation concentration of both WSF and WSPI. The colour is an important characteristic that affects the appearance of the finished products. The colour values (L*, a*, b* and DE*) in WSF and WSPI varied significantly ($p < 0.05$) (Figure 3). A positive b* value indicates yellowness of defatted WSF. The results obtained were similar to the observations of [33], who stated that the L*, a* and b* value of sesame protein isolates as 44.25, 5.48 and 17.07 respectively. Based on the results obtained, as defatted white sesame flour (WSF) and white sesame protein isolates (WSPI) had higher L* values (whiteness) when compared with a*, b* values, they can be used in the product development without greatly altering the colour of the final product.

The bulk density and tapped density of defatted WSF and WSPI were significantly different (Figure 4). The protein isolate's low bulk density suggests that less quantity of it would need to be packaged in a constant volume, thus, ensuring affordable package. Additionally, low bulk density is of high nutritional value as it promotes easy digestion of food materials; hence, defatted WSF and WSPI would be easily digested. Similar reduction in tapped density of sesame protein isolates compared with defatted flour was reported by [34]. Tapped density is a significant factor related to the packaging, transport and commercialization of powders. Optimizing its value can be useful in terms of weight and the amount of material that will fit into a container [14].

The LGC of defatted flours and protein isolates was carried from 2% to 20% flour concentration (Table 3). Slight gel formation at 10% and good gel formation from 12 % to 20% flour concentrations were observed in WSF. In WSPI slight gel formation was observed at 18 % and 20% flour concentrations. The difference in LGC of defatted WSF and WSPI might be due to the relative proportion of protein, carbohydrates and lipids and the interaction between such components may affect functional properties. LGC is the ability of protein to form gels and provide a structural matrix for holding water, sugars, flavors and food ingredients is advantageous in food applications and in new product development, thereby providing an added dimension to protein functionality. The low gelation concentration observed may be advantageous when using these isolates to make curd or as an additive to other gel-forming materials in food products [12]. The LGC in WSPI was contrasted with the findings of [30] who observed LGC in sesame protein isolates at 5%.

Table 3. Least gelation concentration of WSF and WSPI

Flour concentration (% w/v)	WSF	WSPI
2 %	-	-
4%	-	-
6%	-	-
8%	-	-
10%	±	-
12%	+	-
14%	+	-
16%	+	-
18%	+	±
20%	+	±

Note: least gelation concentration (LGC) of defatted oil seed cakes and their protein isolates (- = not gelled; ± = slightly gelled; + = gelled). WSF- White sesame flour, WSPI- White sesame protein isolates.

Fatty acid profile: The fatty acid composition of WSF and WSPI are depicted in Figures (5, 6, 7). The total fat content in WSF and WSPI was 12.46% and 4.42%. As per the results obtained, the most abundant SFA in defatted WSF was stearic acid (9.64%), followed by palmitic acid (9.29%). The highest SFA in WSPI was palmitic acid comprising 9.31% of the total fatty acids and followed by stearic acid (6.14%).

The most abundant MUFA and PUFA in defatted WSF and WSPI were oleic acid (37.04%, 40.58%) and linoleic acid (41.69%, 41.93%) respectively. The high percentage of linoleic acid in defatted WSF and WSPI is desirable as it is an essential fatty acid and has a hypo-cholesterolemic effect [11]. The SFA composition of WSPI is lower than the defatted WSF. The changes in fatty acid composition of defatted WSF and WSPI might be due to isolation process, which was used to extract protein isolates. The fatty acids composition of defatted sesame flour was comparable with the results of [25]. With significant levels of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), both WSF and WSPI exhibit promising health advantages when integrated into food formulations.

