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# Extraction and Separation of Guar Seed Proteins

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The solubility of guar meal protein was determined in various aqueous solvents using different acids and bases. The total proteins were characterized by the techniques of gel filtration, polyacrylamide gel electrophoresis, and ultracentrifugation. They were found to consist of three fractions, two of high molecular weight and one of low molecular weight. Neither the total proteins nor the fractions showed any hemagglutinin activity. Maximum trypsin inhibitor and proteolytic activity was observed with the lowest molecular weight fraction.

Guar bean, *Cyamopsis tetragonoloba*, is a commercially important crop of India and serves as the raw material for the production of the galactomannan gum (guar gum). Guar meal left after the extraction of the gum contains 38–55% protein and some antinutritional factors and objectionable flavors (Subramanian and Parpia, 1975; Ambegaokar et al., 1969; Couch et al., 1966). This investigation was undertaken to characterize the nature of guar proteins and explore the possibilities of obtaining a protein isolate free from deleterious constituents.

Since there were no reports on the solubility and the fractionation profiles of guar seed protein, studies were undertaken on these aspects. In addition, guar meal and protein fractions were assayed for trypsin inhibitor (Couch et al., 1966; Sumathi and Pattabiraman, 1976), hemagglutinin, and proteolytic activities (D'Souza, 1972) which have been reported to be present.

## MATERIALS AND METHODS

**Preparation of Defatted Guar Meal.** Guar seeds of the variety FS-277 obtained from the Haryana Agricultural University, Hissar, India were used. The seeds were broken into splits and grits in an electric blender and the gum splits separated from the protein-rich germ fraction (grits) by passing through a 12-mesh sieve (British Standard Sieve). The grits were ground, solvent extracted thrice with *n*-hexane, and once again ground to 60-mesh size. The moisture content of the flour was 8% and the protein content 50%.

**Nitrogen Solubility Experiments.** To 2 g of the meal 20 mL of the aqueous solvent was added and the pH of the suspension adjusted to the desired value by the addition of 1 N HCl or 1 N NaOH. The suspension was then shaken for 1 h at room temperature (about 28 °C) and centrifuged at 4000 rpm for 20 min, and the pH of the supernatant was noted. Aliquots of 10 mL were taken for nitrogen estimation by the microKjeldahl method. The percentage of the total meal nitrogen extracted was calculated. The solvents used were water, 0.5 and 1.0 M NaCl, 0.1 and 0.2 M $CaCl_2$, and 2% sodium hexametaphosphate (SHMP) solutions in distilled water. A comparative study was also made to find out the efficiency of extraction using some acids such as HCl, $H_2SO_4$, and $H_3PO_4$ and alkalies such as NaOH, $Ba(OH)_2$, and $Ca(OH)_2$. In the case of the mineral acids, 2 g of the meal was suspended in 20 mL of water and the pH adjusted in the acidic range with the respective acids. In the case of the bases, solid calcium and barium hydroxides and 40% sodium hydroxide solution were added to obtain the desired pH.

**Ammonium Sulfate Fractionation.** Guar meal was extracted with 1 M NaCl solution (1:10), the clear extract was dialyzed extensively against 1 M NaCl solution, and the dialyzate was diluted to about 1% protein concentration. Solid $(NH_4)_2SO_4$ was added to the protein solution and in each case the volume made up to 10 mL so as to obtain 10, 20, ...60% $(NH_4)_2SO_4$ concentration. After thorough mixing and dissolution of $(NH_4)_2SO_4$, the samples were kept in a water bath at 30 °C for 30 min and centrifuged to remove the precipitates, and the absorbance values of the supernatant were read at 280 nm in a Carl-Zeiss Spectrophotometer.

**Gel Filtration.** Sephadex G-200 (Pharmacia, Sweden), which had been equilibrated with 1 M NaCl, was packed into a 2.0 × 85.0 cm column. Four milliliters of the total 1 M NaCl extract of guar meal containing about 100 mg of protein was loaded onto the column and allowed to be absorbed. The protein was eluted with 1 M NaCl solution, 3.0-mL fractions collected in an automatic fraction col-

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lector, and the absorbance measured at 280 nm.

