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Effect of Detoxification Treatments on the Proteins of Guar Meal

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Guar meal was subjected to various detoxification pretreatments, and their effect on the proteins was determined by chemical and physicochemical methods. Ethanol treatment removed the high and low molecular weight protein fractions and decreased the carbohydrate content. These two protein fractions were also not observed in the proteins of the autoclaved samples, while there was no appreciable change in the carbohydrate content. Autoclaving also reduced the available lysine content of the total meal protein. Acid treatment dissociated the high molecular weight protein fractions and reduced both the available lysine and carbohydrate contents.

Guar meal is a byproduct of the guar gum industry and contains 45% protein. Guar proteins are known to cause toxicity to animals (Ambegaokar et al., 1969; Kawatra et al., 1969; Subramanian and Shantha, 1968). Extensive studies on the effect of various treatments such as heat, extraction with aqueous alcohols, methanol, ethanol, and 2-propanol, or dilute hydrochloric acid on the nutritional quality of guar meal have been carried out (Kawatra et al., 1974; Subramanian and Shantha, 1968; D'Souza, 1972). Such treatments help to eliminate the antigrowth factors. HCl extracts of guar meal were found to be toxic to the rats, and detailed studies were carried out to isolate the toxic factors. A toxic chromoprotein fraction was obtained from the extract and its properties were studied (Khopkar, 1976). However, data on the changes that may occur in the chemical and physicochemical properties of the total proteins in the detoxified meals due to such treatments are meager. Extraction of raw guar meal proteins in various solvents and isolation and characterization of the major guar protein of the untreated meal have been reported earlier (Nath et al., 1978, 1980). In this investigation proteins of guar meals, subjected to ethanol or acid extraction or autoclaving, were characterized by various techniques.

MATERIALS AND METHODS

A commercial variety of guar seed known as "Durgapur Safed", having a uniform light greyish appearance, procured from a gum processing factory was used in the study. The seeds were split in the dry state in an emery disk sheller and classified to yield three fractions, viz., the gum splits rich in galactomannan, the germ fraction rich in protein, and the fibrous husk (skin or hull) fraction. The germ fraction was flaked and defatted with food-grade hexane solvent. The defatted material having a crude protein content of ~57% was used in the studies.

Preparation of Detoxified Meals. Known quantities of the defatted guar flakes were processed variously as described below.

(a) The flakes were autoclaved in open steam at 1 kg/cm^2 pressure for 20 min, dried in a current of hot air at 60 °C, and powdered (–36, +44 BSS). The yield on the raw material was 92.5%, and the material had a crude protein content of 56.2%.

(b) The flakes were extracted with 70% ethanol at room temperature (RT; 28 °C) by steeping overnight, keeping the flakes:solvent ratio as 1:5. The extract was filtered and the flakes were dried in hot air and powdered as above. The yield was 70% and the crude protein content of the product 73.2%. Most of the nonprotein nitrogenous constituents, soluble sugars, saponins, and minerals are extracted by the aqueous ethanol.

(c) The flakes were extracted with 7 volumes of 0.25 N HCl at RT for 4 h, filtered, washed free of acid, dried in a current of hot air, and powdered. The yield was 26% and the crude protein content 61.3%.

(d) A higher concentration of 1.0 N HCl was used in steeping the flakes for 30 min in the acid at RT. The meal to acid ratio was 1:5 (w/v). The flakes were washed free of acid, dried in hot air, and powdered. The yield was 23% on raw material and the protein content 62.9%.

Acid extraction procedures c and d result in considerable loss of raw material, including protein, nonprotein nitrogenous compounds, sugars, saponins, and minerals.

Protein Solutions. The proteins from the various meals were extracted in 1 M NaCl at pH 7.0 by stirring for 1 h at room temperature (~28 °C), followed by centrifugation at 3700g for 20 min. Nearly 80–85% of the total proteins in the samples was extracted. By use of Visking Sausage dialysis tubing, the extracts were dialyzed exhaustively against 1 M NaCl for 24 h. For the polyacrylamide gel electrophoretic experiments, the 1 M NaCl extract was dialyzed against 0.02 M phosphate buffer of pH 7.5.

Protein Concentration. An absorptivity of 8.2 at 280 nm for 1% solution was used for determining the concentration of the dialyzed 1 M NaCl extract of the untreated and 70% ethanol treated meals (Nath et al., 1980). The protein concentration of the dialyzed extract of the heat-treated and acid-treated meals was estimated by the micro-Kjeldahl method (AOAC, 1975) using 6.25 to convert nitrogen to the protein value since the wavelength of maximum absorption (λ_{max}) of these protein solutions was found to shift below 280 nm.

