

Full Length Research Paper

Isolation and proximate determination of protein using defatted sesame seed oil cake

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Sesame seed was used to extract oil and the cake, which has high amount of protein, was wasted after the extraction. Hence, the present study was done to isolate protein from defatted sesame seed oil cake and the nutrition content was determined. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) was used to isolate the protein; among the three BSS (British Standard Sieve) of the sesame seed flour cake, 52 BSS shows high protein content of 45.9% while 72 and 32 BSS contains 30.3 and 33.4% of protein, respectively. Low fat content of 4.4% was observed in 52 BSS which showed higher fiber content of 3.8%. The moisture content of 52 BSS was found to be 6.8% and others of 7.0 and 7.2%, respectively.

Key words: Protein isolation, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE), British standard sieve.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is called the “queen of the oilseed crops” because of its high yield of oil and quality of the seed, and it is the oldest crop grown for edible oil. Sesame is grown primarily in less developed tropical and subtropical areas of Asia, Mediterranean, and South America. Current world production is estimated at about 2,000,000 metric tons annually, placing sesame behind soybean, peanut, cotton seed, sunflower, linseed and rapeseed, in the quantity of world oilseed production. India produces nearly 21.4% of the world sesame crop, followed by China at 19.6% and Sudan at 13.5%. Sudan is the major world exporter (El Tinay et al., 1976). Asia and Africa produce nearly 90% of the world supply of sesame. Most of the seed is consumed in the countries where it is produced; less than 5% of world production enters export trade (Lyon, 1972).

Dehulled sesame seeds are very small, sweet and oleaginous and are used directly for food in the orient. Fried sesame seed may be mixed with sugar to form a sweetmeat or soup ingredient. A peanut butter counterpart is made from paste of roasted sesame seeds and is called tachini (Tahena). The pastel bar is a candy made of toasted sesame seed, honey and sugar, and dates back to the days of Babylonia. Sesame is also used in high protein snack foods (Mekongsee et al., 1974) and granola (Brasnett et al., 1975). Sesame seed is used extensively as a garnish on specially breads, buns and rolls. Sesame protein is high in methionine which is unusual for most plant protein (Johnson et al., 1979; Dagher et al., 1967) and the defatted meal prepared from dehulled seeds does not contain undesirable pigments (Dagher et al., 1967). These unique properties render sesame seed an excellent protein source.

The protein factor of total nitrogen times 6.25 is generally applied to sesame protein. Although this factor is high for some other oil seed proteins, it is nearly appropriate

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for sesame since Prakish and Nandi (Prakash et al., 1978) have shown that α -globulin, which comprises 65 to 80% of sesame protein, is 15.9% nitrogen. In general, Indian varieties tend to be lower in protein and higher in oil than Sudanese varieties which generally appear in the export market. Most oil seeds show a negative correlation between oil content and protein content; sesame is no exception. For each 1% average increase in protein content, there is a corresponding average decrease in oil content of 0.85% (Kinman and Stark, 1954).

Hull material accounts for 15 to 20% of the whole sesame seed (Krishnamoorthy et al., 1960; Shamanthaka et al., 1969; Ramachandra et al., 1970) and contains large quantities of oxalic acid, calcium, and other minerals as well as fiber. Since the hull has an intense bitter taste and oxalic acid binds to calcium rendering it nutritionally unavailable, it is desirable to remove the hull if the seed is used in human foods. Black varieties of sesame contain higher levels oxalic acid and fiber, and lower protein levels white varieties. When properly dehulled, oxalic acid content is reduced from 2.5 to 3.0% to less than 0.25%. Expeller pressed, dehulled sesame will contain greater than 56% protein, and dehulled, pre-pressed and solvent-extracted meal is generally utilized as animal feed and oftentimes as fertilizer. Only in India is sesame meal extensively used in human foods, although interest is continually increasing in food uses of sesame protein.

