# Extraction of Sesame Seed Protein and Determination of its Molecular Weight by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

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## ABSTRACT

Sesame seeds are an important source of edible oil and protein. Studies were made on protein solubility of defatted sesame seed meal in aqueous solution over various pHs or in various salt solutions. Maximum solubility was found in alkaline solution, and the proteins were almost insoluble at acid solution. The solubility of protein in NaCl or $CaCl_2$ solution was increased upon increasing the salt concentrations up to 1 M. In $Na_2SO_3$ or $Na_2HPO_4$ solution, the solubility of protein was higher at lower salt concentrations but decreased at higher salt concentrations at pH 8. Extractable sesame seed proteins in salt solution separated into seven fractions electrophoretically (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). The mol wt of the seven fractions were 51,000, 31,000, 28,500, 25,500, 21,800, 20,500, and 17,900.

## INTRODUCTION

Sesame (*Sesamum indicum*, L.) is an oilseed of the Pedaliaceae family. The plant has been cultivated in India for more than 2000 years and is grown extensively in tropical and subtropical areas (1). The valuable components of sesame seed are oil and protein. Oil-free sesame meal contains 40-50% protein which is valued in feeds and human food because of the high methionine content. This material has not been used in human foods to any great extent chiefly because of some undesirable characteristics, such as high fiber content, dark color, presence of oxalate (2), and, to some extent, selenium (3). Therefore, the meal must be modified before use for human consumption. Extraction of protein from the meal is one of the solutions to the problem. According to studies by Jones and Gersdorff (4), two globulin fractions differing in precipitability with ammonium sulfate, in solubility, elemental composition, and distribution of nitrogen were extracted by 10% sodium chloride.

This paper is concerned with the extraction of protein from sesame seed meal and determination of its mol wt by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

## EXPERIMENTAL PROCEDURES

### Materials

All reagents used in this study were analytical grade. Reagents for SDS-polyacrylamide gel electrophoresis were purchased from Bio Rad Laboratories, Richmond, Calif., and proteins for standard calibration (lysozyme, trypsin, egg albumin, and bovine albumin) were purchased from Sigma Chemical Co., St. Louis, Mo.

### Preparation of Sesame Meal

Sesame seed, Venezuela 51 variety cultivated in Brazil, was obtained from the Institute of Agriculture in Campinas, SP. Brazil. The sesame meal was prepared by milling in a Stein laboratory mill, model $L_2$, and was defatted by several extractions with hexane for 30 min with stirring at room temperature until the oil content was less than 1%. Residual solvent was removed and the meal dried in air. The dried meal was separated into two fractions by passing through a 50 mesh screen. The fine fraction was used for extraction of protein. All operations were performed at room temperature.

### Extraction of Proteins

Extraction of proteins from sesame meal was carried out at room temperature for 30 min using a distilled water to meal ratio of 15:1 (v:w). The pH of extraction was adjusted to 2, 3, 4, 5, and 6 by adding 0.5 M of hydrochloric acid or adjusted to 7, 8, 9, 10, 11, and 12 by adding 0.5 M sodium hydroxide. The pH was rechecked and readjusted after 30 min. Five g samples were used for protein extraction, and the volume of solution was brought to 75 ml after final pH adjustment. After centrifugation at 8200 x g for 20 min, the suspensions were filtered through Whatman filter paper no. 1 to remove insoluble materials. A 20 ml aliquot of each extract was used for nitrogen determination by the Kjeldahl method (5). Protein solubility profiles of sesame meal in several different salt solutions, such as $Na_2SO_3$, $Na_2HPO_4$, NaCl, and $CaCl_2$, were carried in varied concentrations of salt solutions at pH 8.0 adjusted by the addition