Gel Filtration. Sepharose 6B-100 which had been equilibrated with 1 M NaCl solution was packed into a column, 2 × 85 cm (bed volume 240 mL). The total amount of protein loaded from the different samples was the same and eluted with 1 M NaCl solution; 3-mL fractions were collected in an automatic fraction collector and the absorbance was measured at 280 nm.

Sedimentation Velocity. The experiments were performed at room temperature (~28 °C) in a Spinco Model E analytical ultracentrifuge equipped with RTIC unit and phase-plate Schlieren optics. With the untreated meal, sedimentation velocity experiments were done at 59 780 rpm with a 1% protein solution, using a standard 12-mm duralminium cell centerpiece. With the acid-treated meals, the experiments were conducted at 50 740 rpm with a 0.5% protein solution because of the low extractability of the proteins, using a standard 18-mm duralminium centerpiece. $s_{20,w}$ values were calculated by the standard method

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Table I. Yield, Protein Content, and Available Lysine Content of Guar Meals and Carbohydrate in NaCl-Extracted Protein

guar meals	yield, %	protein content,[a] % (N × 6.25)	av lysine content of the protein in meals,[a] g/16 g of N	carbohydrate content of the extracted protein, %, in glucose equiv
untreated	100	56.8	3.41	2.55
ethanol treated	70	73.2	3.44	2.20
autoclaved	93	56.2	3.09	2.50
0.25 N HCl treated	26	61.3	2.80	2.25
1.0 N HCl treated	23	62.9	3.20	2.05

[a] Data represent the average of triplicate determinations; standard error is in the order of ±0.05–0.10%.

(Schachman, 1959).

Polyacrylamide Gel Electrophoresis. Electrophoresis was carried out in a Shandon electrophoretic apparatus. Gels (7.5%) in a 0.02 M phosphate buffer of pH 7.5 were prepared by the standard procedure. Electrophoresis was carried out for 2.5 h at 4 mA/tube. To make comparisons meaningful, we loaded an approximate amount of the protein (50–80 μg/10 μL) in all the cases.

Absorption Spectrum. The absorption spectrum of the meal proteins in 1 M NaCl was recorded in the range 240–300 nm in a Perkin-Elmer double-beam recording spectrophotometer, Model 124. The protein concentration of the solutions used was in the range 0.3–0.7 mg/mL.

Protein Estimation. Protein was estimated by the micro-Kjeldahl method (AOAC, 1975). A factor of 6.25 was used to convert nitrogen to protein.

Available Lysine Estimation. The available lysine content in the various meals was estimated by the modified method of Carpenter and Ellinger (1955).

Carbohydrate Estimation. The carbohydrate content of the protein in the dialyzed, NaCl extracts was estimated by the method of Montgomery (1961). Aliquots of 0.5% protein in 1 M NaCl solution were used for the estimation. The carbohydrate content is expressed in glucose equivalents.

RESULTS AND DISCUSSION

Chemical Composition of the Meals. The protein content of the guar meal and the available lysine and carbohydrate content of the proteins are given in Table I. In all cases, except the autoclaved meal, the protein content had increased. The treatments obviously leached out nonprotein constituents of guar meal and thus increased the protein content. Since autoclaving did not involve leaching, the protein content was nearly the same as that of the untreated meal.

The available lysine content was unaffected by ethanol treatment. Autoclaving the meal reduced the value by ~10%. The acid-treated meals also showed a decrease in the available lysine content by 18 and 6% for the 0.25 and 1.0 N HCl treated meals, respectively. Surprisingly the 1.0 N HCl treated meal had a higher available lysine content than the 0.25 N HCl treated meal. This could be due to the fact that the meal had been exposed to the acid at 0.25 N concentration for a much longer period of 4 h. Possibly, 1.0 N HCl could cleave the sugar–amino bond. Heating food materials induces the Maillard reaction involving the ε-amino groups of the protein (Hodge, 1953). Autoclaving guar meal could also induce Maillard reaction wherein the ε-amino groups of the protein could react with the sugars present in the meal, thereby lowering the value of available lysine. However, the reason for the acid to decrease the available lysine content of the meal was not clear.

