Bull. Fac.Agric.,Cairo Univ., 61: 263-273(2010).

EFFECT OF SOAKING AND GERMINATION OF FENUGREEK (*Trigonella foenum-graecum* L.) SEEDS: CHANGES IN UPON CHEMICAL AND AMINO ACID COMPOSITION, ANTINUTRIENTS, PROTEIN DIGESTIBILITY AND MINERALS EXTRACTABILITY

(Received:12.4.2010)

By
A. A. S. Alhussain

King Saud University, Faculty of Food and Agriculture Science, Department of Food Science and Nutrition, Riyadh, Saudi Arabia

ABSTRACT

The chemical composition, antinutritional factors, protein digestibility, total and extractable minerals and amino acids composition of Fenugreek seeds (raw, soaked and germinated) from two different origins (Saudi and Yemeni cultivars) were determined. Raw fenugreek seeds of the two cultivars contained high amount of dietary nutrients especially protein. Soaking of fenugreek seeds had no effect on the chemical composition of both cultivars. However, germination significantly ($P \leq 0.05$) increased the total protein and fiber, decreased the dry matter and carbohydrates, while ash and oil remained unchanged. The total energy of the seeds significantly ($P \leq 0.05$) decreased after soaking and after germination of both cultivars. Soaking seeds significantly ($P \leq 0.05$) showed lower level of antinutrional factors of both cultivars compared to germination. *In vitro* protein digestibility and extractability of minerals were also increased appreciably due to a reduction in antinutrient contents. Raw seeds of both cultivars were rich in glutamic and aspartic acids. Soaking of the seeds had a slight effect on some amino acids but the germination of the seeds for 2 days significantly ($P \leq 0.05$) increased the amino acid composition of both cultivars.

Key words: *amino acids, antinutrients, fenugreek, germination, minerals, soaking,*

1. INTRODUCTION

Legume seeds are important sources of energy and protein in many parts of the world for animal and human nutrition. However, their nutritional value may be limited in part by the presence of undesirable components known as antinutritional factors. These factors include protease inhibitors, lectins, phenolic compounds, phytates and indigestible carbohydrates of the rafinose family (Flammang *et al*., 2004). The content of these components may vary for different legumes, and this variation may be reflected on the efficiency of nutrient utilization. Fenugreek *(Trigonella foenum-graecum* L.*)* is one of the oldest cultivated medicinal plants. It has been historically utilized mainly as whole seed as a potential protein source with high nutritive value. Fenugreek is an annual herb belonging to the legume family; it is widely grown in India, Egypt, and Middle Eastern countries (Flammang *et al*., 2004). The seeds are known to have hypoglycaemic effect (Neeraja and Rajyalakshmi 1996) and hypocholesterolemic properties (Khosla *et al.*, 1995). Fenugreek seeds can be a good supplement to cereals because of its high protein, lysine, soluble and insoluble dietary fiber besides being rich in calcium, iron and beta-carotene (Hooda and Jood, 2005).

Various traditional recipes prepared from wheat-fenugreek blends, are mainly consumed by the diabetic and hypercholesterolemic people (Khosla *et al.*, 1995). However, the seeds are bitter in taste due to the presence of bitter saponins, which limit their acceptability in foods. It has been possible to debitter fenugreek seeds by employing various processing methods such as soaking, germination, and roasting. 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 (Khosla *et al.*, 1995). Baked and extruded products from wheat-fenugreek flour blends have been developed with improved characteristics organoleptic (taste, flavor, color, texture, etc.) and nutritional (Hooda and Jood, 2005). Salini and Sudesh (2004) reported that the addition of 10% of fenugreek flour to wheat flour

increased protein content, fibre, total calcium and total iron. Thus fenugreek can be incorporated to prepare acceptable biscuits, and may also be mixed with cereals as a supplement for some limiting amino acids to improve their protein quality.

This investigation aimed to study the effects of processing methods (soaking and germination) on chemical composition, antinutritional factors and *in vitro* protein digestibility of fenugreek seeds from two different origins (Saudi and Yemeni cultivars).

