Nutritional Quality of Aqueous Alcohol Extracted Guar (*Cyamopsis tetragonoloba*) Meals

Razia Tasneem* and N. Subramanian

Oilseeds Technology Discipline, Central Food Technological Research Institute, Mysore 570 013, India

Detoxification of guar meal was done by repeated extraction of the defatted meal with aqueous ethanol, methanol, or 2-propanol. The nutritive quality of the detoxified meals as compared to that of defatted meal was evaluated by both in vivo and in vitro techniques. The free polyphenol content of the detoxified meals was lower, while their trypsin inhibitor activity was higher than that of the defatted meal. Detoxification treatments resulted in a significant improvement in the in vitro digestibility of proteins. The amino acid composition and available lysine content of all the samples were comparable. The rat bioassays indicated that the nutritive value of the meal proteins improved significantly by the detoxification treatments. Weanling rats fed on diets containing detoxified meals gained 35–46 g of body weight in 4 weeks, while those fed the defatted meal diet lost weight. The values for protein efficiency ratio (PER), net protein ratio (NPR), and net protein utilization (NPU) of the detoxified guar meals were in the range 2.2–2.6, 3.8–4.4, and 55–59, respectively.

INTRODUCTION

Guar meal, the protein rich (~50%) byproduct of the guar gum industry, contains antinutritional factors such as trypsin inhibitors (Nath et al., 1978; Kaur and Bhatia, 1984; Kochar, 1985; Ramamani, 1986; Rajput et al., 1987), saponins (D'Souza, 1972; Subramanian and Parpia, 1975; Curl et al., 1986), hemagglutinins (D'Souza, 1972; Ramamani, 1986), polyphenols (Bajaj et al., 1978, Kaushal and Bhatia, 1982), and some unidentified objectionable flavor substances. Attempts have been made to eliminate or inactivate these factors and improve the nutritive value of the proteins. These involved preparation of protein isolate, heat treatment, solvent extraction, acid treatment, fermentation, etc. (Kawatra et al., 1969, 1974; D'Souza, 1972; Subramanian and Parpia, 1975; Khopkar and Rege, 1984; Misra et al., 1984). The results of an earlier investigation, wherein guar meal was detoxified by six different methods involving heat, dilute HCl leaching, or aqueous alcohol extractions revealed that the protein content in acid- or alcohol-washed samples increased by 8–15% and the proteins, especially from aqueous alcohol extracted meals, had excellent functional properties (Tasneem et al., 1982). Thus, guar meal has potential for use in food systems as a protein ingredient, provided it is suitably preprocessed. In the present investigation, guar meal was detoxified by extraction with 80% ethanol, 70% methanol, or 80% 2-propanol (Tasneem et al., 1982), and the samples have been evaluated for their nutritive quality by in vitro and in vivo methods.

MATERIALS AND METHODS

Guar seed (variety, Durgapur Safed) was procured from an established guar gum industry. Casein, hydrogenated fat, shark liver oil, sugar, and corn starch were purchased locally. Male albino rats of Wistar strain were purchased from the Animal House of this Institute. The sources of the chemicals were pepsin, trypsin, and N^{α}-benzoyl-L-arginine *p*-nitroanilide hydrochloride (BAPA) from Sigma Chemical Co. and methyl chloroformate and 1-fluoro-2,4-dinitrobenzene (FDNB) from E. Merck; the other chemicals were of analytical grade purchased from BDH, India, or Sarabhai M. Chemicals.

Detoxification of Guar Meal. Processing of guar seeds to defatted meal and the detoxification of meal by extraction with 80% ethanol, 70% methanol, or 80% 2-propanol were done according to methods reported earlier (Tasneem et al., 1982).

Polyphenol Estimation. Extraction of polyphenols was carried out by refluxing the meal with 80% ethanol, meal to solvent ratio being 1:100, for 2 h. The method of Swain and Hillis (1959) was used for the estimation of polyphenols in the extracts. A standard curve, prepared with gallic acid, was used for quantitation.