In those areas where sesame is primarily processed for its oil content, the seed is not dehulled; rather, the entire seed is crushed. However, in area such as India where the meal is an important food, seed dehulling is an important process step for three reasons: 1) the removal of the hull reduces the content of oxalic acid which is associated with the outer epidermal layer; 2) the protein content of the meal is increased since the hull is primarily composed of fiber (Villegas et al., 1968), and 3) dehulling improves enzymatic digestibility (Shamanthaka et al., 1947).

An unusual feature of sesame is that it generally contains 2 to 3% oxalic acid and 1 to 2% calcium, which are primarily in the hull. The simultaneous presence of large amounts of calcium and oxalic acid makes it highly probable that the two exist as calcium oxalate. It has been assumed that 1/2 to 2/3 of the calcium in sesame exists as the oxalate salt (Dey, 1951). Calcium bound as the oxalate is present primarily in the hull, since dehulling results in low levels of residual oxalate. Dehulling improves nutritional and flavor characteristics of the meal, as well as reducing the fiber content, increasing the protein content and rendering a glossy white color.

Sesame meal and isolated protein have particularly high contents of methionine, 2.5 to 4.0% and total-sulfur containing amino acids, 3.8 to 5.5% (Lyon, 1972). Lysine is the first limiting amino acid. Lysine is deficient in almost all varieties; although sesame varieties with darker seed

coats possess higher lysine. Isoleucine is the only other amino acid lower than the quantity in the FAO reference protein. Tryptophane, which is limiting in other proteins, is present in generous quantities in sesame.

The aim of this study is to: separate the protein by SDS; investigate the functional properties of food-grade flour from sesame seed oil cake; determine the nutritional value and to predict its utilization in food formulation.

MATERIALS AND METHODS

Sample collection

By-product of sesame seed oil, that is, sesame seed oil cake was procured from the local market near Thanjavur. Sesame oil cake was defatted and powdered using mixer juicer. It was dried in hot air oven at 60°C for overnight and sieved by three different mesh screens including 30, 52 and 72 BSS. These three different fractions are taken for further processing. The works were done in triplicates to get concordant value.

Isolation of protein from defatted sesame oil cake

The protein of defatted sesame meal was obtained by alkaline extraction at room temperature by varying the pH from 6.8 and 10.0 according to the method of Taha et al. (1987). For each extraction 50 g of defatted sesame meal and 1 L of water was used along with NaOH (0.2 M). The mixture was stirred at low speed (1200 rpm) for one hour at 30°C and subsequently centrifuged at 3000 rpm for 20 min to remove the insoluble carbohydrate residue. The supernatant was collected and the pH was adjusted to 4.5 with 1 N H₂SO₄ to precipitate the proteins. The precipitate was creamy white in colour. Further, it was centrifuged at 5000 rpm for 15 min to recover the proteins and 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 air dried. The average yield of three replicates was reported.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE)

SDS PAGE was performed by the method of Laemmle (1970) with minor modifications. The separation of protein was performed in 7% separating gel and 5% stacking gel.

Separating gel

This contain 30% Bis/acrylamide, 0.1% SDS, 1.5 M tris (pH 8.8), 10% SDS, 10% APS, and tetra methylethylene-diamine (TEMED).

Stacking gel

This contain 30% Bis/acrylamide mix, 1.0 M (pH 6.8), 10% SDS, 10% APS, and TEMED.