2. MATERIALS AND METHODS

2.1. Materials

The fenugreek (*Trigonella foenum-graecum* L.) seeds of Saudi and Yemeni cultivars were obtained from the local market at Riyadh, Saudi Arabia. All chemicals used in this study were of analytical grade.

2.2. Sample preparation

The seeds of the cultivars were manually cleaned. The cleaned seeds were steeped in thrice the quantity of water for 12 h at 37 ^{o}C with 1 h air rest after 6 h of steeping. For each air rest, the steeping water was changed. After steeping, the seeds were rinsed twice in distilled water and then dried at 55-60 ^{o}C or sterilized by soaking in 1% sodium hypochlorite for 20 min. before it was drained prior to germination. The steeped seeds were spread on wet jute bags and covered with a moist cotton cloth and left to sprout in the dark at room temperature (25 ± 3 ^{o}C) for 1, 2 and 3 days as described by Obizoba and Atii (1994). After germination, the seeds were dried in a Gallenkamp oven (BS model OV-160; Manchester, UK) at 50 ^{o}C for 24 h. Rootlets and shoots of the seeds were separated from the kernels by rubbing the seeds in a sieve (Endecotts Ltd, London, UK) of 0.6 mm mesh size. The soaked and sprouted seeds were separately milled into fine flour with a hammer mill (Gibbons Electric, Essex, UK) to pass through a 0.4 mm mesh size screen and stored at 4 ^{o}C before analysis.

2.3.Proximate composition determination

The (soaked and germinated)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 difference.

2.4.Total energy (calorific value) determination

Energy was calculated as described by Osborne and Voogt (1978) using the Atwater factors; 1g of carbohydrates (C.) provides (4Kcalories), 1g of protein (P.) provides (4Kcalories) and 1g fat (f.) provides (9Kcalories).

2.5. Total mineral determination

Minerals were determined according to dry-ashing method described by Chapman and Pratt (1961). The amounts of iron, zinc, manganese and copper were determined using atomic absorption spectroscopy (Perkin–Elmer 2380). Ammonium vanadate was used to determine phosphorus, by the ammonium molybdate method of Chapman and Pratt (1982). Calcium and magnesium were determined by a titration method described by Chapman and Pratt (1961). Sodium was determined by flame photometer (CORNIG EEL), according to AOAC (1995) method.

2.6. HCl-extractable mineral determination

Hydrochloric acid extractability of minerals was performed according to the Chauhan and Mahjan (1988) method. About 1.0 g sample was extracted using 10 ml of 0.03 N HCl with shaking at 37 ^{o}C for 3 h. Thereafter, the extract was filtered and the clear filtrate obtained was dried at 100 ^{o}C and then placed in a muffle furnace at 550 ^{o}C for 4 h. Thereafter, the samples were cooled and about 5 ml of 5 N HCl were added and boiled gently for 10 min and then cooled and diluted to 100 ml with distilled water. Minerals were determined as follows :

$$\text{Mineral extractability \%} = \frac{\text{Mineral extractable in 0.03NHCl (mg/100g)}}{\text{Total minerals (mg/100g)}} \text{X100}$$

2.7. Determination of amino acids

The amino acid composition of the samples was measured on hydrolysates using amino acid analyser (Sykam-S7130, Tokyo, Japan) based on high performance liquid chromatography technique. Sample hydrolysates were prepared following the method of Moore and Stain (1963). The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as grams/100 grams.

2.8. Phytic acid determination

Phytic acid content was determined according to the method of Wheeler and Ferrel (1971) using 2.0 g dried sample. A standard curve was prepared expressing the results as $Fe(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

2.9. Polyphenols determination

Total polyphenols were determined according to the Prussian blue spectrophotometric method (Price and Butler, 1977) with a minor

modification. Sixty milligrams of ground sample were shaken manually for 1 min in 3.0 ml methanol. The mixture was filtered. The filtrate was mixed with 50 ml distilled water and analysed within an hour. About 3.0 ml of 0.1 M $FeCl_3$ in 0.1 M HCl were added to 1 ml filtrate, followed immediately by timed addition of 3.0 ml freshly prepared $K_3Fe(CN)_6$. The absorbance was monitored on a spectrophotometer (Pye Unicam SP6-550 UV, London, UK) at 720 nm after 10 min from the addition of 3.0 ml of 0.1 M $FeCl_3$ and 3.0 ml of 0.008 M $K_3Fe(CN)_6$. A standard curve was prepared, expressing the result as tannic acid equivalents; *i.e.* the amount of tannic acid (mg/100g) which gives a color intensity equivalent to that given by polyphenols after correction for blank.

