EFFECT OF SOAKING AND GERMINATION ON NUTRIENT AND ANTINUTRIENT CONTENTS OF FENUGREEK (*TRIGONELLA FOENUM GRAECUM* L.)

SHALINI HOODA and SUDESH JOOD[1]

Department of Foods and Nutrition
CCS Haryana Agricultural University
Hisar-125004, India

Received for Publication July 1, 2002
Accepted for Publication October 7, 2002

ABSTRACT

Fenugreek seeds (raw, soaked and germinated) were analyzed for their chemical composition. Raw fenugreek seeds contained higher amount of dietary fiber 46.50% followed by 42.12% in soaked seeds and 32.50% in germinated seeds. Soaking reduced the level of total soluble sugars, reducing sugars, nonreducing sugars, dietary fiber and improved the protein and starch digestibility and availability of minerals. Germinated fenugreek seeds had significantly higher contents of total protein (29%) and total lysine (6.48 g/ 100 g protein) compared to unprocessed seeds. Germination decreased dietary fiber and starch thereby raising the level of sugars. In vitro starch and protein digestibility and availability of Ca, Fe and Zn were also increased appreciably due to reduction in antinutrient contents (phytic acid and polyphenols) after 48 h germination.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is undoubtedly one of the oldest cultivated medicinal plants. It is an erect annual herb native to Southern Europe and Asia, belonging to the family Leguminosae. Over 80% of the total world's production of this seed is contributed by India, one of the major producers and exporters of fenugreek legume in the world.

The seeds are known to have hypoglycemic (Neeraja and Rajyalakshmi 1996; Shashi Kala 1997) and hypocholesterolemic (Khosla *et al.* 1995) properties. Fenugreek seeds can be a good supplement to cereals because of its

[1] Corresponding Author: Department of Foods and Nutrition, CCS Haryana Agricultural University, Hisar-125 004, India. FAX: 0091 1662 34952, EMAIL: ramjood@rediffmail.com

Journal of Food Biochemistry 27 (2003) 165–176.

high protein (25%), lysine (5.7 g/16 g N), soluble (20%) and insoluble (28%) dietary fiber besides being rich in calcium, iron and beta-carotene (NIN 1987). Various traditional recipes of North India namely *Laddo, Methi, Suhali*, etc. prepared from wheat-fenugreek blends, mainly consumed by the diabetic and hypercholesterolemic people (Sharma 1986; Shashi Kala 1997).

However, the seeds are bitter in taste due to presence of bitter saponins, which limit their acceptability in foods (Sharma 1986; Udayasekhera and Sharma 1987). It has been possible to debitter fenugreek seeds by employing various processing methods such as soaking, germination, roasting, etc. (Sharma 1986; Shashi Kala 1997). As fenugreek seeds are rich in mucilaginous fiber and other dietary essentials, their use can be exploited as functional and nutritional foods as well as therapeutic agent. By keeping these facts in view various value-added baked and extruded products from wheat-fenugreek flour blends have been developed. Organoleptic (taste, flavor, color, texture, etc.) and nutritional characteristics have also been studied (Hooda 2002). This paper reports the effect of processing methods include soaking and germination on nutrients, *in vitro* digestibility, *in vitro* availability and antinutrient contents of fenugreek seeds.

MATERIALS AND METHODS

Materials

The fenugreek seeds of Pusa early bunching variety were obtained from the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar, India.

Processing

Soaking. Fenugreek seeds were first cleaned and freed from broken seeds, dust and other foreign materials and then soaked in tap water for 12 h at 37C. A seed to water ratio of 1:5 (w/v) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice in distilled water and then dried at 55-60C.

Germination. The soaked seeds were germinated in sterile petri dishes lined with wet filter paper for 48 h at 37C with frequent watering. The sprouts were rinsed in distilled water and dried at 55-60C. The dried samples of raw, soaked and germinated seeds were ground to fine powder in an electric grinder and then stored in plastic containers for further use.

Chemical Analysis

Proximate Composition and Total Lysine. The treated and untreated samples were analyzed for moisture, protein, fat, ash and crude fiber by adopting standard methods (AOAC 1995). Total carbohydrate was calculated by subtraction from 100. Total lysine was estimated as per the method described by Miyahara and Jikoo (1967).

