ASEAN Food Journal 14 (3): 175-180 (2007)

Studies on the Production of Protein Isolates From Defatted Sesame Seed (*Sesamum indicum*) Flour and Their Nutritional Profile

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Abstract: The proximate analyses of the locally available commercial varieties of white and black sesame indicated that they contained 26.94%-27.65% protein, 47.79%-48.21% fat, 8.16%-8.95% carbohydrates and 5.56%-5.59% ash. Minerals like calcium, magnesium, phosphorus and potassium were present in significant quantities. Protein isolates were prepared from the defatted sesame meal using alkaline extraction method. The data indicated that about 28%-30% proteins were recovered and contained more than 90% proteins with all functional properties.

Keywords: Processes, sesame seeds, protein isolates, defatted sesame flour, physicochemical characters, functional profile, NSI, WAC

INTRODUCTION

Sesame (*Sesamum indicum* L) also known as gingely, beniseed, sim sim and til is an important annual seed crop. It is one of the earliest condiment and crop grown for edible oil. It is consumed directly as sweetmeat and snack. The seed has been called "queen of the oil seed crop" due to its high oil yield and its quality. The oil is mainly used for cooking purposes and a small percentage is also used in pharmaceuticals, cosmetic and perfumery industries. It is also used in the manufacture of soaps, paints and insecticides. The oil can be readily hydrogenated to medium melting fats for use in margarines, shortenings and vanaspathi (Padua, 1983). Fried sesame seed with sugar is used as soup ingredient. The paste of roasted seed is similar to peanut butter and is used in bakeries. The dehulled and defatted meal can be used in food products as a protein and tryptophan/methionine supplement (Serna-Saldivar *et al.*, 1999; Eleazar *et al.,* 2003; Albo, 2001). It is precisely these amino acids that distinguish from other oilseeds. The seed contains about 40-50% oil, 20-25% protein, 18-20% carbohydrates and 5-6% ash (Paredes *et al.,* 1994). Though the seed contains anti nutritional factors like phytates and oxalates, they are significantly minimized during processing. Defatted sesame meal contains more sugar and is generally utilized as animal feed and often as manure. This meal has a great potential in combating the protein calories malnutrition because of its high quality and quantity protein. However there is a need to process the meal carefully for human consumption. Hence this work was undertaken with the primary objective to characterize the chemical constituents and antinutritional factors of black and white sesame seeds and prepare defatted sesame meal for its value addition as commercially used protein isolates.

MATERIALS AND METHODS

Seed

Varieties of white and black sesame seeds were procured from the local market. They were

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thoroughly cleaned and made free from dirt, foreign matter and other stubbles. They were stored in airtight containers till further use.

Preparation of Defatted Sesame Meal

The seeds were soaked in water at ambient temperature overnight and the hulls were completely removed by floatation technique through hand rubbing. The seeds were later dried at 60°C for 12 h. Cold extraction method with petroleum ether was used for 72 h followed by n-Hexane for another 24 h to extract the oil from the seed. The ratio of seed to solvent was 1:4(w/v). The solvent was changed at 12 h interval. The defatted seeds were washed at the end with n-Hexane thrice and then desolventized.

Isolation of Proteins From Defatted Sesame Meal

Batch: 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 lot, one kilogram defatted sesame meal and 20 litres of water was used along with NaOH (0.2M)/KOH (0.2M) / Ca $(OH)_2$/ NaCl /$NaHCO_3$/deionised water as appropriate for the various extractions. The mixture was stirred at low speed (1200rpm) 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 1N H_2SO_4 to precipitate the proteins. The precipitate was creamy white in color. 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 freeze dried at 0.6096 mm H_2O and –40°C. The average yield of three replicates is reported.

Counter Current

The counter current extraction is a prerequisite for continuous large-scale production of protein isolates. NaOH (0.2M), which was the most suitable solvent in single step extraction, was used in the counter current extraction procedure. The experimental conditions were solvent to meal ratio 25:1(v/w), temperature of extraction 30°C and period of extraction 1 h at low speed. Four gram samples each was taken in four stopered Erlenmeyer flasks (250 ml) containing glass beads and designated as I, II, III and IV. Four stages of extraction were used namely A, B, C and D. Fresh 100 ml solvent was added in flask I and the extraction was carried out as above. After extraction, the contents were centrifuged at 6000 rpm for 10 min and the supernatant was collected. The supernatant was then added to flask II and likewise continued for flasks III and IV. All the supernatants were analyzed for their nitrogen content. Similarly the experiments were conducted for all four levels as indicated below:

A	I	II	III	IV
B	II	III	IV	I
C	III	IV	I	II
D	IV	I	II	III

The nitrogen contents in all the four levels were calculated and computations done for the total protein recovery. The main advantage of this method was the low solvent requirement.