Protein loading dye

This contain 1.25 ml Tris pH 6.8, 4 ml 10% SDS, 2.0 ml glycerol,

2.0 mg bromophenol blue, and distilled water to 10.0 ml. SDS-PAGE of total seed protein was carried out in polyacrylamide slab gels in a discontinuous buffer system according to the method of Laemmle (1970). Vertical gel slabs were prepared in a glass sandwich which was tightened by a set of plastic clips lined with a band of foamed silicon rubber. Separation gel was put into the space between a set of glass plates (up to 2 cm from the top). Small amount of distilled water (120 µl) was gently added to prevent gel surface from air and promote fixation. The setup was left for 30 min so that gel was fixed. The separating gels contained 15% by weight of acryl amide and 0.135% by weight of N,N-ethylene-bis-acryl amide in 1 MTris-HCl buffer (pH 8.8) with 0.27% SDS. The gels were polymerized chemically by the addition of 20 µl by volume of tetra methylethylenediamine (TEMED) and 10% ammonium per sulfate (APS). During the fixation of separation gel, stacking gel was prepared. Stacking gel consisted of 4.5%. The stacking gel was polymerized chemically in the same way as for the separation gel. When separation gel was fixed, distilled water was removed from its top and stacking gel solution poured on it. Combs were fixed into the stacking gel. Combs were put with special care and it was confirmed that there was no air bubble at the bottom of the combs. The set up was left for 15 min so that the stacking solution became gel. Combs, clips and gaskets were removed from glass plates carefully and it was confirmed that there was no any air bubble at this stage. Gel plates were freshly used for electrophoresis but it was also possible that these would be wrapped in aluminum foil and could be used even for one week.

The electrode buffer contained tris-glycine (9.0 g tris HCl and 43.2 g glycine per 3 L buffer solution at a pH 8.9) with 3.0 g (0.1%) SDS. 15 µl of protein supernatant were applied into the stacking gel sample wells with a micro syringe, followed by 20 µl of reservoir buffer containing bromophenol blue which served as the tracking dye. Electrophoresis was carried out at 70 mA until the bromophenol blue marker reached the bottom of the gel (approximately two and a half hour). In order to check the reproducibility of the method, two separate gels were run under similar electrophoretic conditions. After electrophoresis, the gels were stained with silver staining solutions.

Protein determination

The test portion (0.7 to 2.2 g) of the sample was weighed and placed in a digestion flask (Gerhardr-Turbotherm). It was then mixed with 0.7 g mercuric oxide, 10 g of powdered potassium sulphate or anhydrous sodium sulphate, 1 g of copper sulphate and 25 ml of concentrated sulphuric acid. The digestion tubes were placed in digestion chamber and the sample was digested until the solution was cleared (2 h for test portions containing organic material). The sample was allowed to cool down and 20 ml of water added to it.

Oil evaluation

5 g of sample was taken in a thimble. This was extracted in soxhlet fat extractor with hexane for six hours. After extraction completed the oil flask was dried in an air oven for three hours at 100 to 105°C. Cooled in a dessicator and weighed.

Crude fibre content

4 g of defatted sample was weighed and added to 200 ml of 1.25% sulphuric acid held in a 500 ml beaker, and glass rod was dipped in the beaker and boiled for 30 min on a hot plate. Any loss in volume

during boiling was made up with distilled water. The hot solution was filtered through a cotton cloth and the residue was washed with distilled water; to this residue, 200 ml of 1.25% sodium hydroxide solution was added and boiled for 30 min in water. The liquor was filtered through a cotton cloth and the residue washed with distilled water until the washing was no longer alkaline. The residue was dried at 105°C for 3 h and weighed again.

Moisture content

The low flat bottomed dishes were heated at 100°C in an oven, and were kept on asbestos sheet before it was passed to the desiccators (1/2 h). The value was noted and the same process was repeated till a constant weight occurred (with maximum difference of 0.02 g). 5 g of protein powder was weighed and placed in oven and was thermostatically controlled at 100 to 150°C for a stipulated time (10 to 12 h). The dishes were cooled in a dessicator for half-an hour and weighed successfully till it showed no further loss.

Ash content

A quantity of 4 g of sample was weighed in silica crucible and ignited in muffle furnace at approximately 800°C for five hours (dull red) until it resulted to light clay ash or to constant weight. It was then cooled and weighed at room temperature.

SDS-PAGE

The electrophoretic patterns of the 30, 52 and 72 BSS isolates produced after the fractionation showed that the 52 BSS was enriched in protein (Figure 1).