Sugars, Dietary Fiber and Starch Digestibility. Total soluble sugars were extracted by refluxing in 80% ethanol (Cerning and Guilbot 1973). Quantitative determination of total soluble sugars was carried out according to the colorimetric method (Yemm and Willis 1954). Reducing sugars were estimated by Somogyi's modified method (Somogyi 1945). Nonreducing sugars were determined by calculating the difference between total soluble sugars and reducing sugars. Total, soluble and insoluble dietary fiber constituents were determined by the enzymatic method given by Furda (1981). *In vitro* starch digestibility was assessed by employing pancreatic amylase and incubating at 37C for 2 h. Liberated maltose was measured colorimetrically by using dinitrosalicylic acid reagent (Singh *et al.* 1982).

Total and Available Minerals. Calcium, iron and zinc in acid digested samples were determined by Atomic Absorption Spectrophotometer 2380, Perkin Elmer (Shelton, Conn.) according to the method of Lindsey and Norwell (1969). Available calcium and zinc were extracted by the method of Kim and Zemel (1986). Ionizable iron in the samples was extracted according to the procedure of Rao and Prabhavathi (1978). Ionizable iron was determined as described by AOAC (1995).

Protein Digestibility. Protein digestibility *(in vitro)* was assessed by employing pepsin and pancreatin (Akeson and Stahmann 1964). The nitrogen contents of the sample and the undigested residue were determined by the microkjeldahl method (AOAC 1995). The digested protein of the sample was calculated by subtracting residual protein from total protein of the sample.

$$\text{Protein digestibility (\%)} = \frac{\text{Digested protein}}{\text{Total protein}} \times 100$$

Antinutritional Factors. Phytic acid was determined by the method of Haug and Lantzsch (1983). Total polyphenols were extracted by the method of Singh and Jambunathan (1981), and estimated as tannic acid equivalent according to Folin-Denis procedure (Swain and Hills 1959).

RESULTS AND DISCUSSION

Moisture content of unprocessed (raw) fenugreek flour increased from 13.70 to 14.20%, respectively on dry weight basis (Table 1). There was significant ($P<0.05$) increase in protein (25.90 to 29.00%) in germinated fenugreek flour. This might be due to reduction of seed nitrates into plant protein or ammonium compounds during germination (Youngs and Varner 1959), crude fiber (6.88 to 9.42%) and ash (2.88 to 3.15%) contents, respectively in raw, soaked and germinated fenugreek flour. However, crude fat decreased by about 22% of the original amount on germination. These changes agree with those mentioned by El-Mahdy and EL-Sebaiy (1983) for germinated fenugreek seeds. Carbohydrate contents of fenugreek flour ranged from 43.74 to 38.83%, respectively in raw, soaked and germinated fenugreek seed flour. Fenugreek seeds are very rich source of lysine, which are comparable with the egg protein (FAO 1973) and most commonly used legumes (Gopalan *et al.* 1978). Lysine content significantly ($P<0.05$) increased on germination as a result of bioconvertion during germination. Other research workers also demonstrated that germination increased the lysine, methionine and tryptophane content of legumes (Wang 1977; Ahmed and Fields 1979).

Total (4.01%), reducing (0.35%) and nonreducing sugar (3.76%) contents were found in raw fenugreek flour (Table 2). All the sugar contents decreased on soaking for 12 h. Losses of sugars during soaking could be from simple diffusion of sugars after being solubilized. Soaking has been known to reduce the level of sugars in various pulses (Jood *et al.* 1988, 1998) whereas, germination caused significant increase in the content of sugars for 48 h. The increase in sugar content of soaked seeds during germination may be because of mobilization and hydrolysis of seed polysaccharides, leading to more available sugars. A similar trend in sugar content of fenugreek and other legume seeds during germination has been observed earlier (El-Mahdy and EL-Sebaiy 1983; Jood *et al.* 1998).

The total, soluble and insoluble fiber contents of raw fenugreek were higher compared to soaked and germinated seeds (Table 2). In case of all fiber contents, significant variations were found among raw, soaked and germinated seed flour. Raw fenugreek seed flour contained 18.80% soluble fiber, which decreased on germination by about 47%. This decrease is accompanied by a drop in galactan content. The breakdown of mucilage during germination was previously reported by El-Mahdy and EL-Sebaiy (1983), Neeraja and Rajyalakshmi (1996). An enzyme α-galactosidase from germinated fenugreek seeds partially attacks galactomannan to yield galactose. The decrease in the polysaccharide and mucilage content may be attributed to their breakdown and utilization by the growing sprouts.