2.10. Tannin content determination

Quantitative estimation of tannins was carried out using the modified vanillin–HCl method (Price *et al*., 1978). A 200 mg sample was extracted using 10 ml of 1% (v⁄v) concentrated HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 ml) was added to the extract (1 ml) and the absorbance of the colour developed after 20 min at 30 ^oC was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, *i.e.* amount of catechin (mg/100g) which gives a colour intensity equivalent to that given by tannins after correcting for blank. Then tannin content was calculated and expressed in mg/100g.

2.11. Trypsin inhibitor activity determination

Trypsin inhibitor activity of raw, soaked and germinated fenugreek samples (0.2 g) was determined using N-α-benzoyl-DL-arginine-*p*-nitroaniline (BAPA) as a substrate according to Kakade *et al.* (1974) method. The trypsin inhibitor units were expressed in terms of the trypsin units inhibited per millilitre of extract. The trypsin unit was defined as an increase of 0.01 absorbance units, at 410nm, of the reaction mixture under the experimental conditions.

2.12. Protein digestibility determination

Protein digestibility *(in vitro)* was assessed by employing pepsin and pancreatin mehod of 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 samples was calculated by subtracting residual protein from total protein of the sample.

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

2.13. Statistical analysis

Each sample was analyzed in triplicate and the values were then averaged. Data were assessed by the analysis of variance (ANOVA) and Duncan-multiple range test with a probability $P \leq 0.05$, as described by Snedecor and Cochran (1987).

3. RESULTS AND DISCUSSION

3.1. Effect of seed soaking and germination on chemical composition and total energy of fenugreek cultivars

Table (1) shows the effect of seed soaking and germination on the chemical composition (%) of Saudi and Yemeni fenugreek cultivars. The dry matter contents of unprocessed (raw) fenugreek flour of the cultivars were 93.13 and 92.25% for Saudi and Yemeni cultivars, respectively. Soaking of the seeds in water significantly ($P \leq 0.05$) decreased the dry matter to 92.75 and 90.36% for the two cultivars, respectively. Germination of the seeds for different periods of time significantly ($P \leq 0.05$) reduced the dry matter of the two cultivars. Germination of the seeds for 3 days significantly ($P \leq 0.05$) reduced the dry matter to 80.38 and 80.33% of the two cultivars, respectively (Table 1). Soaking Saudi seeds had significant effect on ash, oil, protein fiber and carbohydrates (NFE), however the order is different in Yemni seeds. In the mean time, germination for 3 days significantly ($P \leq 0.05$) increased the protein content from 30.28 to 32.93% and from 31.62 to 35.15% for Saudi and Yemeni fenugreek seeds, respectively. This might be due to the reduction of seed nitrates into plant protein or ammonium compounds during germination (Sulieman *et al.*, 2008). Crude fiber and ash were respectively increased to 11.54 and 5.20% for Saudi cultivar and to 10.35 and 6.60 for Yemeni cultivars after 3 days germination. However, crude oil was not significantly affected by germination of Saudi seeds. Carbohydrate (NFE) content of raw fenugreek flour was 43.08 and 40.29% for Saudi and Yemeni cultivars, respectively. Soaking of the seeds in water decreased carbohydrate content to 42.13 and 35.54% for the cultivars, respectively**.** For both cultivars carbohydrate content significantly ($P \leq 0.05$) decreased with increasing germination time and reached 25.76 and 24.21% after 3 day germination of the cultivars, respectively. This decrease could be attributed to leaching of soluble components into soaking water prior to sprouting and enzymes activities during sprouting (Obizoba and Atii, 1994; El Maki *et al*., 1999) that could be responsible for the changes in nutrients. Similar