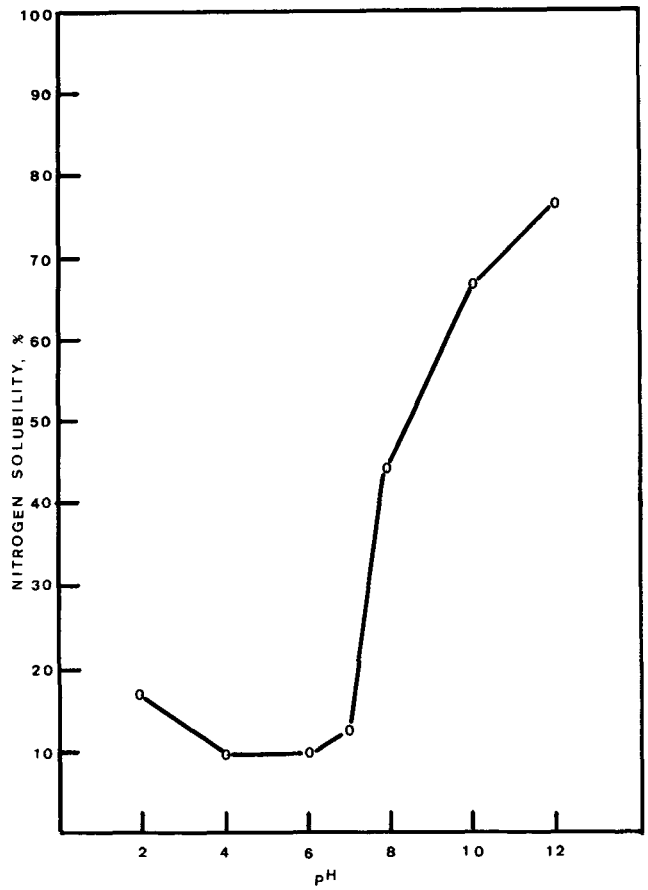


FIG. 1. Protein solubility profile of sesame seed meal in aqueous solution at various pHs.

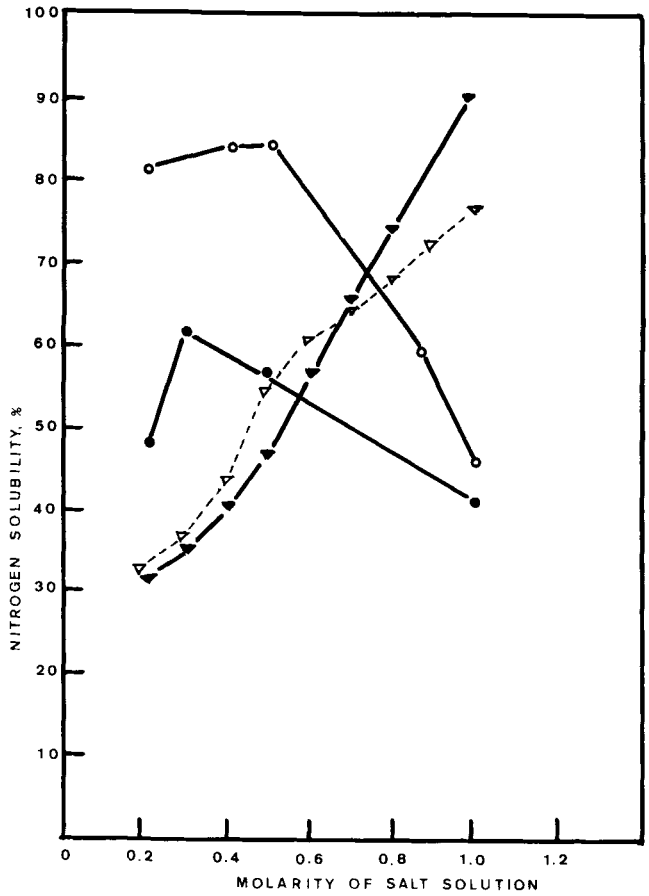


FIG. 2. Protein solubility profile of sesame seed meal in various salt solutions at pH 8. —●—●— = $Na_2HPO_4$, —○—○— = $Na_2SO_3$, —△—△— = $CaCl_2$, and —▲—▲— = NaCl.