TABLE 1.
PROXIMATE COMPOSITION AND LYSINE CONTENT OF FENUGREEK FLOUR (ON DRY MATTER BASIS)[1]

Fenugreek	Moisture (g/100 g)	Fat (g/100 g)	Protein (g/100 g)	Crude Fiber (g/100 g)	Ash (g/100 g)	Carbohydrates (g/100 g)	Total Lysine (g/100 g protein)
Raw	13.70 ± 1.01	6.90 ± 0.46	25.90 ±1.26	6.88 ± 0.78	2.88 ± 0.06	43.74 ± 0.95	5.86 ± 0.98
Soaked	13.96 ± 1.03	6.45 ± 0.26	26.00 ± 1.05	6.95 ± 0.42	2.92 ± 0.07	43.74 ± 0.72	5.92 ± 0.62
Germinated	14.20 ± 1.12	5.40 ± 0.26	29.00 ± 1.35	9.42 ± 0.32	3.15 ± 0.08	38.83 ± 0.96	6.48 ± 0.42
CD ($P<0.05$)	0.92	0.78	1.20	0.85	0.26	1.13	0.89

[1]Values are means ± SD of three independent determinations
CD = Critical differences at 5% level of significance. Differences of two means between treatments exceeding this value are significant.

TABLE 2.
SUGAR AND DIETARY FIBER CONTENTS OF FENUGREEK FLOUR (%, ON DRY MATTER BASIS)[a]

Fenugreek	Sugars			Dietary fiber		
	Total	Reducing	Non-reducing	Total	Soluble	Insoluble
Raw	4.01 ± 0.42	0.35 ± 0.01	3.76 ± 0.45	46.50 ± 1.26	18.80 ± 0.89	29.70 ± 1.02
Soaked	3.67 ± 0.39	0.25 ± 0.05	3.42 ± 0.39	42.12 ± 1.39	16.00 ± 1.00	26.12 ± 0.98
Germinated	12.15 ± 0.98	3.09 ± 0.15	8.06 ± 0.72	32.50 ± 1.02	10.00 ± 0.79	22.50 ± 0.15
CD ($P<0.05$)	1.20	0.15	0.32	1.23	1.02	1.89

[a]Values are means ± SD of three independent determinations
CD = Critical differences at 5% level of significance. Differences of two means between treatments exceeding this value are significant.

Raw fenugreek seed flour contained higher total mineral content such as 72.50% calcium, 12.60% iron and 6.95% zinc contents (Table 3). Soaking and germination did not show any significant change in total minerals. Comparatively lower contents of minerals when soaked in water might be due to leaching out of some minerals into the soaking water (Nolan and Duffin 1987; Jood and Kapoor 1997). However, soaking improved the availability of all the minerals. It ranged from 48.50 to 69.20%, 39.62 to 62.49% and 49.17 to 70.20% for calcium, iron and zinc, respectively in raw, soaked and germinated fenugreek. However, phytic acid in plant foods forms complexes with essential dietary minerals such as Ca, Fe, Zn and Mg makes them biologically unavailable for absorption. The phytase activity increased on germination causing catabolism of phytic acid. Phytases, or myo-inositol hexaphosphate phosphohydrolases, are enzymes that hydrolyze myo-inositol 1,2,3,4,5,6,-hexakis (dihydrogen phosphate) to myo-inositol and inorganic phosphate and thereby increasing the *in vitro* availability of divalent minerals (Srivastava 1994; Jood and Kapoor 1997).

Raw fenugreek seed flour contained higher amount of phytic acid (588.20 mg/100 g) and lower amount of polyphenols (148.26 mg/100 g) than treated samples (Table 4). In the case of fenugreek seed, phytic acid and polyphenol contents significantly ($P<0.05$) decreased on soaking and germination, which ultimately caused significant increase in protein and starch digestibility. *In vitro* starch and protein digestibility of raw, soaked and germinated fenugreek seed flour ranged from 42.50 to 53.69 mg maltose released/g meal and 58.50 to 65.60%, respectively (Table 4). Germination may mobilize starch, seed proteins are metabolized and antimetabolites are catabolized, thereby resulting in improved digestibility of starch and protein of pulses. Sprouting causes mobilization of protein with the help of protease leading to the formation of peptides, oligopeptides and free amino acid (Kataria 1986; Jood *et al.* 1988). However, protein digestibility of fenugreek is lower compared to other pulses, possibly due to gum in the seeds (El-Mahdy and EL-Sebaiy 1983).

