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Low-Phytate Protein Concentrate and Isolate from Sesame Seed

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The solubility of sesame meal protein in different solvents was investigated. Sodium hydroxide solution (0.04 M) proved to be the most suitable solvent for sesame protein, extracting about 90% of the meal nitrogen. A countercurrent extraction of the meal protein using 0.04 M NaOH and a solvent to meal ratio of 25:1 resulted in a total extraction of 91.7% of the meal nitrogen. The solubility pattern of phytates and proteins of sesame meal was investigated. At pH 5.4, 50% of the phytates present in the meal was removed, while only 17.5% of the protein was dissolved. A sesame seed protein isolate was prepared by dissolving the protein by the countercurrent procedure and precipitating it at pH 5.4. The resulting protein isolate contained 91.4% protein and was almost free of phytates. A three-stage countercurrent extraction procedure was used to extract the meal at pH 5.4, resulting in a protein concentrate with 68.2% protein and a low phytate content.

The gap between the nutritional requirements and actual consumption of protein by the majority of the population in developing countries is widening rapidly. Although food supply and population must be brought into balance, it is unlikely in the coming years that reduction in birth rates will have much effect on the critical decline of the per capita food supply. Exploitation of unconventional and novel sources of food seems to be one of the adequate approaches to solve this imbalance.

Over the past years major attention has been directed to the use of oilseeds as cheaper yet adequate protein foods. Modern research and technology has clearly demonstrated how increasing varieties of protein foods can be made available for human consumption (Waggle and Kolar, 1979; Forsythe and Briskey, 1977).

Sesame seed is a rich source of edible oil as well as protein. It contains about 25% protein that is rich in sulfur amino acids and tryptophan. However, the problems involved in the use of sesame protein as a source of food is its high content of both oxalic acid and phytic acid (Johnson et al., 1979).

The present work has been designed to investigate a procedure for the preparation of sesame seed protein products with a high protein and a low phytate content.

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MATERIALS AND METHODS

Materials. Local sesame seeds (*Sesamum indicum*), variety Giza 25, were obtained from the Ministry of Agriculture. Since oxalic acid of sesame seed exists mainly in the hulls (Johnson et al., 1979), a flotation technique was used to separate the hull portion from the rest of the kernel. Finely ground sesame seed was blended with hexane in a Waring Blendor until a suspension was formed. The blend of sesame seed with hexane was allowed to settle overnight in a beaker. The top layer was siphoned and discarded, and the lower layer containing the protein fraction was reextracted six times with hexane in a Waring Blendor. The defatted meal was spread to dry at room temperature and then ground and sieved to pass an 80-mesh screen.

Methods. Moisture, lipids, crude fiber, and ash were determined according to standard procedures (AOAC, 1980). Nitrogen was also determined by the Kjeldahl procedure according to AOAC methods (1980) and the protein calculated as N × 6.25. Nitrogen solubility was determined according to Lyman et al. (1953), and phytates were estimated according to Wheeler and Ferrel (1971). Samples were hydrolyzed with constant-boiling aqueous HCl in sealed ampules (20 mg/10 mL of acid solution) at 110° C for 24 h. The amino acids for each hydrolysate were then determined according to Moore et al. (1958), through the use of an automated amino acid analyzer constructed