Trypsin Inhibitor Activity. The trypsin inhibitor activity was determined by the method of Kakade et al. (1969) with a synthetic substrate, BAPA. A trypsin standard was run in the absence of meal extract. Protein (N × 6.25) content of the meal extract was determined by the micro-Kjeldahl method and the activity was expressed as number of trypsin units inhibited (TUI) per milligram of protein.

Hemagglutinating Activity. The hemagglutinating activity of the samples was tested by the serial dilution method of Liener and Hill (1953) with 0.9% saline extract of the meal and 2% suspension of trypsinized (Lis and Sharon, 1972) rabbit erythrocytes.

Amino Acid Analysis. Amino acid analysis was performed with a LKB 4150 α-amino acid analyzer equipped with a programmer and an integrator, previously calibrated with a standard mixture of amino acids, by using acid hydrolysates of the meals that were prepared according to the method of Moore and Stein (1963). The hydrolysate containing about 3 μg of nitrogen was loaded, and analysis was performed. Nitrogen content of the hydrolysate was estimated by the micro-Kjeldahl method.

Tryptophan content of the various meals was estimated by the microbiological assay (Barton-Wright, 1952) with *Lactobacillus arabinosus* 17/5 and a standard curve for tryptophan in the range 0.2–2.0 μg.

Available Lysine Estimation. Available lysine content of the samples was estimated by the modified method of Carpenter and Ellinger (1955) with acid hydrolysates containing DNP-lysine. This method essentially involves an extra separation step with methoxycarbonyl chloride to obtain a blank value for non-lysine interfering color (Bruno and Carpenter, 1957).

Chemical Score. The chemical score of the processed guar meal proteins was calculated as suggested by FAO/WHO (1973).

In Vitro Digestibility. In vitro digestibility was determined according to the method of Villeges et al. (1968) with some modifications as detailed below. After peptic and pancreatic digestions, the digest was diluted with water and the undigested proteins and any intermediary products stable to enzyme hydrolysis were precipitated by adding an equal volume of 10% trichloroacetic acid solution (TCA) (Birk and Bondi, 1955). The dispersion was centrifuged, and nitrogen in

* Address correspondence to this author.

0021-8561/90/1438-1926$02.50/0

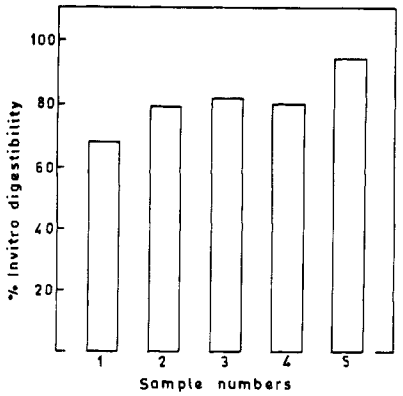

Figure 1. In vitro digestibility of processed guar meal proteins and casein. (1) Defatted guar meal; (2) 80% ethanol extracted guar meal; (3) 70% methanol extracted guar meal; (4) 80% 2-propanol extracted guar meal; (5) casein.

the supernatant was estimated by the micro-Kjeldahl method and corrected for the blank. The in vitro digestibility was calculated as a percent of the total nitrogen present in the TCA supernatant.

All the above determinations were made in triplicate and average values obtained.

Net Protein Ratio (NPR) and Protein Efficiency Ratio (PER). The method of Pellett and Young (1980) was used for the determination of NPR and PER of the various samples. The basal diet contained 10% hydrogenated fat, 10% sugar, 2% salt mixture, 2% vitamin mixture, 10% protein (derived from casein/guar samples), and the rest corn starch. Young weanling rats of Wistar strain, 21–23 days old, were allotted to the various groups of 10 rats each by the randomized block design. Each rat was housed in an individual cage, and the diet was mixed with water and 2 drops of shark liver oil to pasty consistency and given ad libitum. Water was provided separately. A weekly and 10-day record of food consumption and body weights of rats was maintained. The NPR and PER values were calculated after feeding for 10 and 28 days, respectively.