changes were observed in malted cereal grains (Traoré *et al*., 2004). Figure (1) shows the effect of soaking and germination on total energy of fenugreek seeds of two different sources (Saudi and Yemeni). The total energy values of the seeds were 354.64 and 348.84 Kcal/100gm for Saudi and Yemeni cultivars, respectively. The total energy of the cultivars was slightly decreased after soaking of the seeds for 12 h in water. However, germination decreased the total energy with increasing the sprouting time for both cultivars. In this respect, the reduction in NFE and protein could be attributed to their utilization during sprouting. Reduction of some nutrients of the seeds resulted in a concomitant increase in other nutrient. Changes in nutrients occurring during sprouting depend on the type of legume and on the sprouting conditions such as time, temperature and light cycle (Frias *et al.*, 1995). Sulieman *et al.* (2008) reported similar changes in total energy of lentil seeds during sprouting.

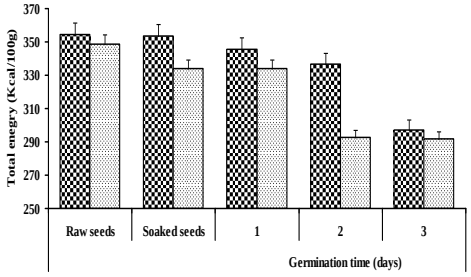

Fig. (1): Total energy (Kcal/100g) of raw, soaked and germinated fenugreek cultivars. Columns from left to right for each treatment represent Saudi and Yemeni cultivars. Error bars indicate the standard deviation of triplicate samples.

3.2. Effect of soaking and germination on antinutritional factors and protein digestibility of fenugreek cultivars

Figure (2) shows the effect of soaking and germination on trypsin inhibitor activity of fenugreek cultivars. As shown in Figure (2), trypsin inhibitor activity (TIA) decreased in fenugreek seeds during germination and soaking. Soaking of the seeds for 12 h at 25 ^{o}C decreased TIA by 4.71% for Saudi cultivar and by 5.13% for Yemeni cultivar. Trypsin inhibitor activity declined continuously as germination proceeded up to 3 days for both cultivars. The activity of the raw seeds was 9.34 and 10.71 units/mg sample for Saudi and Yemeni cultivars, respectively and after sprouting for 3 days it significantly ($P \leq 0.05$) declined to 4.27 and 5.34 units/mg sample of the cultivars, respectively.

The effect of germination on several compounds remains controversial; since different results have been obtained that depend on the legume species being studied and the germination conditions employed (Vidal-Valverde *et al*., 1994). Weder and Link (1993) observed that sprouting for 72 h did not alter the TIA in lentils, whereas Vidal-Valverde *et al* (1994) observed reductions of 24 and 28% in TIA of two lentil varieties after 6 days of germination. The results obtained by different groups are difficult to correlate, mainly because of differences in the methods of determination and in the source of the enzyme tested and possible confusion by the inhibitory activity of other non protein compounds.

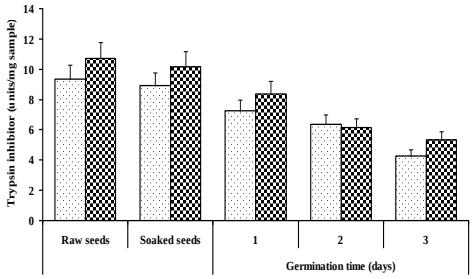

Fig. (2): Trypsin inhibitor (unit/mg sample) of raw, soaked and germinated fenugreek cultivars. Columns from left to right for each treatment represent Saudi and Yemeni cultivars. Error bars indicate the standard deviation of triplicate samples.