Analytical Methods

Physico-Chemical Composition and Nutritional Analyses: The moisture, crude protein, crude fat, fibre, ash, minerals (Zn, Fe, Cu, Mn, P, Ca and Mg), available carbohydrates and free amino acids were analyzed using AOAC (1995) methods. Phytate was evaluated by the Latta and Eskin (1980) method.

Nitrogen Solubility Index: This was determined using the method of AOCS (1989). About 5g soybean sample was added to 200 ml water at 30°C and stirred at 120 rpm for 2 h. The contents were later centrifuged at 1500 rpm for 10 min and the supernatant was taken for

nitrogen analyses using the AOAC (1995) method.

Water Absorption Capacity and Oil Absorption Capacity: The water and oil absorption capacities were determined by the method of Sosulski *et al.* (1976).One gram of the sample was mixed with 10 ml distilled water or refined soybean oil (specific gravity 0.9092) in a conical graduated centrifuge tube and allowed to stand at ambient temperature (30±2°C) for 30 min, then centrifuged for 30 min at 2000 rpm. Water or oil absorption capacity was expressed as percent water or oil bound per gram isolate.

Gelation: This was determined by a modified method of Coffman and Garcia (1977). About one gram sample was prepared in 5 ml distilled water in test tubes, which were heated to 90°C for 1 h in a water bath. The heated dispersions were cooled rapidly under running tap water and then at 10±2°C for 2 h. The gel strength was determined by inverting the tube. For determining the least gelation concentration, appropriate sample suspensions of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) were prepared. The least gelation concentration is that where the sample from the inverted test tube did not fall down or drop.

pH: This was measured in 10% (w/v) protein samples in distilled water. Each suspension was mixed thoroughly and the pH was measured with a hand held pH meter (Manufactured by E. Merck).

RESULTS

Nutritional profile of sesame seeds: The chemical characteristics of the black and white sesame seeds were assessed and presented in Table 1.

Table 1: Physicochemical characteristics of the sesame seeds

Parameters	Sesame seeds	
	White	Black
Moisture content (wb)%	10.50	11.00
Crude protein, %	27.65	26.94
Crude fat, %	47.79	48.21
Total carbohydrates, %	8.95	5.59
Ash, %	5.56	5.59
Crude fiber, %	3.80	3.90
Minerals		
Calcium, ppm	640.0	700.0
Magnesium, ppm	560.0	360.0
Phosphorus, ppm	6190.0	5870.0
Zinc, ppm	0.6	0.8
Manganese, ppm	1.0	0.8
Iron, ppm	2.2	8.6
Copper, ppm	0.2	0.2
Potassium, ppm	42.0	54.2
Available free amino acids, mg/g	5.5	5.3
Phytate P, mg/g	450.0	475.0
Nitrogen Solubility Index, %	75.2	71.5
Water Absorption Capacity, %	240.0	200.0

Chemical Composition of Defatted Sesame Flours

The chemical composition and nutritional properties of defatted sesame meal from both the varieties were analyzed and the results are presented in Table 2.

Table 2: Nutritional properties of sesame meal

Characteristics	Defatted sesame flour	
	White seeds	Black seeds
Moisture content (wb), %	8.87	10.40
Crude protein, %	51.45	47.63
Crude fat, %	1.10	1.98
Total carbohydrates, %	26.84	25.72
Available free amino acids, mg/g	4.95	3.60
Phytic Phosphorus, mg/g	167.00	175.00
NSI, %	17.60	20.70
WAC, %	320.00	360.0

Isolation of Proteins Through Batch Mode

Effect of Extractants: Different types of extractants namely deionized water, NaOH, KOH, Ca $(OH)_2$; NaCl and $NaHCO_3$ were used for isolation of proteins from the defatted sesame meal. The results are given in Table 3.