Net Protein Utilization (NPU). For NPU determination, 28-day-old rats (eight per group, allotted by randomized block design), maintained on a stock diet for 1 week, were fed the test diet for 10 days, and the body nitrogen, after the carcass was dried thoroughly at 110 °C, was determined by the Kjeldahl method. The NPU values were calculated from the body nitrogen content and nitrogen intake of rats (Pellett and Young, 1980).

RESULTS AND DISCUSSION

Antinutritional Factors. The polyphenol content, trypsin inhibitor activity, and hemagglutinating activity of the detoxified samples were compared with those of the defatted meal. Neither the defatted meal nor the aqueous alcohol extracted samples showed any hemagglutinating activity. Nath et al. (1978) also observed that neither the meal extract nor the protein fractions, resolved on Sepharose 6B-100, had any hemagglutinating activity. The detoxification treatments lowered the polyphenol content of the meal by 50–75% (Table I), and this is mainly due to the leaching out of polyphenols during the aqueous alcohol extractions. It was observed that 70% methanol and 80% ethanol were more efficient than 80% 2-propanol in removing the polyphenols from the meal. The trypsin inhibitor activity in the alcohol-extracted meals was higher than that in the defatted meal. In soybean (Gertler et al., 1967) and field bean (Ramamani et al., 1979), also, extraction with aqueous alcohol did not reduce their trypsin inhibitor activities. The higher activities of the detoxified guar meals could be explained on the basis of the molecular size of the inhibitor. The trypsin inhibitor of guar meal, being a macromolecule (Couch et al., 1966) would tend to concentrate in the extracted meals as a result

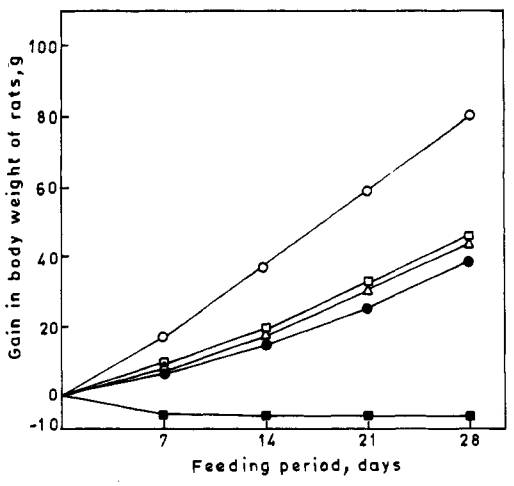

Figure 2. Growth curves of rats fed casein or processed guar meal diets. (○) Casein diet; (□) 70% methanol extracted guar meal diet; (△) 80% ethanol extracted guar meal diet; (●) 80% 2-propanol extracted guar meal diet; (■) defatted guar meal diet.

Table I. Free Polyphenol Content and Trypsin Inhibitor Activity of Processed Guar Meals

sample	free polyphenol, %	trypsin inhibitor activity, TUI[a]/mg of protein
defatted guar meal	0.36	7.6
80% ethanol extracted guar meal	0.11	9.0
70% methanol extracted guar meal	0.10	8.9
80% 2-propanol extracted guar meal	0.20	10.4

[a] TUI, trypsin units inhibited.

of leaching out of other low molecular weight and nonprotein nitrogenous substances such as small peptides and free amino acids.

Amino Acid Composition. The proteins of defatted and detoxified guar meals showed close similarity in their amino acid profiles (Table II). All the samples were rich in glutamic acid, aspartic acid, and arginine and deficient in methionine, cysteine, lysine, threonine, isoleucine, and valine. The aqueous ethanol and methanol extracted meals showed a slight increase in the content of most of the essential amino acids. Also, the detoxification treatments showed a slight increase in the availability of lysine, which could be due to the denaturing effect of alcohols on the proteins. Alcohols might alter the three-dimensional structure of proteins and increase lysine availability. The chemical scores of defatted and aqueous ethanol and methanol extracted meals were comparable, while that of 80% 2-propanol extracted meal was low. Sulfur amino acids were the most limiting in all samples.