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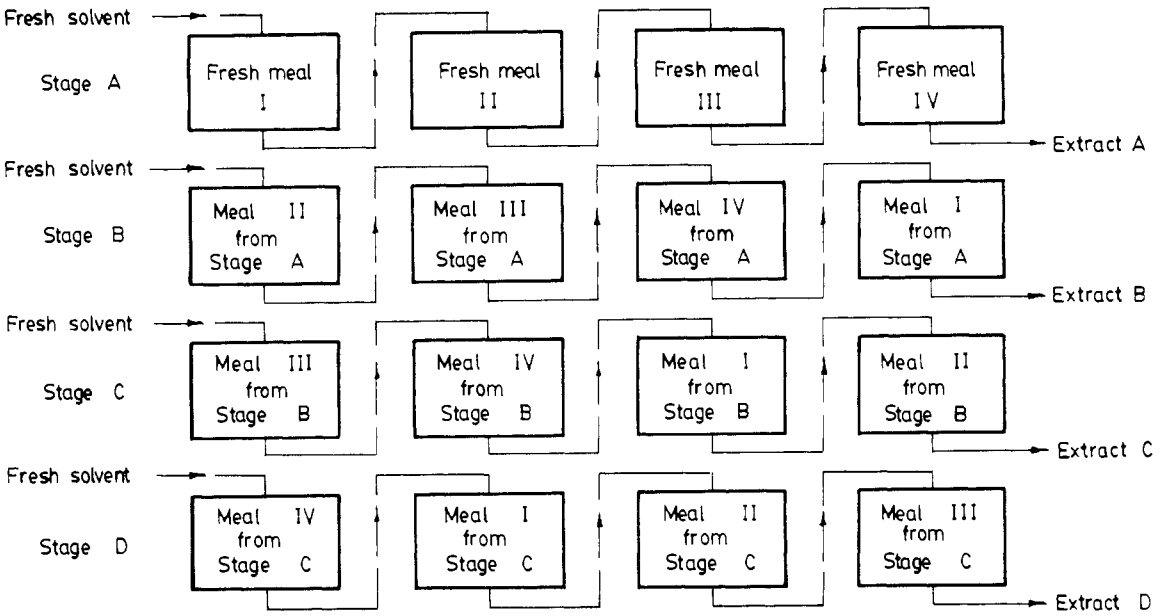

Figure 1. Scheme of countercurrent extraction for preparation of protein isolate from defatted meal.

essentially as described by Spackman et al. (1958).

(1) Single-Step Extraction of Proteins. Dehulled defatted sesame meal was extracted with distilled water, sodium chloride solutions (0.5, 1.0 M), sodium carbonate solutions (0.2, 0.5 M), sodium bicarbonate solutions (0.2, 0.5 M), sodium carbonate–bicarbonate buffers (pH 10.0–10.6), and sodium hydroxide solutions (0.02–0.07 M). Exactly 1 g of meal and 10 g of glass beads were placed in a 250-mL Erlenmeyer flask, and aqueous solutions were added to give solvent to meal ratios ranging from 50:1 to 200:1. Extraction was carried out at 30 °C by shaking the flasks vigorously for 30–120 min. The mixtures were then centrifuged at 6000*g* for 10 min. Aliquots (1 mL) of the supernatant solutions were taken for nitrogen determination. Protein solubility was calculated on the assumption of full recovery of total volume.

(2) Countercurrent Extraction of Proteins. A schematic representation of the countercurrent extraction procedure followed is illustrated in Figure 1.

Four 5-g samples of dehulled defatted sesame meal were weighed in 250-mL beakers designated I–IV. In each of the four stages (A–D) the first sample was extracted with 125 mL of 0.04 M NaOH (solvent to meal ratio 25:1). Details of the procedure have been described previously by El-Nockrashy et al. (1977).

(3) Solubility Pattern of Phytates and Proteins. Five grams of dehulled defatted sesame meal was extracted with 50 mL of distilled water, while the mixture was adjusted to pH 4.0–6.0 (for each pH adjustment a fresh quantity of meal and water were used). Extractions were carried out for 10 min in an Omni mixer, after which the solutions were centrifuged for 30 min at 6000*g*.

Phytates and proteins were determined on aliquots of the supernatant solutions, and percentages of total recovery were calculated at different pH values.

RESULTS AND DISCUSSION

In the present study, sesame seed protein isolate and sesame seed protein concentrate were prepared. The procedures used result in products high in protein and low in phytate. Preparation of sesame protein isolate involved two major steps: (i) a countercurrent extraction procedure using the optimum conditions that were established through single-step extraction; (ii) precipitation of the proteins at the pH value for maximum solubility of phytates. Sesame protein concentrate was prepared by a three-stage countercurrent extraction of the meal at the pH value that resulted in highest removal of phytates.

Table I. Solubility of Sesame Seed Protein in Aqueous Solutions

extractants[a]	concn, M	pH	protein sol, %
distilled water			12.26
sodium chloride	0.5		49.12
	1.0		76.90
sodium bicarbonate	0.2		44.50
	0.5		72.31
sodium carbonate	0.2		86.90
	0.5		84.72
sodium carbonate–sodium bicarbonate buffer		10.0	76.89
		10.2	77.90
		10.4	78.52
		10.6	79.61
sodium hydroxide	0.02		86.41
	0.03		87.10
	0.04		89.63
	0.05		86.00
	0.06		85.72
	0.07		84.74

[a] Extraction was by shaking for 90 min at 30 °C. Calculations were made on the assumption of full recovery of total volume.