CONCLUSION

It may be inferred from the present study that nutritional quality of fenugreek seeds can be improved through processing methods and bitterness can also be reduced to some extent. Therefore, the use of processed fenugreek flour can be exploited in functional and nutritional foods as well as a therapeutic agent in various bakery products.

TABLE 3.
TOTAL AND PERCENT AVAILABILITY OF MINERALS OF FENUGREEK FLOUR (ON DRY MATTER BASIS)[1]

Fenugreek	Calcium		Iron		Zinc	
	Total (mg/100 g)	Available (%)	Total (mg/100 g)	Available (%)	Total (mg/100 g)	Available (%)
Raw	72.50 ± 1.20	48.50 ± 1.00	12.60 ± 1.00	39.62 ± 0.43	6.95 ± 0.29	49.17 ± 0.39
Soaked	70.60 ± 1.33	51.25 ± 1.11	11.00 ± 0.49	42.15 ± 1.02	6.80 ± 0.38	55.19 ± 1.20
Germinated	71.22 ± 1.42	69.20 ± 1.23	11.20 ± 0.89	62.49 ± 0.98	6.85 ± 0.42	70.20 ± 1.31
CD ($P<0.05$)	1.25	1.01	0.79	1.00	0.25	1.12

[1]Values are means ± SD of three independent determinations.
CD = Critical differences at 5% level of significance. Differences of two means between treatments exceeding this value are significant.

TABLE 4.
PHYTIC ACID, POLYPHENOLS AND *IN VITRO* STARCH AND PROTEIN DIGESTIBILITY OF FENUGREEK FLOUR (ON DRY MATTER BASIS)[1]

Fenugreek	Phytic acid (mg/100 g)	Polyphenols (mg/100 g)	*In vitro* starch digestibility (mg maltose released/g meal)	*In vitro* protein digestibility (%)
Raw	588.20 ± 4.37	148.26 ± 4.32	42.50 ± 1.32	58.50 ± 1.10
Soaked	535.10 ± 5.20	130.15 ± 3.90	46.49 ± 1.25	60.75 ± 2.00
Germinated	340.30 ± 5.34	84.49 ± 4.29	53.69 ± 1.49	65.50 ± 1.62
CD ($P<0.05$)	6.42	4.23	1.56	1.42

[1]Values are means ± SD of three independent determinations.
CD = Critical differences at 5% level of significance. Differences of two means between treatments exceeding this value are significant.

REFERENCES

AKESON, W.E. and STAHMANN, M.A. 1964. A pepsin-pancreatin digestibility index of protein quality evaluation. J. Nutr. *83*, 257–259.

AHMED, M.H. and FIELDS, M.L. 1979. Evaluation of the protein quality and available lysine of germinated and fermented cereals. J. Food Sci. *44*, 456–458.

AOAC. 1995. Official methods of analysis. Association of Official Analytical Chemists, Washington, DC.

CERNING, J. and GUILBOT, J. 1973. Changes in carbohydrate composition during maturation of wheat and barley kernel. Cereal Chem. *50*, 220–222.

EL-MAHDY, R. A. and EL-SEBAIY, L.A. 1983. Changes in carbohydrates of germinating fenugreek seeds (*Trigonella faenum* graecum L.). J. Sci. Food Agric. *34*, 951–956.

FAO. 1973. Energy and protein requirements. WHO Technical Report, Serial No. 522.

FURDA, I. 1981. Simultaneous analysis of soluble and insoluble dietary fibre. In *The Analysis of Dietary Fibre in Food*, (W.P.T. James and O. Theander, eds.) pp. 163–172, Marcel Dekker, New York.

GOPALAN, C., RAMASASTRY, B.V. and BALASUBRAMANYAN, S.C. 1978. *Nutritive Value of Indian Foods*. NIN, ICMR, Hyderabad, India.

HAUG, W. and LANTZSCH, H.J. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. *34*, 1423–1426.

HOODA, S. 2002. Nutritional evaluation of fenugreek supplemented wheat products. M.Sc. Thesis. Haryana Agricultural University, Hisar, India.

JOOD, S., BISHNOI, S. and SHARMA, A. 1998. Chemical analysis and physicochemical properties of chickpea and lentil cultivars. Nahrung *42*, 71–74.