Table (2) shows the effect of soaking and germination on antinutritional factors and protein digestibility of fenugreek cultivars. Raw fenugreek seed flour of Saudi cultivar contained 205.88, 134.74 and 91.40 mg/100g phytic acid, polyphenols and tannin, respectively while Yemeni cultivar contained 274.76, 151.28 and 109.43 mg/100g of the antinutrients, respectively. For both cultivars phytic acid, polyphenols and tannin contents significantly ($P \leq 0.05$) decreased on soaking and early stages of germination, which ultimately caused significant increase in protein digestibility. However, tannin content of both cultivars started to increase after two days of germination and exceeded that of the raw seeds after 3 days of germination. This could be a result of solubilization of tannin when the seeds were soaked in water and migration of tannin to the outer layer as a result of germination, as indicated by the browning of the germinated seeds (Ahmed

Table (1): Effect of seed soaking and germination on the proximate composition (%) of two fenugreek cultivars.

Treatments	Cultivar											
	Saudi						Yemeni					
	Dry matter	Ash	Oil	Protein	Fiber	NFE	Dry matter	Ash	Oil	Protein	Fiber	NFE
Raw seeds	93.13^{a} (±0.89)	4.67^{b} (±0.08)	6.80^{a} (±0.15)	30.28^{c} (±0.17)	8.36^{b} (±0.08)	43.08^{a} (±0.38)	92.25^{a} (±0.63)	4.23^{c} (±1.08)	6.80^{a} (±0.13)	31.62^{d} (±1.02)	9.31^{b} (±0.11)	40.29^{a} (±0.38)
Soaked seeds	92.75^{b} (±1.06)	4.62^{b} (±0.13)	6.88^{a} (±0.39)	30.77^{c} (±0.93)	8.35^{b} (±0.05)	42.13^{a} (±0.23)	90.36^{b} (±0.15)	4.33^{b} (±0.29)	6.87^{a} (±0.21)	32.49^{c} (±0.64)	9.13^{c} (±0.15)	35.54^{c} (±0.21)
1-day sprouts	89.82^{c} (±0.62)	4.55^{b} (±0.43)	6.81^{a} (±0.13)	31.30^{b} (±0.48)	7.38^{d} (±0.11)	39.78^{b} (±0.48)	88.20^{c} (±0.20)	4.20^{c} (±1.13)	6.07^{b} (±0.84)	32.88^{c} (±0.10)	8.10^{c} (±0.10)	36.95^{b} (±0.51)
2-day sprouts	82.36^{d} (±0.15)	4.77^{b} (±0.06)	6.93^{a} (±0.40)	31.50^{b} (±0.89)	8.10^{c} (±0.10)	37.06^{c} (±0.61)	82.25^{d} (±0.44)	3.68^{c} (±0.40)	6.33^{b} (±0.15)	34.15^{b} (±2.02)	9.37^{b} (±0.15)	24.72^{d} (±0.61)
3-day sprouts	80.38^{e} (±0.08)	5.20^{a} (±0.87)	6.95^{a} (±0.15)	32.93^{a} (±0.71)	11.54^{a} (±0.12)	25.76^{d} (±0.62)	80.33^{e} (±0.72)	6.60^{a} (±0.69)	6.02^{b} (±0.10)	35.15^{a} (±0.13)	10.35^{a} (±0.08)	24.21^{d} (±0.28)

Values are means of triplicate samples (± SD). Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range tests. NFE, Nitrogen free extract (carbohydrates).

Table (2): Effect of seed soaking and germination on the antinutritional factors content (mg/100g) and percent *in vitro* protein digestibility (IVPD) of two fenugreek cultivars.