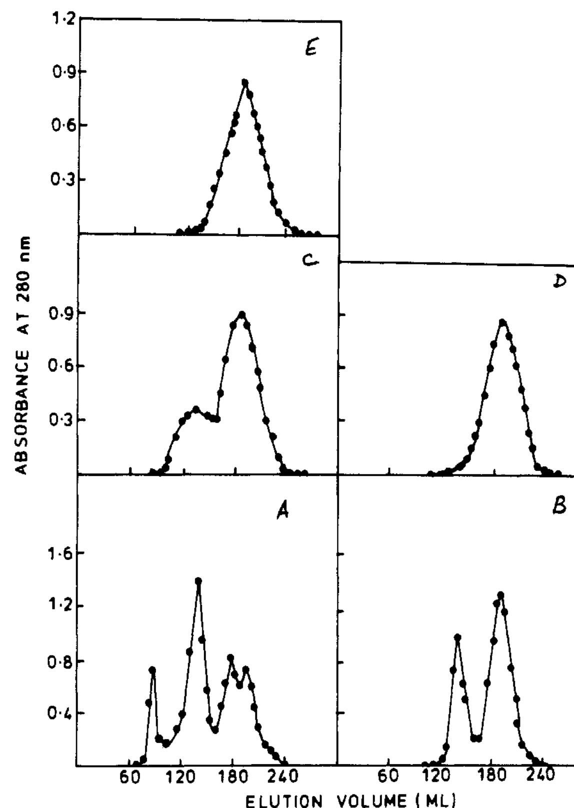

Figure 1. Gel filtration patterns of untreated and treated guar meal proteins on Sepharose 6B-100 (1 M NaCl; 2.0 × 85 cm column). (A) Untreated guar meal. (B) Ethanol-treated guar meal. (C) Autoclaved guar meal. (D) 0.25 N HCl treated guar meal. (E) 1.0 N HCl treated guar meal.

The NaCl-extracted proteins of the untreated meal had a carbohydrate content of 2.55%. With the autoclaved meal there was no difference in the carbohydrate content. The ethanol- and acid-treated meals gave a lower carbohydrate content. The values represent the amount of carbohydrate present in the bound form with the protein, as the free sugars in the NaCl extracts are removed during dialysis. The decreased available lysine content and an altered absorption spectrum of the autoclaved meal suggested the possibility of protein–sugar interaction. However, the carbohydrate content of the extracted proteins of the autoclaved meal did not indicate any such interaction. The available lysine content was estimated in the meals whereas the carbohydrate content was estimated in the dialyzed extracts of the meal. It is possible that on heating the sugars are bound to the low molecular weight proteins or peptides and these dialyze out, thus leading to no change in the carbohydrate content of the meal.

Gel Filtration. With the untreated meal, four peaks were observed eluting at 88, 138, 176, and 198 mL, respectively (Figure 1A). V_e/V_0 values of the four peaks were 1.05, 1.64, 2.10, and 2.36, respectively. The proportions of the peaks eluting at 138, 178, and 198 mL were greater than that of the first peak. The gel filtration pattern of the ethanol-treated meal showed the presence of only two peaks eluting at 140 and 190 mL (Figure 1B). The peak eluting at 190 mL was in a greater proportion than the peak eluting at 140 mL. The autoclaved meal also gave only two peaks eluting at 138 and 188 mL, the latter being in a larger proportion than the former (Figure 1C). Both the 0.25 and 1.0 N HCl treated meals showed the

presence of only one broad peak eluting at 190 mL (parts D and E of Figure 1).

These data show that guar proteins undergo definite changes due to treatment of the meal. In the case of the ethanol-treated guar meal, the high molecular weight protein (V_eV_0 = 1.05) and the low molecular weight (V_e/V_0 = 2.10) proteins were missing. In the autoclaved sample these two proteins were also missing. Further, in the heat-treated meal the proportion of the protein with V_e/V_0 = 1.64 (138-mL peak) was lower than that with the ethanol-treated meal. It is possible that ethanol washing preferentially leached out the high and low molecular weight protein fractions.