Table II. Solubility of Sesame Seed Protein in Aqueous Sodium Hydroxide Solutions (0.04 M)[a]

solvent: meal	time of extractn, min	protein sol, %	solvent: meal	time of extractn, min	protein sol, %
50:1	30	89.0	100:1	90	89.8
50:1	60	89.0	100:1	120	88.6
50:1	90	89.8	200:1	30	90.4
50:1	120	90.1	200:1	60	89.0
100:1	30	89.8	200:1	90	89.2
100:1	60	91.6	200:1	120	89.4

[a] Calculations were made on the assumption of full recovery of total volume.

Single-Step Extraction of Proteins. During the study of the solubility of sesame protein in different solvents, it became clear that sesame protein was more soluble in sodium hydroxide than in other solvents (Table I). Table II elucidates the optimum conditions for the extraction of proteins from sesame meal where 0.04 M NaOH at a solvent to meal ratio of 100:1 and extraction time of 60 min resulted in ca. 92% total extraction of the meal nitrogen.

Table III. Countercurrent Extraction of Sesame Seed Protein

stage	sample no.	meal N extr from ea sample, %	total meal N extr at ea stage, %
A	I	84.6	53.6
	II	84.6	
	III	38.9	
	IV	6.3	
B	II	100.7	25.1
	III	76.1	
	IV	53.3	
	I	84.6	
C	III	83.3	10.2
	IV	75.9	
	I	95.4	
	II	100.7	
D	IV	80.9	2.8
	I	100.3	
	II	102.1	
	III	83.3	

Sodium hydroxide solutions of normalities ranging from 0.02 to 0.2 M have been recommended by investigators working on the isolation of proteins from rapeseed (El-Nockrashy et al., 1977), sunflower seed (Taha et al., 1981), peanuts (Abbasy et al., 1981), and cottonseeed (El-Nockrashy and Frampton, 1967). All these procedures require rather large volumes of solvent to acquire a satisfactory degree of extraction.

Countercurrent Extraction of Proteins. Countercurrent extraction is a prerequisite for a continuous large-scale production of protein isolates. The advantage of using low solvent to meal ratio is that comparatively small volumes of solvent have to be used for a high output.

Sodium hydroxide (0.04 M), which proved to be the most suitable solvent in single-step extraction, is used in the countercurrent extraction procedure (Figure 1). Table III gives typical results of the countercurrent extraction of proteins from dehulled defatted sesame meal at a solvent to meal ratio of 25:1. At lower solvent to meal ratios, difficulties result at the later steps of stage A owing to the high solvent retention by the meal. Calculations are based on actual recovery of the meal nitrogen in the extracts.

In stage A, the meal retains 39% of the solvent. This solvent retention is reduced to form 8.0–10.0% in the following stages. Out of 500 mL of 0.04 M NaOH, a total of 416 mL of the extract was recovered. The highest amount of protein extracted was in the final extract of stage A, where 53.9% of the total nitrogen present in the meal dissolved. The final extracts of stages B–D resulted in 25.1, 10.2, and 2.8% extraction of the meal nitrogen, respectively. At the end of stages A–D, 91.7% of the meal nitrogen was extracted.

It is obvious that the countercurrent extraction of the proteins as described in this paper offers great advantages over single-step or multistage extraction. The advantages are (i) a high degree of extraction of proteins, (ii) least requirement of solvents and chemicals, which means optimum utilization of the dissolving capacity of the solvent, and (iii) easy adaptability to either a semicontinuous or a fully continuous process.

Solubility Pattern of Phytates and Proteins. In this study the aim was to establish the pH value at which the maximum amount of phytates is dissolved from sesame meal. Sesame seeds contain among the highest levels of phytates found in nature. The defatted sesame meal contains substantial amount of phytic acid, which reaches 5% compared to 1.5% in soybean meal (de Boland et al., 1975). Phytates are reported to reduce the biological availability of zinc, calcium, magnesium, perhaps iron, and complexes with proteins, rendering them less soluble