RESULTS AND DISCUSSION

Proximate analysis of three different fractions of sesame seed oil cake

The sesame seed contains about 50% oil and 20 to 25% protein (Vaughan, 1970). The residue sesame oil cake contains an average of 32% crude protein, 8 to 10% oil, total oil and albuminoids of 40 to 42% (Mehta, 2000) and rich in essential amino acids namely methionine and cystine (Johri et al., 1988).

Defatted sesame meal contains more than 5% phytic acid compared to defatted soybean meal at 1.5% (de Boland et al., 1975). Phytate reduces the biological availability of zinc, calcium, magnesium and perhaps iron, and complexes with protein rendering it less soluble (Smith and Circle, 1972). Sesame meal can cause nutritional problems when used in chicken feed (Lyon, 1972).

Moisture

The moisture content of the sesame seed oil cake of 52 BSS fractions have low moisture content of 6.8, while the

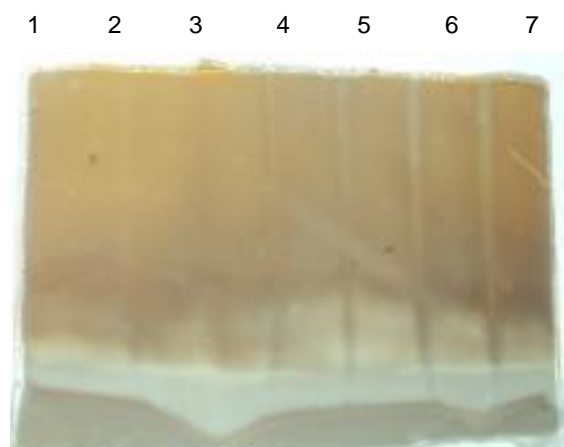


Figure 1. SDS PAGE of 52 BSS sesame seed oil cake.

other two fractions show the similar results of 7.0 and 7.2. The very low moisture content of the seed was suggestive of its long shelf life and keeping quality; and this might be an advantage since most spoilage microorganisms do not thrive well on food items that are low in moisture content (Tresler et al., 1980).

Fat

52 BSS had low fat content while comparing to other mesh sizes like 72 and 30 BSS (Table 1). The fat, protein, ash, crude fiber and calcium content of both whole and dehulled white, and Indian black sesame seeds showed the seeds to be rich in these nutrients (Ensminger and Ensminger, 1994); while Obiajunwa et al. (2005) reported the mineral value and certified range of sesame seed, indicating the seed to be rich in calcium, potassium, iron and phosphorous.

Fiber

72, 52 and 30 BSS had fiber content of 3.0, 3.8 and 3.5%, respectively (Table 1). The result shows that the 52 BSS fractions have high fiber content of 3.8%, while the 72 and 32 BSS have 3.5 and 3.0%.

Protein

Of the three BSS of the sesame seed flour cake, 52 BSS shows the high protein content of 45.9% while the 72 and 32 BSS contains 30.3 and 33.4% of the protein content. So this meal has a great potential in combating the protein calories malnutrition because of its high quality and quantity protein. The high protein content of 45.9%

Table 1. Proximate compositions of isolated protein from sesame seed oil cake.

Observation	72 BSS (%)	52 BSS (%)	30 BSS (%)
Moisture	7.0	6.8	7.2
Fat	4.8	4.4	5.0
Fiber	3.0	3.8	3.5
Protein	30.3	45.9	33.4
Ash	7.5	6.9	7.0

can be used to supplement low protein flours from cereals for infant feeding.

It also leads to an increase in protein content, reduction in fiber content and improvement in the functional characteristics of the protein (Inyand and Nwadiuka, 1992; Eknem, 1996). Since sesame is an oil seed, extraction of oil from the sesame seed meal led to increases in other constituents in the flour. The major increase was in protein content which ranged from the 20.0 in the meal from seed cake to 45.9 in the extracted flour. This shows that sesame flour is a good source of protein.