In Vitro Digestibility. Detoxification treatments increased the in vitro digestibility of guar meal proteins from 68% to values ranging from 70% to 82% (Figure 1), although these values were low compared to that of casein. The improvement in the digestibility of legume proteins, in general, seems to parallel the destruction of trypsin inhibitors and hemagglutinins (Liener, 1962; Liener and Kakade, 1980). However, our results on the in vitro digestibility and trypsin inhibitor activities of processed guar meals did not show any correlation. There are also other instances wherein the destruction of trypsin inhibitor did not reflect the improvement in protein digestibility. Kakade et al. (1973) reported that the apparent protein digestibility of soybean was not affected by selectively removing the trypsin inhibitors from the bean. According

Table II. Amino Acid Composition (Grams/16 g of N) of Processed Guar Meals

amino acid	defatted guar meal	80% ethanol extracted guar meal	70% methanol extracted guar meal	80% 2-propanol extracted guar meal
aspartic acid	8.9	10.1	10.3	9.1
threonine	2.7	2.9	2.9	2.7
serine	4.4	4.8	4.6	4.3
glutamic acid	21.9	23.7	21.3	21.8
proline	3.5	2.9	2.6	3.4
glycine	4.0	4.2	4.1	4.3
alanine	3.1	2.6	2.6	2.8
cysteine	0.7	0.6	0.5	0.5
valine	3.3	3.2	3.3	3.0
methionine	0.9	1.0	1.2	0.9
isoleucine	2.7	2.8	2.9	2.5
leucine	5.4	5.6	5.5	5.1
tyrosine	3.2	3.1	2.8	2.7
phenylalanine	3.5	4.1	4.4	3.5
histidine	2.3	2.7	2.6	2.5
lysine	3.6	4.2	4.1	3.7
arginine	12.2	13.6	11.4	12.7
tryptophan	1.3	1.2	1.3	1.2
available lysine	3.0	3.2	3.4	3.2
chemical score	46	46	48	40
limiting amino acids	sulfur amino acids	sulfur amino acids	sulfur amino acids	sulfur amino acids

Table III. PER and NPR of Guar Meals and Casein

group	protein source	av protein intake, g		av wt gain, g		NPR (mean ± SE)	PER (mean ± SE)
		10 days	28 days	10 days	28 days		
A	defatted guar meal	1.1	6.6	−10.7	−5.5	negative value	negative value
B	80% ethanol extracted guar meal	4.2	17.3	11.9	43.4	4.4[a] ± 0.2	2.5[a] ± 0.1
C	70% methanol extracted guar meal	4.6	17.7	13.7	46.3	4.3[a] ± 0.2	2.6[a] ± 0.1
D	80% 2-propanol extracted guar meal	4.5	15.5	10.6	34.7	3.8 ± 0.2	2.2 ± 0.1
E	casein	5.4	21.4	24.5	80.9	5.7 ± 0.2	3.8 ± 0.1

[a] Values are not significantly different at 5% level (Harter, 1960).

Table IV. NPU of Guar Meals and Casein

group	protein source	av N intake, g	av carcass N, g	NPU (mean ± SE)
A	defatted guar meal	0.58	1.45	negative value
B	80% ethanol extracted guar meal	1.61	2.44	59[a] ± 3
C	70% methanol extracted guar meal	1.61	2.30	58[a] ± 3
D	80% 2-propanol extracted guar meal	1.54	2.36	55[a] ± 3
E	casein	1.78	2.75	71 ± 3
F		0.08	1.57	

[a] Values are not significantly different at 5% level (Harter, 1960).

to Ramamani et al. (1979) aqueous ethanol extraction of field beans eliminated the adverse effect on the digestibility of the proteins, although such extraction had very little effect on trypsin inhibitor and hemagglutinating activities.