The proteins of 0.25 or 1.0 N HCl treated meal appeared to consist of only the low molecular weight protein fraction. This could be due to one or all of the following reasons: (1) the high molecular weight proteins of guar meal are leached out in acid; (2) they are denatured and become insoluble; (3) the high molecular weight proteins are dissociated by the action of acid. To test the alternatives, we conducted the following experiments. The untreated meal proteins were extracted in 1 M NaCl solution and dialyzed against 1 M NaCl solution. An aliquot of the solution was adjusted to 1 N HCl concentration by the slow addition of 2 N HCl with vigorous shaking. In spite of vigorous shaking, there was considerable precipitation. The precipitate was removed by centrifugation, and the supernatant was analyzed by the sedimentation velocity technique. The precipitate was suspended in 1 M NaCl solution and the pH adjusted to 7.0 when it dissolved completely. This solution was also used for the sedimentation velocity experiment. Also, the supernatant left after acid precipitation of the protein was readjusted to pH 7.0 and analyzed in the ultracentrifuge.

The 1 M NaCl extract of the untreated meal gave four peaks, with $s_{20,w}$ values of 1.5, 6.5, 12, and 15 S. The relative proportions of the 1.5S, 6.5S, 12S, and 15S protein fractions were 53, 8, 33, and 6%, respectively. The supernatant of the acid-precipitated protein extract gave only the slow moving peak characteristic of the low molecular weight fraction. The precipitate redissolved in a 1 M NaCl solution of pH 7.0 also gave a similar pattern. These results suggest that 1 N HCl causes dissociation of the high molecular weight protein fractions. To determine if this dissociation was reversible, we adjusted the supernatant left after acid precipitation of the protein to pH 7.0 and used it for sedimentation velocity experiments. The pattern was characteristic of a low molecular weight protein fraction and was identical with that of the supernatant. Thus, HCl appeared to dissociate the high molecular weight proteins irreversibly.

Polyacrylamide Gel Electrophoresis. The polyacrylamide gel electrophoretic pattern of the untreated guar meal proteins in 0.02 M phosphate buffer of pH 7.5 consisted of four bands, 1, 2, and 3 having poor mobility and 4 having a good mobility (Figure 2). The fourth band appeared as a diffuse stained region and was characteristic of low molecular weight proteins. The changes observed in the pattern of the ethanol-treated meal proteins were (a) bands 3 and 4 were missing and (b) another band with a higher mobility was present, indicating degradation of the low molecular weight protein. This suggests that one of the high molecular weight proteins had either been removed or been dissociated to low molecular weight protein. With the autoclaved meal, band 3 was missing. This would suggest that the high molecular weight protein was either dissociated or insolubly denatured. With the 0.25 and 1.0 N HCl treated guar meals, the polyacrylamide gel electrophoretic patterns showed only a diffuse stained region halfway down the gel, characteristic of low molecular weight proteins.

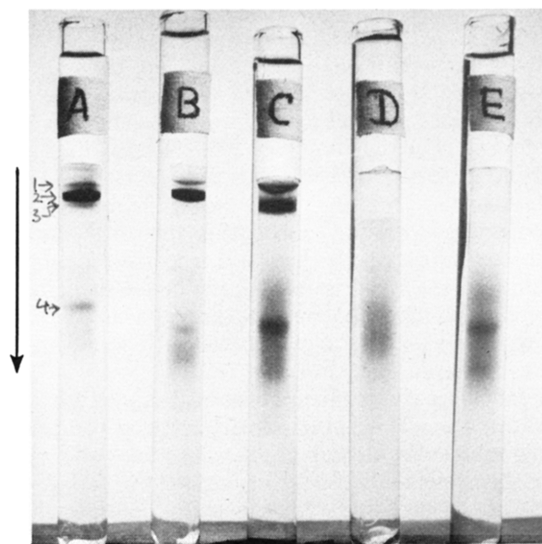

Figure 2. Polyacrylamide gel electrophoretic pattern of the treated and untreated guar meal proteins in 0.02 M phosphate buffer, pH 7.5; 7.5% gels. The arrow shows the direction of migration. (A) Untreated guar proteins. (B) Ethanol-treated guar proteins. (C) Autoclaved guar proteins. (D) 0.25 N HCl treated guar proteins. (E) 1.0 N HCl treated guar proteins.