**Polyacrylamide Gel Electrophoresis.** Electrophoresis was carried out in a Shandon electrophoresis apparatus. Gels (7.5%) in 0.02 M phosphate buffer of pH 7.5 were prepared by the standard procedure. The same buffer was used as the running buffer. Tubes 0.5 × 7.5 cm were used, and electrophoresis was carried out for 2.5 h at 4 mA/tube. The sample was prepared by extracting guar meal in 1 M NaCl and dialyzing the protein extract extensively against 0.02 M phosphate buffer of pH 7.5 with several changes of the buffer.

**Ultracentrifugation.** Using a protein concentration of 1% in 1 M NaCl ultracentrifugation was performed at room temperature (~28 °C) at 59780 rpm in a Spinco Model E Analytical Ultracentrifuge equipped with RTIC Unit and phase plate Schlieren optics. $s_{20,w}$ was calculated by the standard procedure (Schachmann, 1959).

**Proteolytic Activity.** Proteolytic activity was determined using casein as the substrate by the method described by Chrispeels and Boulter (1975). Casein (E. Merck, Germany) was dissolved in 25 mM citrate phosphate buffer of pH 5.7 at a concentration of 1%. The assay mixture containing 1 mL of casein, 0.2 mL of enzyme, and 0.8 mL of water was incubated for 2 h at 37 °C in a thermostatic water bath. The reaction was stopped by the addition of 1 mL of 12% trichloroacetic acid, and the proteins were allowed to precipitate in the cold for 1 h. The supernatant obtained by centrifugation was read at 280 nm. Guar meal extract as a source of protease was prepared by shaking 1 g of guar meal in 15 mL of 25 mM citrate phosphate buffer of pH 5.7 for 1 h and taking the clear centrifuged extract. The protein content of the extract was determined.

**Trypsin Inhibitor Activity.** The total proteins of guar meal and the protein fractions, obtained by passing the dialyzed 1 M NaCl extract through Sephadex G-200 equilibrated with 1 M NaCl, were tested for trypsin inhibitor activity by the procedure of Kakade et al. (1969). The 1 M NaCl extract of the meal and the other fractions were dialyzed extensively against 0.1 M phosphate buffer of pH 7.6. The activity was also tested in the 25 mM citrate phosphate buffer extract of pH 5.7, after dialysis against 0.1 M phosphate buffer of pH 7.6. The citrate phosphate buffer of pH 5.7 selectively extracts only the low molecular weight protein fraction. The protein content of the extracts was determined by Lowry's method (1951).

**Hemagglutinin Activity.** The hemagglutinin activity of guar meal was tested by the serial dilution method of Liener and Hill (1953). The sample was prepared by extracting guar meal with 0.9% saline overnight at 4 °C and taking the clear extract. Serial dilutions of the extract were also prepared (1-, 2-, 4-, 16-, etc. fold dilutions). Aliquots of a 0.2-mL suspension of trypsinated (Lis and Sharon, 1973) rabbit blood cells were pipetted into ten test tubes (10 mm × 70 mm). Then 0.5 mL of the extract was added in the first tube. In the next tube 0.5 mL of the twofold diluted extract was added. Thus in every succeeding tube, the serially diluted extract was added. In another set of control tubes containing 0.2 mL of trypsinated red blood cell suspension, 0.5 mL of 0.9% saline was added. All the tubes were gently shaken and kept in the cold overnight and then observed visually for agglutination of the red blood cells by gentle tapping.

## RESULTS AND DISCUSSIONS

**Solubility.** The solubility profiles of guar protein in different solvents show a U-shaped pattern, suggesting the existence of only one isoelectric point typical of other plant proteins such as groundnut and soybean (Figure 1)

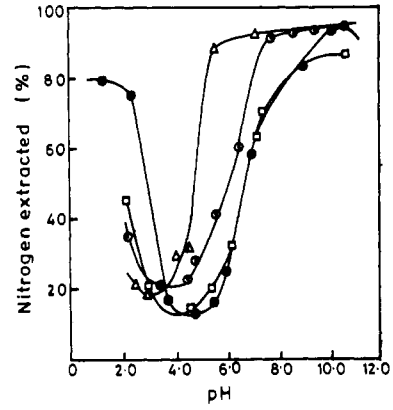


**Figure 1.** Nitrogen solubility vs. pH profiles: (—●—) water, (—⊙—) 0.5 M NaCl solution, (—△—) 1.0 M NaCl solution, (—□—) 2% sodium hexametaphosphate solution.