Evaluation of Nutritive Value by in Vivo Methods. The growth curves of rats maintained on guar meals and casein (control) are shown in Figure 2. The biological parameters, PER, NPR and NPU of the samples, are given in Tables III and IV, respectively. Defatted guar meal did not support growth. Instead, the animals lost body weight as well as body nitrogen; one rat died during the fourth week of feeding. The loss in body weight and nitrogen resulted in negative values for the biological parameters. Growth retardation and significantly high mortality in young rats fed raw guar meal have been reported in several studies (Kakade et al., 1969; Kawatra et al., 1969; D'Souza, 1972; Misra et al., 1984). These could be attributed to low diet consumption and the presence of antinutritional factors in the meal. The consumption of defatted meal diet, 63.4 g in 4 weeks, in our study, was the lowest among the different groups and was only about 32% compared to casein diet intake. The beany odor and bitter taste together with the sticky texture might be responsible for the low diet consumption.

Extraction of the meal with aqueous alcohols resulted in significant improvement of its nutritive value as indicated by rat bioassays. The results indicated that the treated meals promoted growth of rats, the gain in body weight being 39–44 g in 4 weeks. Among the three detoxified samples, the aqueous methanol and ethanol extracted meals showed comparable growth response of rats, and PER and NPR and were better than 2-propanol-extracted meal; the NPU values of these three detoxified meals were, however, comparable.

Although the nutritive value of guar meal was significantly improved by the aqueous alcohol extractions, the treated meals were inferior to casein as indicated by rat bioassays. The lower proportion of total essential amino acids as well as the deficiency of selected essential amino acids, e.g., sulfur amino acids, in guar proteins as in other legumes (FAO, 1970) might be responsible for this difference. Also, any residual antinutritional factors present might affect the biological utilization of proteins.

In summary, the results of this investigation indicate that the aqueous alcohol extracted guar meals, unlike the defatted meal, promoted satisfactory growth of weanling rats. The nutritive value of the detoxified meals, as indicated by growth response of rats and biological parameters, was, however, inferior to casein. The lower proportion of total essential amino acids and the deficiency

of selected essential amino acids in guar proteins compared to casein together with the residual antinutritional factors might be responsible for this difference. In the case of defatted guar meal, the very low consumption of diet together with the antinutritional factors might be responsible for the growth-retarding effects. The beany odor, bitter taste, and sticky texture of the defatted meal might be responsible for the low diet consumption. With the detoxified samples the consumption of diet was appreciable, and this contributed to satisfactory growth response of rats. Evaluation of nutritive quality by in vitro techniques such as in vitro digestibility, amino acid analysis and available lysine did not, however, reveal any significant difference among guar samples.

ACKNOWLEDGMENT

We are thankful to Mrs. Indira A. S. Murthy for her help in the statistical analysis of the biological parameters and Dr. M. C. S. Sastry for carrying out amino acid analysis of the samples.

LITERATURE CITED

Bajaj, K. L.; Madhu Rani; Arora, K. J. *Plant Biochem. J.* **1978**, *5*, 140.

Barton-Wright, E. C. In *The Microbiological assay of the vitamin B complex and amino acids*; Sir Issac Pitman and Sons: London, 1952; p 99.

Birk, Y.; Bondi, A. *J. Sci. Food Agric.* **1955**, *6*, 549.

Bruno, D.; Carpenter, K. J. *Biochem. J.* **1957**, *67*, 130.

Carpenter, K. J.; Ellinger, G. M. *Biochem. J.* **1955**, *61*, XI.

Couch, J. R.; Creger, C. R.; Bakshi, Y. J. *Proc. Soc. Expt. Biol. Med.* **1966**, *123*, 263.

Curl, C. L.; Price, K. R.; Fenwick, G. R. *Phytochemistry* **1986**, *25*, 2675.