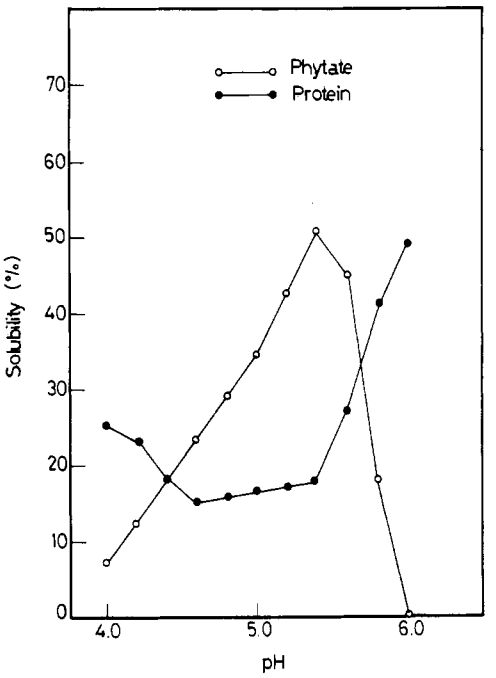

Figure 2. Solubility curves of phytate and protein present in sesame meal.

Table IV. Analysis of Sesame Seed Protein Products[a]

	meal	protein concentrate	protein isolate
protein, %	53.4	68.2	91.4
oil, %	0.8	0.8	0.0
ash, %	3.2	1.3	0.06
fiber, %	3.0	2.8	0.03
N-free extr, %	39.6	26.9	8.5
mg phytate P/g product	14.50	1.81	0.65

[a] Values are given on a moisture-free basis.

(Smith and Rackis, 1957). There is evidence that phytate–protein complexes are less subject to proteolytic digestion (O'Dell and Boland, 1976).

Figure 2 shows the solubility pattern of sesame meal phytate and protein at pH 4.0–6.0. Maximum solubility of phytate is at pH 5.4, where 50% of the phytate present in the meal is dissolved. Above and below pH 5.4 the amount of phytate extracted decreases. O'Dell and Boland (1976) reported that sesame meal phytate is not readily soluble in neutral or alkaline aqueous solutions. Protein solubility shows a minimum at pH 4.6–5.4 where 15–17.5% of the meal protein is extracted. Rivas et al. (1981) reported that at pH 4.0–6.5 less than 10% N is extracted from sesame flour, whereas more than 90% is extracted at pH 11.0.

In view of the previous findings, a recommended procedure for the preparation of sesame seed protein isolate includes a step for dissolving the protein from the dehulled defatted meal by the countercurrent extraction procedure and then precipitating the proteins at pH 5.4. The precipitated proteins are then washed with acetone and then ether and air-dried. Sesame seed protein concentrate can be prepared by a three-stage countercurrent extraction of the meal at pH 5.4, washing the resulting protein concentrate with acetone and ether and air-drying.

Analysis of Sesame Seed Protein Products. The compositions of the dehulled defatted sesame meal, sesame seed protein concentrate, and sesame seed protein isolate are given in Table IV.

The data reveal total protein contents of 54.3, 68.2, and 91.4%, respectively, for sesame meal, sesame protein

Table V. Amino Acid Analysis of Sesame Seed Protein Products[a]

	meal	concentrate	isolate	req of human adults[b]
lysine	3.28	2.49	2.67	5.5
histidine	2.81	2.49	2.87	
arginine	14.10	12.80	12.20	
threonine	4.08	2.69	4.20	4.0
valine	4.36	3.46	5.45	5.0
methionine	2.64	2.80	3.10	
cystine	1.29	1.16	1.30	3.5
isoleucine	4.36	2.97	4.70	4.0
leucine	6.91	5.17	5.83	7.0
phenylalanine tyrosine	4.89	3.24	5.13	6.0

[a] Amino acids were calculated as grams/16 g of nitrogen. [b] FAO/WHO *Energy and Protein Requirements*, FAO Nutrition Meeting Report Series No. 52; Food and Agricultural Organization: Rome, 1973.

concentrate, and sesame protein isolate and an increase in the protein content (27.2 and 71.2%, respectively) for sesame protein concentrate and sesame protein isolate over the sesame meal. Values for oil in the three products are negligible. The protein isolate showed negligible ash and fiber contents. Naturally, with the increase in the protein content of the concentrate and isolate, the nitrogen-free extract decreased. Sesame meal contains 14.5 mg of phytate P/g of meal, whereas sesame protein concentrate contains only 1.81 mg of phytate P/g of concentrate. The sesame protein isolate can be considered almost free of phytate (0.65 mg of phytate P/g of isolate). The procedures used for the preparation of the sesame protein concentrate and isolate resulted in 87.5 and 95.5% reduction of the phytate content, respectively, over the meal.