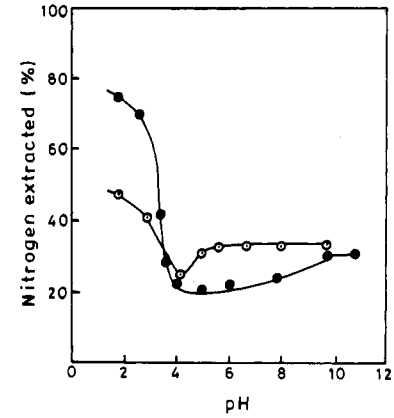


**Figure 2.** Nitrogen solubility vs. pH profiles in $CaCl_2$ solutions: (—●—) 0.1 M $CaCl_2$, (-⊙-) 0.2 M $CaCl_2$.

(Fontaine et al., 1944; Smith and Circle, 1938). In water the isoelectric point of guar protein was found to be around pH 4.7, similar to that reported by D'Souza (1972). At the isoelectric point about 12.5% of the total meal nitrogen remained in solution, whereas at pH 9.5 about 90% of the nitrogen was solubilized. The solubility patterns in 0.5 and 1.0 M NaCl solutions showed higher solubility than in water. As the molarity of NaCl was increased the isoelectric point was found to shift toward the acidic side. In 1 M NaCl, the isoelectric pH was at 3.0. The solubility in 2% SHMP was high, similar to the solubility profile in water (Figure 1). In this case, at pH 9.0 about 80% of the total nitrogen was in solution.

The solubility of guar meal protein in 0.1 and 0.2 M $CaCl_2$ solutions at pH 9.0 was very low, being 27 and 33%, respectively (Figure 2). At lower pH values the solubility was much higher. At pH 3.0, the solubility was 55 and 40% in 0.1 and 0.2 M $CaCl_2$ solutions, respectively.

Amongst the three mineral acids used for protein extraction in the pH range of 2 and 6, phosphoric acid and hydrochloric acid gave higher extractability than sulfuric acid (Figure 3). This could be due to the better solubilizing action of the phosphate and chloride ions compared to the sulfate ions. Smith and Circle (1938) have investigated the dispersion behavior of soy meal nitrogen over a wide range of pH in several solvents. They found that phosphoric and hydrochloric acids were better extractants than sulfuric acid.

The solubility profiles of guar meal proteins in sodium, calcium, and barium hydroxide are given in Figure 3. It is seen that over the entire pH range of 6 to 12, sodium hydroxide was much more efficient in solubilizing the protein than the other two bases. The solubility in the case

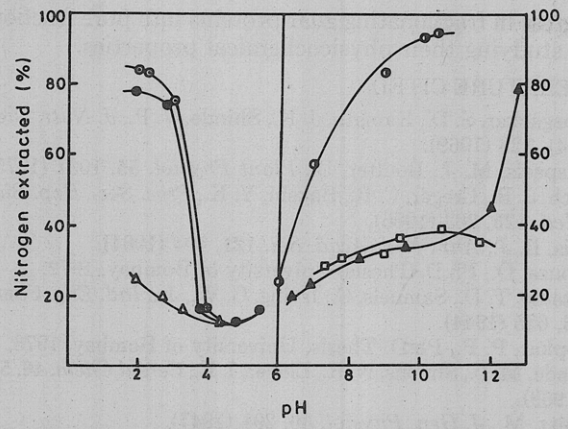


**Figure 3.** Nitrogen solubility vs. pH profiles in different acids and bases: (—⊙—) phosphoric acid, (—●—) hydrochloric acid, (—△—) sulfuric acid, (—◑—) sodium hydroxide, (—□—) calcium hydroxide, (—▲—) barium hydroxide.