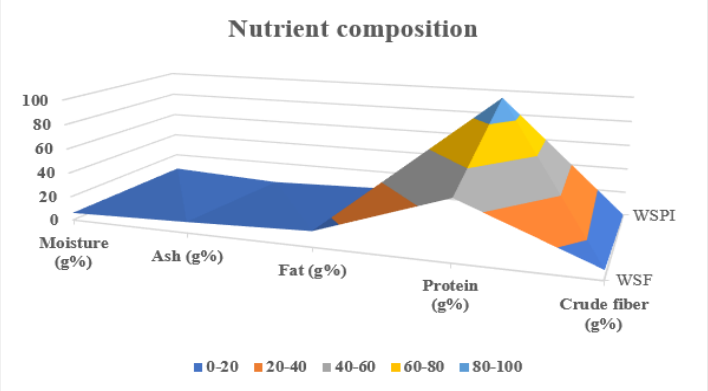

Figure 2. Nutrients estimation of defatted WSF and WSPI

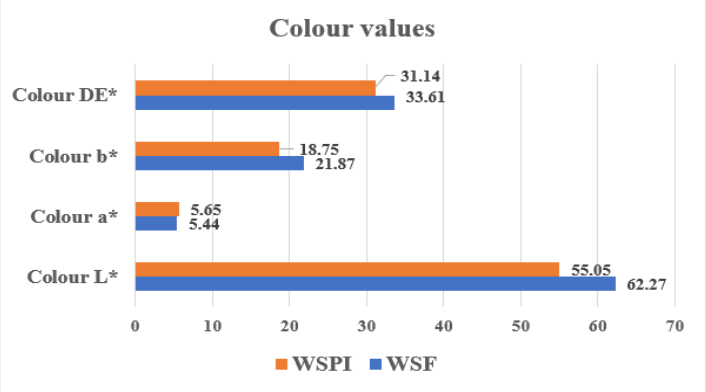

Figure 3. Colour values of defatted WSF and WSPI

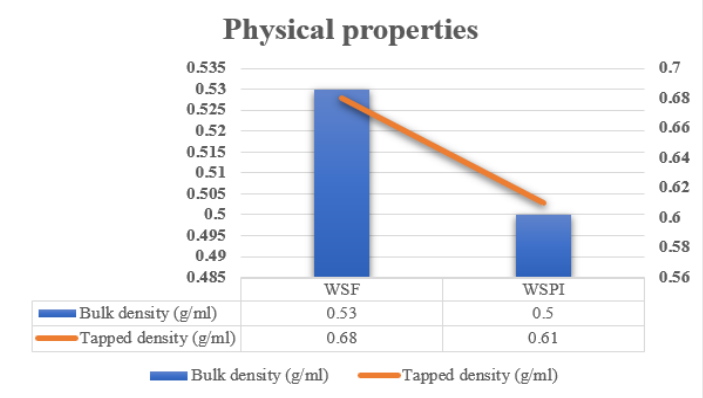

Figure 4. Physical properties of defatted WSF and WSPI

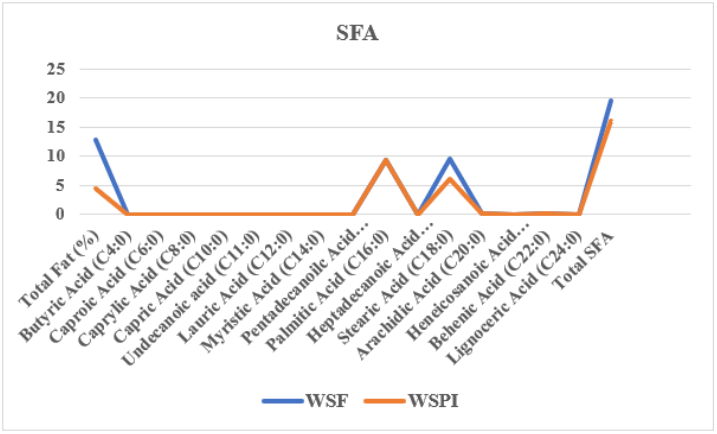

Figure 5. SFA composition of defatted WSF and WSPI

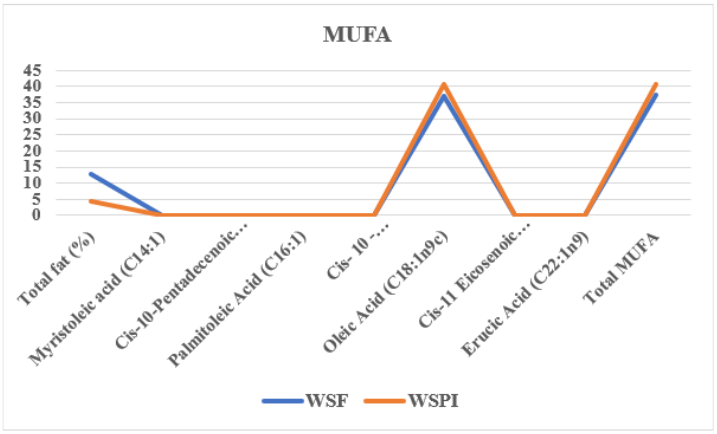

Figure 6. MUFA composition of defatted WSF and WSPI

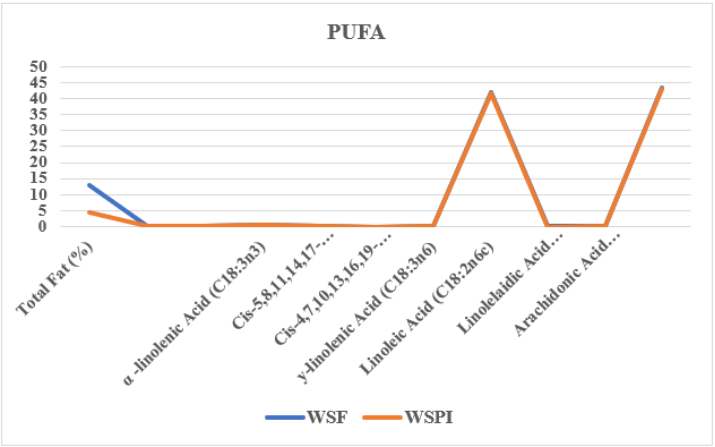

Figure 7. PUFA composition of defatted WSF and WSPI

CONCLUSION

In this study, white sesame protein isolates were prepared from defatted white sesame flour through alkaline extraction method. The outcomes of the present study indicated that defatted flours and protein isolates exhibited remarkable nutritional, functional and physical properties. High protein content in the WSPI, along with its good emulsion and foaming properties, indicate their potential as a food ingredient in formulation of protein rich foods. The WSPI had low content of SFA and high content of PUFA and MUFA, when compared with the defatted WSF. The defatted WSF and WSPI may find important applications as emulsifiers and stabilizers in food systems and can also be used in different food formulations with proper pretreatment to decrease the antinutritional factors and thereby improve the nutritional quality of the product.

Future scope of the study: Further research aimed at expanding the usage of protein isolates for development of low cost value added products and testing their efficacy in improving the nutritional status of various age groups can be validated.

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Declarations

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

Table 1. Nutrient composition of defatted white sesame flour and protein isolate

	Moisture (g%)	Ash (g%)	Fat (g%)	Protein (g%)	Crude fiber (g%)
WSF	6.10±0.55^{a}	9.03±0.08^{a}	12.76±0.45^{a}	48.37±0.34^{a}	7.64±0.08^{a}
WSPI	5.95±0.56^{b}	1.27±0.00^{b}	4.42±0.17^{b}	93.83±0.34^{b}	0.34±0.00^{b}
Grand Mean	6.03	5.15	8.59	71.10	3.99
SE of Mean	0.27	1.73	1.86	10.16	1.63
C.D	1.27	0.13	0.78	0.78	0.13
CV%	9.29	1.13	4.02	0.48	1.48

Note: Values are expressed as Mean ± SD. Level of significance (p <0.05). WSF- White sesame flour, WSPI- White sesame protein isolates. Values with similar superscripts within columns are statistically similar at 0.05% level.

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