Treatments	Cultivar							
	Saudi				Yemeni			
	Phytate	Polyphenols	Tannin	IVPD	Phytate	Polyphenols	Tannin	IVPD
Raw seeds	205.88^{a}(±2.01)	134.74^{a}(±2.61)	91.40^{d}(±2.40)	74.73^{d}(±0.77)	274.76^{a}(±3.75)	151.28^{a}(±1.06)	109.43^{c}(±1.83)	74.60^{c}(±1.08)
Soaked seeds	62.25^{e}(±5.95)	116.95^{b}(±2.75)	81.57^{e}(±3.43)	97.06^{a}(±2.74)	60.86^{e}(±2.25)	130.74^{b}(±3.55)	97.90^{d}(±3.10)	87.31^{a}(±0.31)
1-day sprouts	181.21^{b}(±5.70)	106.47^{c}(±2.78)	101.47^{c}(±0.83)	89.57^{b}(±0.62)	265.93^{b}(±4.06)	121.85^{c}(±5.70)	85.80^{e}(±1.65)	87.12^{a}(±0.47)
2-day sprouts	102.17^{d}(±2.25)	71.34^{e}(±1.85)	107.80^{b}(±2.10)	83.79^{c}(±0.80)	150.86^{d}(±3.54)	85.17^{e}(±4.25)	115.50^{b}(±2.00)	75.16^{b}(±1.53)
3-day sprouts	173.67^{c}(±3.18)	90.48^{d}(±2.38)	116.33^{a}(±0.83)	81.88^{c}(±1.27)	264.94^{c}(±3.11)	105.29^{d}(±2.15)	125.95^{a}(±0.95)	73.82^{c}(±0.57)

Values are means of triplicate samples (± SD). Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range tests.

et al., 1996), which ultimately affected the protein digestibility of the cultivars.

In vitro protein digestibility values of raw and soaked Saudi cultivar were 74.73 and 97.06%, respectively, while those of Yemeni cultivar were 74.60 and 87.31, respectively. The significant increase in protein digestibility after soaking of the seeds is due to significant reduction in antinutrients of both cultivars. Germination of both fenugreek cultivars for one day significantly ($P \leq 0.05$) increased the protein digestibility to 89.57 and 87.12% for Saudi and Yemeni, respectively. However, germination for longer periods increased the protein digestibility but with a low rate due to progressive increase in antinutrients of the cultivars with time. The improvement in protein digestibility after soaking and germination could be ascribed to the reduction in antinutrients and increase in soluble proteins brought about by proteolytic activity of enzymes inherent in the germinated seedling (Parrish *et al*., 1990; Obizoba, and Atii, 1994; Larrson and Sandberg, 1995; Arbab and El-Tinay, 1997; Idris *et al*., 2005).

3.3. Effect of soaking and germination on total and extractable minerals of fenugreek cultivars

Tables (3) and (4) show the effect of soaking and germination on the total and extractable major and trace minerals of Saudi and Yemeni fenugreek seeds, respectively. Soaking and germination of fenugreek seeds of both cultivars significantly ($P \leq 0.05$) reduced total Ca and Mg but increased total P and Na. However, HCl extractability of major minerals of both cultivars increased significantly ($P \leq 0.05$) after soaking and germination of the seeds. Total Ca contents of raw seeds were 70.13 and 65.28 mg/100g for the two cultivars, respectively . After soaking these values decreased to 68.98 and 64.88 mg/100g, respectively. Germination of the seeds of the cultivars decreased Ca content with time and it reached after, 3 days germination, 61.96 and 61.78 mg/100g, for the cultivars, respectively. Extractable Ca percentage of raw seeds were 46.51% and 53.43% for the two cultivars, respectively while after soaking in water for 12 h it was increased to 49.87 and 55.95%, respectively.Germination of the seeds significantly ($P \leq 0.05$) increased the extractable Ca with time and reached 75.79% and 76.83% of 3-day sprouts of the two cultivars, respectively. For both cultivars, the effect of soaking and germination on the total and extractable Magnesium (Mg) was similar to that of Ca.

Total P of Saudi fenugreek seeds was significantly ($P \leq 0.05$) decreased after soaking in water from 289.23 (raw seeds) to 287.56 mg/100g (soaked seeds)but the extractability was increased from 48.15 to 57.67%. However, for Yemeni cultivar both total and extractable P were significantly ($P \leq 0.05$) increased. Total P increased from 301.32 to 307.56 and the extractable percents increased from 46.45 to 54.32. The variation in total P content between the two cultivars may be attributed to the nature of P and the origin of the cultivars. Germination of the seeds for different periods of time significantly ($P \leq 0.05$) increased both total and extractable P of the cultivars. Total P increased to 321.97 and 323.97 mg/100g, for the cultivars respectively. The extractable P of Saudi and Yemeni cultivars increased to 66.29% and 64.34%, respectively after 3 days germination. For both cultivars the effect of soaking and germination on total and extractable Sodium (Na) were similar to that of P.