Table3: Extraction of proteins with different extractants

Extractant	pH of dispersion	Recovery of proteins, %
Deionised water	6.8	16.6
NaOH, 0.2M	9.0	30.1
KOH, 0.2M	9.3	29.2
Ca $(OH)_2$,0.5%	10.0	23.0
NaCl, 0.5%	7.2	Nil
$NaHCO_3$, 0.2M	8.8	21.8

Effect of Meal to Extractant Ratio: From the earlier experiment it was found that NaOH gave the maximum protein recovery with a meal to extractant ratio of 1:20 (w/v). It was therefore designed to vary the meal to extractant ratio up to 1:60 (w/v). This investigation was carried out with NaOH. The results are presented in Table 4.

Table 4: Extraction of proteins at different meal to extractant ratios

Meal: extractant (w/v)	pH of dispersion	Recovery of proteins, %
1:10	8.8	27.12
1:20	9.0	30.08
1:30	9.2	29.75
1:40	9.4	22.18
1:50	9.6	20.00
1:60	10.0	20.00

Effect of Temperature and Time: Earlier experiments were conducted at ambient temperature for one hour. In this experiment the extraction temperature was varied up to 70°C. The results are given in Table 5.

Table 5: Protein recoveries at different extraction temperatures

Temperature (°C)	Extraction time, min					
	10	20	30	40	50	60
	Protein recovery, %					
30	5	10	16	20	24.0	30.08
40	6	12	17	25	29.9	
50	8	16	23	31		
60	10	22	29.5			
70	15	30				

Counter Current Extraction of Sesame Proteins: Counter current extraction was carried out in four stages with 0.2M NaOH. The results are presented in Table 6.

Table 6: Counter current extraction of sesame protein

Stage	Supernatant	Nitrogen content in each meal fraction,%	Total nitrogen at each stage of extraction, %
A	I	80.0	
	II	75.0	52.0
	III	40.0	
	IV	5.2	
B	I	90.0	
	II	81.0	25.0
	III	54.0	
	VI	81.0	
C	III	82.0	
	IV	73.0	12.0
	I	95.0	
	II	99.0	
D	IV	81.0	
	I	98.0	3.0
	II	97.0	
	III	84.0	

Physicochemical and Functional Properties of Protein Isolates

The protein isolates from one of the processes were analyzed for their nutritional profile and presented in Table 7.

Table 7: Chemical composition and nutritional properties

Characteristics	Values
Protein, %	90.5
Fat, %	ND
Crude fibre, %	ND
Ash, %	7.8
pH	6.4
WAC, ml/100g	192
Oil absorption, ml/100g	378
Gelation at 12% suspension	Very weak gel

DISCUSSION

The results of this work illustrate that the protein content of white seeds (27.65%) were marginally higher than those of black seeds (26.94%). However, a reverse trend was observed with fat content. Both varieties of seeds had significant quantities of minerals present. The NSI values were more than 70% and WAC was in the range of 200-240%. Thus there existed a nutrient variation among these seeds. The results further showed that the protein content of sesame meal from white seeds (51.45%) was higher than the black seeds (47.63%). On contrary the ash content of the black seeds was significantly higher than the white seeds. The phytic phosphorus was reduced significantly from the original seed values to a range of 167-175 mg/g. The NSI values were reduced to 17.6-20.7% and WAC was increased to 320-360% in the meals. The data on protein isolation revealed that NaOH gave the maximum extraction of proteins (30.1%) in one hour at ambient temperature followed by KOH with 29.2% recovery. The pH of dispersion ranged between 6.8 and 10.0. Overall, the alkaline conditions favored the solubilization of proteins. However at higher pH (10.0), the extraction efficiency was lowered due to decrease in solubility of proteins. The results are in accordance with earlier published literature (Taha *et al.*, 1987). The results also indicated that maximum extraction was attained with 1:20 meal extractant ratio and the pH of dispersion was 9.0. Gandhi *et al.* (2000) obtained similar results during isolation of proteins from soy meal. The results showed that the protein recovery was about 30.1% at ambient temperature (30°C) in 1 h of extraction. However, the rate of extraction increased with an increase in temperature from 30°C to 70°C. Higher temperatures of extraction reduced the extraction time considerably. It took about 50 min, 40 min, 30 min, and 20 min, respectively. The data from the counter current extraction substantiated that more nitrogen was extracted in the first stage of the extraction and overall about 92% protein was extracted. The isolated proteins have very high water and oil absorption capacities and form very weak gels. Thus the findings of this investigation lead to a simple process for the production of defatted sesame flours and isolation of proteins from them to yield isolates with good functional and nutritional profiles.

CONCLUSION

Simple processes were developed for making defatted sesame flour and protein isolates from sesame seed and they were characterized for their nutritional profiles.

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