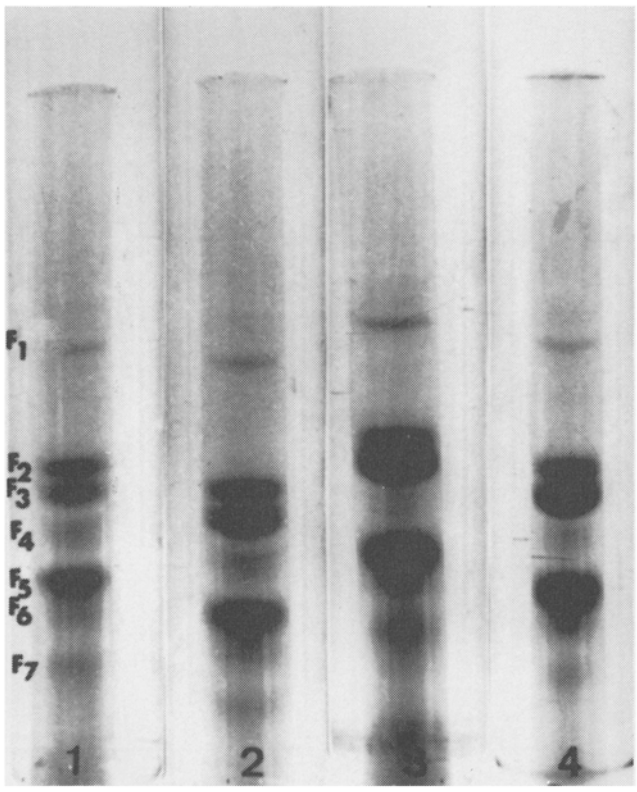


FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sesame seed proteins extracted with various salt solutions at pH 8.0. 1. 0.3 M $Na_2HPO_4$, 2. 0.4 M $Na_2SO_3$, 3. 1 M NaCl, and 4. 1 M $CaCl_2$.

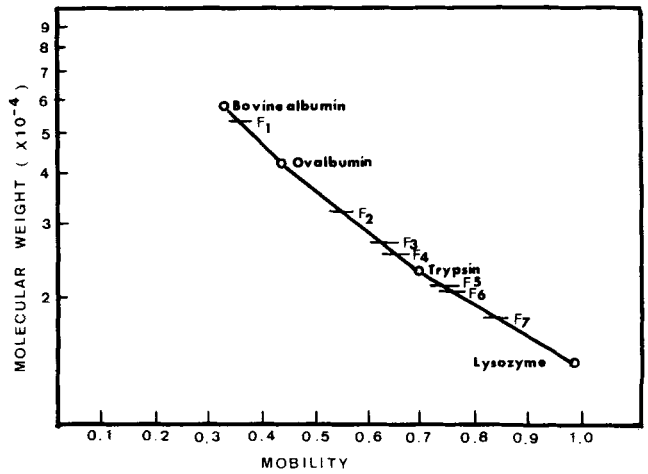


FIG. 4. Comparison of mol wt of standard proteins (lysozyme 14,300; trypsin, 23,300; ovalbumin, 43,000; bovine albumin, 69,000) and 7 different fractions of sesame seed proteins extracted in 0.3 M $Na_2HPO_4$ solution by sodium dodecyl sulfate polyacrylamide gel electrophoresis. $F_1$ = fraction 1, $F_2$ = fraction 2, etc.

of 0.5 M sodium hydroxide.