For both cultivars, trace mineral contents slightly decreased (except Mn) after soaking of the seeds in water and after germination they were fluctuated with time. However, the HCl extractability of such minerals increased progressively and significantly ($P \leq 0.05$) after soaking and with germination time for both cultivars. Total Fe contents of the raw seeds were 12.90 and 13.56 mg/100g for Saudi and Yemeni fenugreek seeds, respectively. After soaking it was decreased to 10.97 and 11.87 mg/100g, respectively. After germination it was decreased to 9.89 and 10.12 mg/10g of 3-day sprouts of the cultivars, respectively. The extractable Fe percents of the raw seeds were 41.25 and 39.25 for the two cultivars, respectively. After soaking these values were significantly ($P \leq 0.05$) increased to 44.53 and 41.53%, respectively. Further increment was observed after germination with a maximum value of 67.46 and 65.46% for 3-day sprouts of the two cultivars, respectively. The effect of soaking and germination on other trace minerals was similar to that of Fe for both cultivars.

Comparatively lower contents of minerals when the seeds were soaked in water might be due to the leaching of some minerals into the soaking water as observed. Reduction in minerals content after germination of the seeds could be due to utilization of such minerals during germination. However, P was increased and the increment in phosphorus content after germination may be due to hydrolysis of phytate by the enzyme phytase, which is released during germination (Jood and Kapoor 1997).

Table (3): Effect of soaking and germination on total (mg/100g) and extractable (%) major and trace minerals of Saudi fenugreek seeds (on a dry weight basis).

Treatments	Minerals							
	Ca		P		Na		Mg	
	Total	Extractability%	Total	Extractability%	Total	Extractability%	Total	Extractability%
Raw seeds	70.13^{a} (±0.32)	46.51^{e} (±0.13)	289.23^{d} (±0.38)	48.15 ^{e} (±0.13)	142.34^{d} (±0.24)	70.32^{e} (±0.51)	166.51^{a} (±0.21)	24.68^{e} (±0.42)
Soaked seeds	68.98^{b} (±0.16)	49.87^{d} (±0.52)	287.56^{e} (±0.45)	57.67 ^{d} (±0.56)	143.62^{d} (±0.29)	74.87^{d} (±0.65)	165.23^{a} (±0.43)	26.57^{d} (±0.51)
1-day sprouts	66.89^{c} (±0.42)	65.67^{c} (±0.61)	307.36^{c} (±0.43)	61.45^{c} (±0.37)	147.64^{c} (±0.33)	76.36^{c} (±0.57)	164.76^{b} (±0.61)	32.98^{c} (±0.62)
2-day sprouts	63.67^{d} (±0.54)	70.26^{b} (±0.67)	316.86^{b} (±0.41)	65.87^{b} (±0.33)	152.95^{b} (±0.42)	79.45^{b} (±0.24)	162.85^{c} (±0.16)	35.46^{b} (±0.67)
3-day sprouts	61.96^{e} (±0.71)	75.79^{a} (±0.56)	321.97^{a} (±0.87)	66.29^{a} (±0.39)	157.73^{a} (±0.25)	82.70^{a} (±0.22)	160.92^{d} (±0.19)	37.34^{a} (±0.78)
Treatments	Fe		Mn		Cu		Zn	
	Total	Extractability%	Total	Extractability%	Total	Extractability%	Total	Extractability%
Raw seeds	12.90^{a} (±0.81)	41.25^{e} (±0.12)	1.23^{a} (±0.02)	5.27^{d} (±0.11)	1.81^{a} (±0.15)	18.26^{e} (±0.78)	1.87^{a} (±0.19)	51.17^{e} (±0.31)
Soaked seeds	10.97^{c} (±0.06)	44.53^{d} (±0.31)	1.48^{a} (±0.11)	7.26^{c} (±0.21)	1.78^{a} (±0.29)	20.17^{d} (±0.57)	1.23^{a} (±0.63)	57.23^{d} (±0.34)
1-day sprouts	11.52^{b} (±0.34)	60.15^{c} (±0.42)	1.31^{a} (±0.53)	9.24^{b} (±0.27)	1.98^{a} (±0.53)	21.89^{c} (±0.98)	1.95^{a} (±0.28)	61.34^{c} (±0.64)
2-day sprouts	10.98^{c} (±0.41)	63.58^{b} (±0.71)	1.36^{a} (±0.26)	9.98^{b} (±0.45)	1.87^{a} (±0.49)	22.67^{b} (±0.32)	1.97^{a} (±0.99)	71.24^{b} (±0.74)
3-day sprouts	9.89^{c} (±0.98)	67.46^{a} (±0.43)	1.39^{a} (±0.37)	10.37^{a} (±0.78)	1.82^{a} (±0.69)	23.99^{a} (±0.38)	2.01^{a} (±0.61)	73.13^{a} (±0.88)