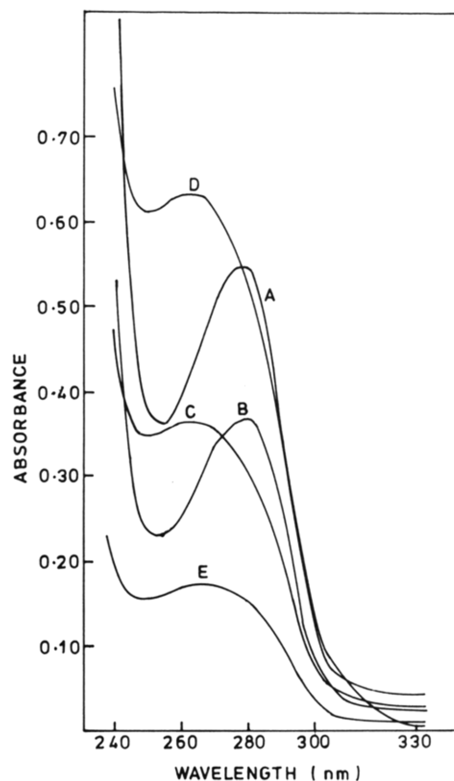

Figure 3. Ultraviolet absorption spectra of the untreated and treated guar meal proteins (in 1 M NaCl solution). (A) Untreated guar proteins. (B) Ethanol-treated guar proteins. (C) Autoclaved guar proteins. (D) 0.25 N HCl treated guar proteins. (E) 1.0 N HCl treated guar proteins.

Absorption Spectra. The untreated meal gave a typical protein spectrum with a maximum at 278–280 nm and a minimum at 255 nm (Figure 3). The spectrum of the ethanol-treated meal was similar to that of the untreated meal. The autoclaved meal gave a broad peak; the max-

imum had shifted to lower wavelengths (260–268 nm) and the minimum occurred at 250 nm. With the 0.25 and 1.0 N HCl treated meals also, the peaks were broad and the maximum had shifted to lower wavelengths. The 1.0 N HCl treated meal gave a broader peak than the 0.25 N HCl treated meal. The maxima were 260–268 and 262–272 nm, respectively. Srikanta and Narasinga Rao (1974) have reported that wet heating of groundnut proteins brought about changes in the UV absorption spectrum similar to those reported here. Heating and acid treatment of the guar proteins may lead to interaction of the proteins with other constituents of the meal, notably the carbohydrates, resulting in the formation of complexes. These complexes may have altered spectrum.

The following changes were observed due to the various pretreatments: (1) ethanol extraction removed the high and low molecular weight protein fractions and did not change the available lysine but decreased the carbohydrate content of the proteins; (2) autoclaving has possibly denatured the high molecular weight protein fractions and reduced the available lysine and carbohydrate content. It was of interest to compare these results with those of nutritional evaluation of such treated guar meals.

Raw unprocessed guar meal when fed to rats at 10% level of protein in the diet caused the death of animals (D'Souza, 1972; Subramanian and Shantha, 1968). Subramanian and Shantha (1968) have reported that defatted guar meal extracted with 70% ethanol gave a good growth response in experimental rats. We have observed that 70% ethanol extraction removed the soluble components in the meal and increased the protein content to 73.2% (Table I). Such processing techniques have the added advantage of eliminating the objectionable flavor of the raw meal. Since the available lysine content, which is a nutritional parameter, did not increase due to ethanol extraction, the good growth response may be due to increased intake of the diet and higher protein content. In the literature, there have been conflicting reports of the effect of heat treatment on the nutritive value and toxicity of guar meal. It has been suggested that even though trypsin inhibitor is present in guar meal (Borchers and Ackerson, 1950; Couch et al., 1966), some other toxic factors besides hemagglutinin and trypsin inhibitors could be responsible for growth inhibition in rats (Borchers and Ackerson, 1950; Tannus and Ullah, 1969; Kawatra et al., 1974). On the other hand, some groups of workers have suggested that when raw guar meal was autoclaved and fed to experimental rats, there was good growth response in the animals (Subramanian and Shantha, 1968; Ambegaokar et al., 1969). Our data show that there was no increase in the protein content in guar meal on autoclaving. On the other hand, available lysine content decreased, which would lower the nutritive value. The reported improvement in the nutrition quality of guar meal may be due to the inactivation of some toxic component. The 1 N HCl or 0.025 N HCl treatment of guar meal yielded a product free from toxicity and gave good growth performance in young rats (D'Souza, 1972; Kawatra et al., 1974; Khopkar, 1976). Khopkar (1976) has also reported that toxicity in guar meal is due to a chromoprotein present in the meal which is soluble in 0.25 N HCl. Experimental rats fed on a diet containing the chromoprotein showed severe toxic symptoms. There are no reports on the mechanism of the detoxification of the protein. It is possible that the toxic protein in the meal is degraded or modified, thereby becoming soluble in HCl, and then is leached out, leaving the meal free of toxicity. However, acid treatment of guar meal results in considerable loss of the material and protein, with a low recovery of the detoxified product.

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