### SDS-Polyacrylamide Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis is based upon the method of Weber and Osborn (6). For 10% acrylamide solution, 22.2 g acrylamide and 0.6 g methylenebisacrylamide were dissolved in water to give 100 ml. Gel buffer consisted of 7.8 g $NaH_2PO_4 \cdot H_2O$. 38.6 g $Na_2HPO_4 \cdot 7H_2O$, and 2 g SDS in 1000 ml water. The 15 ml gel buffer was mixed with 13.5 ml acrylamide solution, 1.5 ml ammonium persulfate solution (15 mg/ml), and 0.045 ml N,N,N'N'-tetramethylethylenediamine; then immediately it was poured into the gel tubes. Before the gel hardened, one drop of water was layered on top of the solution. After polymerization of gels, the water layer was sucked off, then 50 μliter protein sample was layered on the gels. The gel buffer was diluted 1:1 with water to fill the tubes and the two compartments of the electrophoresis apparatus. Electrophoresis was performed at a constant current, 8 ma/gel tube for ca. 4 hr. After electrophoresis, gels were removed from the tubes, and the length of the gel and the distance moved by the dye were measured. The gels were fixed in 50% trichloroacetic acid solution overnight and stained for 2 hr in 0.25% coomassie blue in 20% trichloroacetic acid, then rinsed over a period of 2 days with 7% acetic acid solution to remove background color. The length of the gels after destaining and the position of the blue protein fractions were measured. The mobility of protein fractions were calculated as:

$$\text{Mobility} = \frac{\text{Distance of protein migration}}{\text{Gel length after destaining}} \times \frac{\text{Length before staining}}{\text{Distance of dye migration}}.$$

The mobilities were plotted against mol wt of standard proteins expressed on a semilogarithmic scale.

### Preparation of Protein Sample for Electrophoresis

The protein extract in salt solution or aqueous solution was dialyzed against distilled water for 48 hr at 4 C. After dialysis, the sample was freeze-dried. The samples were incubated at 37 C overnight in 0.01 M sodium phosphate buffer, pH 7.0, containing 1% SDS and 1% β-mercaptoethanol. The protein concentration was 1 mg/ml. After incubation, 0.1 ml sample was mixed with 10 μliter 0.05% bromophenol blue and 0.1 ml 40% sucrose, then subjected to electrophoresis.

## RESULTS AND DISCUSSION

Figure 1 shows the nitrogen solubility profile of sesame seed meal in aqueous solution at various pHs. It can be seen that the protein is substantially insoluble at low pH and very soluble at high pH. The solubility profile of sesame seed protein is remarkably different in various salt solutions. The solubility of protein in various concentrations of salt at pH 8 is shown in Figure 2. NaCl and $CaCl_2$ are efficient compounds for extraction of sesame protein. In NaCl and $CaCl_2$ solution, the solubility of protein increased with increasing concentrations up to 1 M. In contrast, in $Na_2SO_3$ and $Na_2HPO_4$ solution, protein solubility was increased at low concentration and decreased at high concentrations up to 1 M. Therefore, the high solubility characteristics of sesame seed protein in alkaline, NaCl, or $CaCl_2$ solution can be used to prepare a protein isolate, followed by precipitation at a pH ca. 4.

Extractable sesame seed proteins in various salt solutions were electrophoresed, and mol wt of each fraction were determined by SDS-polyacrylamide electrophoresis. The pH for extraction of protein in salt solution was adjusted to 8.0, although a high pH in salt solution extracts more proteins. Actually, there is no difference in the number of protein fractions between high and low pH. Figure 3 demonstrates the SDS-polyacrylamide gel electrophoresis for fractions of sesame seed proteins isolated in various salt solutions (0.3 M $Na_2HPO_4$, 0.4 M $Na_2SO_3$, 1 M NaCl, and 1 M $CaCl_2$) at pH 8. Each protein isolate consists of seven fractions, and the relative mobilities for all fractions were calculated and compared with the standard curve in Figure 4. The mol wt for 7 fractions of sesame seed proteins were in the following order: fraction 1, 51,000; fraction 2, 31,000; fraction 3, 28,500; fraction 4, 25,500; fraction 5, 21,800; fraction 6, 20,500; and fraction 7, 17,900.

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