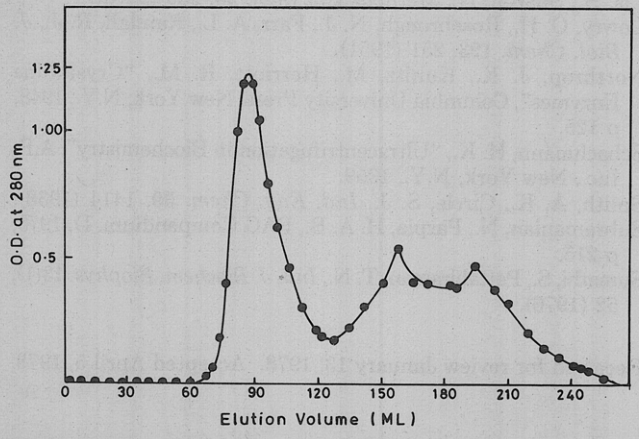


**Figure 4.** Gel filtration pattern of guar proteins in 1 M NaCl solution (column dimensions 2 × 85 cm).

of 0.1 and 0.2 M $CaCl_2$ solution and calcium and barium hydroxides was very low probably because the cations form insoluble complexes with the protein.

**Gel Filtration.** The gel filtration pattern of guar total proteins gave three peaks eluting at 85, 150, and 195 mL (Figure 4). The proportion of the three fractions were 48, 26, and 26%, respectively. Khopkar (1976) has fractionated the acid extract of guar meal on Sephadex G-50 and Sephadex G-100 gels using 0.025 M Tris–HCl buffer of pH 8.8. Sephadex G-100 resolved the acid extract into four fractions whereas Sephadex G-50 into three fractions. Apart from this study, there are no other reports of gel filtration of total proteins of guar.

**Gel Electrophoresis.** The polyacrylamide gel electrophoresis pattern of the total proteins is shown in Figure 5. Three major bands were observed, two of which had poor mobility and one good mobility. The relative mobilities suggest that the two protein fractions were of high molecular weight and the other of low molecular weight. Khopkar (1976) has carried out disc gel electrophoresis of the alkaline and acidic extracts of guar meal, by the method of Davis (1964) in 7.5% gels using Tris–glycine buffer of pH 8.3. The alkaline extract was found to give three major bands and one minor band. The acidic extract showed five bands, indicating the possibility of dissociation of the proteins in acid. The penetration was found to be greater in disc electrophoresis than in the ordinary gel electrophoresis.

**Ultracentrifugation.** Sedimentation velocity pattern of the 1 M NaCl extract showed the presence of three peaks having $s_{20,w}$ values of 9.5, 6.4, and 1.5 S (Figure 6).

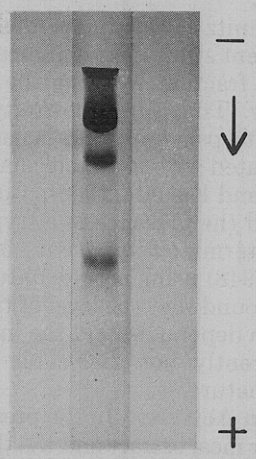


**Figure 5.** Polyacrylamide gel electrophoresis pattern of guar protein in 0.02 M phosphate buffer of pH 7.5. The arrow shows the direction of migration.

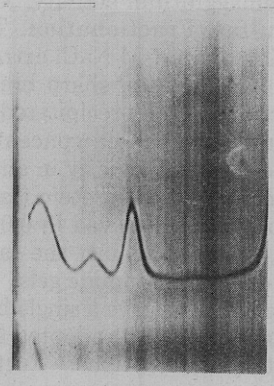

**Figure 6.** Sedimentation velocity pattern of guar proteins in 1 M NaCl solution. Photograph at 30 min of centrifugation at 59780 rpm. Sedimentation proceeds from left to right.

The fraction having $s_{20,w}$ value of 1.5 S gave a broad diffuse peak characteristic of low molecular weight proteins. The relative proportions of the three fractions were 30, 10, and 60%, respectively. Ultracentrifugation was also carried out in 0.02 M phosphate buffer of pH 7.5 without the addition of NaCl. The pattern was essentially the same as in 1 M NaCl, suggesting that dissociation of the proteins had not occurred in low ionic strength buffer solution.