D'Souza, O. Chemistry and technology of seed proteins. Ph.D. Thesis, 1972, University of Bombay, Bombay, India.

FAO. *Amino Acid contents of Foods and Biological Data on Proteins*; FAO Nutritional Studies 24; Food and Agriculture Organization of the United Nations: Rome, 1970.

FAO/WHO. *Energy and Protein requirements*, Report of a Joint FAO/WHO Ad-Hoc Expert Committee; FAO Nutrition Meetings Report Series 52, WHO Technical Report Series 522; Food and Agriculture Organization of the United Nations: Rome, 1973.

Gertler, A.; Birk, Y.; Bondi, A. *J. Nutr.* **1967**, *91*, 358.

Harter, H. L. *Biometrics* **1960**, *16*, 671.

Kakade, M. L.; Simons, N. R.; Liener, I. E. *Cereal Chem.* **1969**, *46*, 518.

Kakade, M. L.; Hoffa, D. E.; Liener, I. E. *J. Nutr.* **1973**, *103*, 1772.

Kaur, P.; Bhatia, I. S. *J. Sci. Food Agric.* **1984**, *35*, 987.

Kaushal, G. P.; Bhatia, I. S. *J. Sci. Food Agric.* **1982**, *33*, 461.

Kawatra, B. L.; Garcha, J. S.; Wagle, D. S. *J. Nutr. Diet.* **1969**, *6*, 91.

Kawatra, B. L.; Garcha, J. S.; Kaur, C.; Singh, R. *J. Food Sci. Technol.* **1974**, *11*, 263.

Khopkar, P. P.; Rege, D. V. *Qual. Plant.—Plant Foods Hum. Nutr.* **1984**, *34*, 135.

Kochar, G. K. *Indian J. Anim. Res.* **1985**, *19*, 45.

Liener, I. E. *Am. J. Clin. Nutr.* **1962**, *11*, 281.

Liener, I. E.; Hill, E. G. *J. Nutr.* **1953**, *49*, 609.

Liener, I. E.; Kakade, M. L. In *Toxic constituents of plant foodstuffs*, 2nd ed.; Liener, I. E., Ed.; Academic Press: New York, 1980; p 7.

Lis, H.; Sharon, N. *Methods Enzymol.* **1972**, *28B*, 360.

Misra, B. K.; Singh, S. P.; Varade, P. B.; Singh, R.; Sikka, K. C. *J. Agric. Food Chem.* **1984**, *32*, 1075.

Moore, S.; Stein, W. H. *Methods Enzymol.* **1963**, *6*, 819.

Nath, J. P.; Subramanian, N.; Narasinga Rao, M. S. *J. Agric. Food Chem.* **1978**, *26*, 1243.

Pellett, P. L.; Young, V. R. In *Nutritional evaluation of protein foods*; The United Nations University: Tokyo, 1980; p 103.

Rajput, L. P.; Narasimha Murthy, K.; Ramamani, S. *J. Food Sci.* **1987**, *52*, 1755.

Ramamani, S. *J. Food Sci. Technol.* **1986**, *23*, 339.

Ramamani, S.; Subramanian, N.; Parpia, H. A. B. *J. Biosci.* **1979**, *1*, 241.

Subramanian, N.; Parpia, H. A. B. In *PAG-Compendium*: World Mark Press: New York, 1975; Vol. D, p 215.

Swain, T.; Hillis, W. E. *J. Sci. Food Agric.* **1959**, *10*, 63.

Tasneem, R.; Ramamani, S.; Subramanian, N. *J. Food Sci.* **1982**, *47*, 1323.

Villegas, A. M.; Gonzaler, A.; Calderon, R. *Cereal Chem.* **1968**, *45*, 379.

Received for review July 25, 1989. Revised manuscript received April 2, 1990. Accepted April 19, 1990.

Registry No. Trypsin inhibitor, 9035-81-8; lysine, 56-87-1.