Table V gives the amino acid analysis of the three sesame protein products as well as the requirements of human adults recommended by the FAO for comparison.

The amino acid analysis pattern shows that the three sesame protein products are first limiting in lysine but are rich sources of both methionine and cystine, which are primarily limiting amino acids in many vegetable protein sources. Johnson et al. (1979) and Lyon (1972) reported the same findings. Sesame meal is second limiting in valine, while the sesame protein isolate is second limiting in leucine. Sesame protein concentrate shows a poor amino acid composition when compared to the FAO requirements for human adults. It is limiting in lysine, threonine, valine, isoleucine, and leucine.

From the previous findings it is concluded that sesame seed protein isolate prepared by the given procedure provide a promising source of protein for incorporation in food, for either chicken or human consumption. It has the advantage of containing a high bland protein rich in sulfur amino acids and a negligible amount of phytate. Sesame protein concentrate has the disadvantage of a poor amino acid pattern, but this can be overcome through its supplementation with other plant proteins.

Registry No. NaOH, 1310-73-2; phytate, 83-86-3.

LITERATURE CITED

Abbasy, M.; Taha, F. S.; Hamouda, A. A. *Grasas Aceites (Seville)* **1981**, *32*, 171.
AOAC *Official Methods of Analysis*, 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.
de Boland, A. R.; Garner, G. B.; O'Dell, B. L. *J. Agric. Food Chem.* **1975**, *23*, 1186.
El-Nockrashy, A. S.; Frampton, V. L. *Biochem. Biophys. Res. Commun.* **1967**, *28*, 675.
El-Nockrashy, A. S.; Mukherjee, K. D.; Mangold, H. K. *J. Agric. Food Chem.* **1977**, *25*, 193.
Forsythe, R. H.; Briskey, E. J. In *Food Proteins*; AVI: Westport, CT, 1977.
Johnson, L. A.; Suleiman, T. M.; Lusas, E. W. *J. Am. Oil Chem. Soc.* **1979**, *56*, 463.
Lyman, C. M.; Chang, W. Y.; Couch, J. R. *J. Nutr.* **1953**, *49*, 679.
Lyon, C. K. *J. Am. Oil Chem. Soc.* **1972**, *49*, 245.
Moore, S.; Spackman, D. H.; Stein, W. H. *Anal. Chem.* **1958**, *30*, 1185.
O'Dell, B. L.; de Boland, A. R. *J. Agric. Food Chem.* **1976**, *24*, 804.
Rivas, R. N.; Deneh, J. F.; Caygill, J. C. *J. Sci. Food Agric.* **1981**, *32*, 565.
Smith, A. K.; Rackis, J. J. *J. Am. Oil Chem. Soc.* **1975**, *79*, 633.
Spackman, D. H.; Stein, W. H.; Moore, S. *Anal. Chem.* **1958**, *30*, 1190.
Taha, F. S.; Abbasy, M.; El-Nockrashy, A. S.; Shoeb, Z. E. *J. Sci. Food Agric.* **1981**, *32*, 166.
Waggle, D. H.; Kolar, C. W. In *Soy Protein and Human Nutrition*; Academic: New York, 1979.
Wheeler, E. L.; Ferrel, R. E. *Cereal Chem.* **1971**, *48*, 312.

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Mössbauer Study of Iron in Soybean Seeds

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Chemical states of iron in soybean seeds cultivated with a nutrient solution containing enriched ^{57}Fe have been studied by Mössbauer spectroscopy. The Mössbauer spectra showed that iron in the seeds was in both trivalent high-spin and divalent high-spin states. Most of the iron in mature seeds was found to be in the ferric state. In immature seeds, the relative area of Mössbauer absorption due to ferrous ions was much larger than in ripe ones. The relative area of ferrous ions increased in the process of germination of the ripe seeds. The Mössbauer parameters were compared with those of ferric phytates, ferritin, and other iron compounds isolated from plants. The ferric ion in the soybean seeds was not identified as a ferric phytate as has been reported for wheat. More data with model compounds are required to specify the iron complexes in the seeds.

Although a number of biogenic iron compounds have been isolated and characterized, little is known concerning the distribution of the whole iron among different chemical species in living organisms. Oxidation–reduction reactions between ferric and ferrous ions are known to be associated with a number of biochemical phenomena such as absorption of iron by roots, transport of iron across inner

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