**Proteolytic Activity.** A value of 8.3 Kunitz units/mg of protein was obtained for the proteolytic (caseolytic) activity of the undialyzed citrate phosphate buffer extract of guar meal. This value was achieved within 15 min of incubation and remained constant up to 2 h of incubation. There are no reports on the proteolytic activity of guar meal (D'Souza, 1972). The dialyzed extract had also the same activity. However, the NaCl extract had lower activity of 5.6 units/mg of protein. This could be due to the fact that citrate phosphate buffer preferentially extracts the low molecular weight fraction. It was observed that peak I of gel filtration pattern had no proteolytic activity. Peak II had 2 units of activity, whereas peak III, which represents the low molecular weight fraction, had 11.4 units.

**Trypsin Inhibitor Activity.** The trypsin inhibitor activity of total proteins and the three fractions obtained by gel filtration on Sephadex G-200 was estimated. The total proteins gave a value of 5.5 trypsin inhibitor units (TIU)/mg of protein and the third fraction (lowest molecular weight fraction) a value of 20.4 TIU/mg of protein. The first two fractions had negligible trypsin inhibitory activity. Sumathi and Pattabiraman (1976) have reported a trypsin inhibitor activity of 2.45 units/mg of protein by

the method of Kunitz (1947) in the dialyzed guar meal extract. The present study clearly indicates that the low molecular weight fraction contains the highest trypsin inhibitory activity. This is in conformity with the many reports that most trypsin inhibitors isolated from various sources are associated with low molecular weight protein fractions (Liener and Kakade, 1969). Couch et al. (1966) have also reported the presence of a trypsin inhibitor in guar meal and determined its activity by the method of Northrop et al. (1948) using urea-denatured hemoglobin substrate. They found that the level of trypsin inhibition was concentration dependent and the inhibitor was heat labile and apparently not dialyzable indicative of a macromolecular nature.

**Hemagglutinin Activity.** In the present study it was observed that guar meal extract and the three gel filtration fractions did not show any hemagglutinin activity. However, D'Souza (1972) had reported that one of the protein fractions isolated from the acid extract of guar meal showed a little hemagglutinin activity.

**Ammonium Sulfate Fractionation.** The ammonium sulfate fractionation of the 1 M NaCl extract of guar gave a S-shaped curve without any sharp breaks. However, three protein fractions which precipitated at 0–20, 20–33, and 33–75% ammonium sulfate concentration, respectively, were tested for homogeneity in an attempt to use this method for separating the protein fractions. Each of these cuts was collected, dissolved in 0.02 M phosphate buffer of pH 7.5, dialyzed against the same buffer, and electrophoresced on polyacrylamide gels. It was observed that none of these fractions gave a single band, suggesting that this procedure could not be adopted for fractionating guar proteins into pure fractions. Further work is in progress in fractionating guar proteins into pure fractions and studying their physicochemical properties.

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# Glycoalkaloid Composition of Wild and Cultivated Tuber-Bearing *Solanum* Species of Potential Value in Potato Breeding Programs

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The glycoalkaloid composition of tubers from a variety of wild and cultivated *Solanum* species has been analyzed qualitatively and quantitatively. These species represent potential breeding stock for the production of new commercial potato varieties, and the glycoalkaloids identified in these species were: tomatine, demissine, $\alpha$- and $\beta$-solamarine, $\alpha$- and $\beta$-chaconine, and $\alpha$-solanine. With the exception of *Solanum acaule*, which runs somewhat high in glycoalkaloid concentration, these clones, based on their glycoalkaloid composition, present no apparent hazard to human health. The conflict in reported data for glycoalkaloid composition of the species *S. acaule* has been resolved.

The introduction of wild *Solanum* species germplasm in potato breeding programs to obtain characteristics such as disease resistance and cold hardiness is being actively studied. The wild Mexican species *S. demissum* has been used for a long time as a source of late blight resistance, and most modern potato cultivars exhibiting such resistance contain germplasm from *S. demissum*. Currently, with the intent of making potatoes a more universal staple, breeders are attempting to develop potato varieties that can be grown in areas of the world where presently available cultivars cannot be grown. Some wild species, because of the environment in which they are found, make good candidates for breeding programs.

The introduction of germplasm from new species must be approached with some caution. Undesirable traits that may not be easily detectable must not be introduced in commercial cultivars. Such undesirable properties should be uncovered in the early stages of a breeding program in

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