Dehulling improves the nutritional and flavor characteristics of the meal, and leads to the production of a glossy white product (Johnson et al., 1979). Seed cake is very high in protein. A portion of this seed cake is used as an animal feed, while the remainder is ground into sesame flour and added to health foods. The seed contains 45 to 55% oil, 19 to 25% protein and about 5% water.

The seeds were also reported to contain 25% protein, which are rich in methionine and tryptophane and one ounce of decorticated or hulled seed contains 6 g of protein, 3.7 g of fiber and 14 g of total fat (Godin and Spensley, 1971). The fat in sesame seed comprises of 38% monounsaturated and 44% polyunsaturated fatty acids (McIntyre, 2002).

The various fractions of sesame protein are as follows: albumin 8.6%; globulins 67.3%; prolamins 1.3% and glutelins 6.7% (Rivas, 1981). The molecular weight of the protein fractions ranges from 17,000 to 51,000. The sesame globulin consists of two fractions (Nath and Giri, 1957) namely α -globulin (60 to 70%) and β -globulin (25%). Hasegwa et al. (1978) have found mostly 13S globulin in protein isolates of sesame meal. Consesamin globulin for sesame has been isolated from sesame. It is rich in acidic amino acids especially glutamine and hydrophobic amino acids (Rajendran and Prakash, 1989).

Sesame is high in protein compared with other plant fruits, seeds and nuts (Nahar et al., 1990; Hernandez-Perez et al., 1994). Since vegetables and fruits are the major contributing sources of protein in the developing countries, the level of crude protein in sesame seed can qualify it as a good source of plant protein, if bio-available and easily digestible by the body.

The major asset of sesame protein is its unique

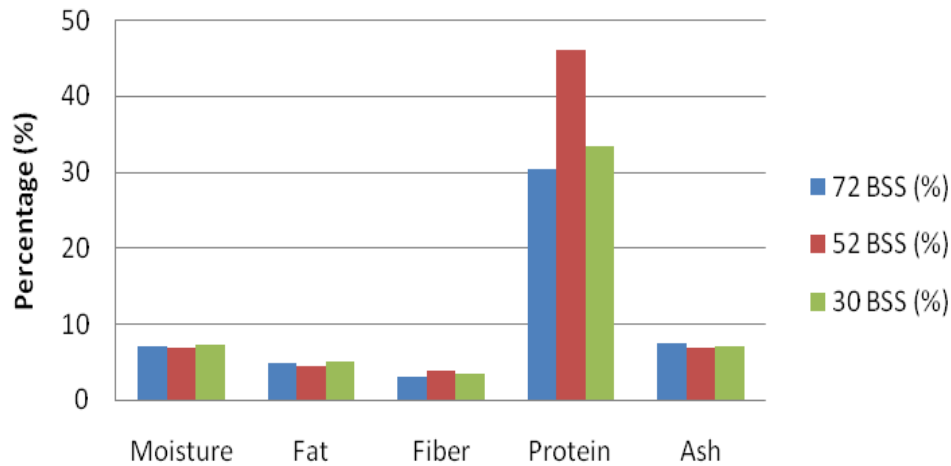


Figure 2. Proximate compositions of isolated protein from sesame seed oil cake.

nutritional character. The dehulled, defatted meal contains >60% protein, which is high in methionine, cystine, cysteine and tryptophane, and is bland and white in color. On the other hand, sesame meal is low in lysine and may contain high amounts of oxalic and phytic acids. High levels of selenium (Jaffe et al., 1964) and lead (Yannai and Hass, 1973) have also been reported.

Fat

A reverse trend was observed with fat content. 52 BSS fractions have low fat content 4.4% than the other two fractions which had 5% (Figure 2).

Ash

The ash content of three fractions is 7 and 7.5. It was comparable with the results (Gandhi and Srivatsava, 2007) (Figure 2).

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