JOOD, S., CHAUHAN, B.M. and KAPOOR, A.C. 1988. Contents and digestibility of carbohydrates of chickpea and blackgram as affected by domestic processing and cooking. Food Chem. *34*, 417–420.

JOOD, S. and KAPOOR, A.C. 1997. Improvement in chickpea and blackgram through processing and cooking techniques. Intern. J. Food Sci. Technol. *48*, 307–312.

KATARIA, A. 1986. Studies on nutrient composition and antinutritional factors of amphidiploids (green gram × black gram). M.Sc. Thesis. Haryana Agricultural University, Hisar, India.

KHOSLA, P., GUPTA, D.D. and NAGPAL, R.K. 1995. Effect of *Trigeonella faenum* Graecum (fenugreek) on serum lipids in normal and diabetic rats. Ind. J. Pharm. *27*, 89–93.

KIM, H. and ZEMEL, M.R. 1986. *In vitro* estimation of the potential bioavailability of calcium from sea mustard, milk and spinach under stimulated normal and reduced gastric acid conditions. J. Food. Sci. *51*, 957–963.

LINDSEY, W.L. and NORWELL, M.A. 1969. A new DPTA-TEA soil test for zinc and iron. Agron. Abstract *61*, 84–92.

MIYAHARA, S. and JIKOO, K. 1967. Examination of the method of calorimetric determination of lysine. J. Jap. Soc. Food. Sci. Technol. *14*, 512–513.

NEERAJA, A. and RAJYALAKSHMI, P. 1996. Hypoglycemic effect of processed fenugreek seeds in humans. J. Food. Sci. Technol. *33*, 427–430.

NIN Report. 1987. Use of fenugreek seed powder in the management of non insulin dependent diabetes mellitus. NIN, ICMR, Hyderabad, India.

NOLAN, K.B. and DUFFIN, P.A. 1987. Effect of phytate on mineral availability. *In vitro* studies of Mg, Ca, Fe, Cu and Zn solubilities in the presence of phytate. J. Sci. Food. Agric. *40*, 70–83.

RAO, B.S.N. and PRABHAVATHI, T. 1978. An *in vitro* method for predicting the bioavailability of iron from foods. Am. J. Clin. Nutr. *31*, 169–174.

SHARMA, R.D. 1986. Effects of fenugrek seeds and leaves on blood glucose and serum insulin response in human subjects. Nutr. Res. *6*, 1353–1363.

SHASHI KALA. 1997. Management of non insulin dependent diabetes mellitus by using traditional medicinal plant products. Ph.D. Thesis. Haryana Agricultural University, Hisar, India.

SINGH, U. and JAMBUNATHAN, R. 1981. Studies on desi and kabuli chickpea (*Cicer arietinum*) cultivars. Level of protease inhibitors, levels of polyphenolic compounds and *in vitro* protein digestibility. J. Food Sci. *46*, 1364–1367.

SINGH, U., KHEDEKAR, M.S. and JAMBUNATHAN, R. 1982. Studies on desi and kabuli chickpea cultivars. The level of amylase inhibitors, levels of oligosaccharides and *in vitro* starch digestibility. J. Food Sci. *47*, 510–516.

SOMOGYI, M. 1945. A new reagent for the determination of sugars. J. Biol. Chem. *160*, 61–62.

SRIVASTAVA, S. 1994. Study on the effects of processing on nutrient and non nutrient composition of *Lathyrus sativus*. M.Sc. Thesis. Haryana Agricultural University, Hisar, India.

SWAIN, J. and HILLS, W.E. 1959. The phenolic constituents *Pramus domestical*. The qualitative analysis of phenolic constituents. J. Sci. Food Agric. *10*, 63–68.

UDAYASEKHERA, P. and SHARMA, R.D. 1987. An evaluation of protein quality of fenugreek seeds and their supplementary effect. Food Chem. *24*, 1–5.

WANG, D. 1977. Enrichment of ingredients for fabricated foods by fermentation and germination of corn and sorghum. Ph.D. Thesis, University of Missouri-Columbia, MO.

YEMM, E.W. and WILLIS, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. *54*, 508–509.

YOUNGS, J.L. and VARNER, J.E. 1959. Enzyme synthesis in the cotyledons of germinating seeds. Arch. Biochem. Biophys. *84*, 71–74.