Values are means (± SD) of triplicate samples. Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range tests.

Table (4): Effect of soaking and germination on total (mg/100g) and extractable (%) major and trace minerals of Yemeni fenugreek seeds (on a dry weight basis).

Treatments	Minerals							
	Ca		P		Na		Mg	
	Total	Extractability%	Total	Extractability%	Total	Extractability%	Total	Extractability%
Raw seeds	65.28^{a} (±0.56)	53.43^{e} (±0.53)	301.32^{e} (±0.68)	46.45^{e} (±0.35)	151.26^{d} (±0.31)	72.73^{e} (±0.51)	165.73^{a} (±0.21)	25.62^{e} (±0.54)
Soaked seeds	64.88^{a} (±0.35)	55.95^{d} (±0.72)	307.56^{d} (±0.72)	54.32^{d} (±0.62)	149.73^{e} (±0.27)	75.17^{d} (±0.65)	164.21^{b} (±0.52)	27.37^{d} (±0.35)
1-day sprouts	64.46^{a} (±0.42)	66.43^{c} (±0.43)	313.36^{c} (±0.63)	57.35^{c} (±0.58)	156.91^{c} (±0.34)	78.52^{c} (±0.57)	163.86^{b} (±0.45)	31.78^{c} (±0.62)
2-day sprouts	62.72^{b} (±0.34)	71.56^{b} (±0.41)	321.86^{b} (±0.51)	62.37^{b} (±0.79)	159.75^{b} (±0.52)	81.64^{b} (±0.14)	160.45^{c} (±0.23)	33.76^{b} (±0.17)
3-day sprouts	61.78^{c} (±0.63)	76.83^{a} (±0.32)	323.97^{a} (±0.54)	64.34^{a} (±0.69)	166.73^{a} (±0.25)	83.75^{a} (±0.44)	159.98^{c} (±0.63)	35.98^{a} (±0.48)
Treatments	Fe		Mn		Cu		Zn	
	Total	Extractability%	Total	Extractability%	Total	Extractability%	Total	Extractability%
Raw seeds	13.56^{a} (±0.71)	39.25^{e} (±0.82)	1.17^{a} (±0.12)	4.46^{d} (±0.21)	1.76^{a} (±0.35)	19.87^{d} (±0.48)	1.37^{a} (±0.89)	49.56^{e} (±0.29)
Soaked seeds	11.87^{c} (±0.26)	41.53^{d} (±0.37)	1.29^{a} (±0.08)	5.98^{c} (±0.32)	1.74^{a} (±0.69)	21.67^{c} (±0.73)	1.03^{a} (±0.36)	55.99^{d} (±0.17)
1-day sprouts	12.52^{b} (±0.81)	58.15^{c} (±0.36)	1.23^{a} (±0.43)	7.82^{b} (±0.58)	1.78^{a} (±0.73)	23.21^{b} (±0.78)	1.45^{a} (±0.34)	59.24^{c} (±0.32)
2-day sprouts	1134^{c} (±0.37)	62.58^{b} (±0.24)	1.31^{a} (±0.32)	9.49^{a} (±0.89)	1.79^{a} (±0.87)	24.37^{a} (±0.72)	1.57^{a} (±0.86)	69.78^{b} (±0.34)
3-day sprouts	10.12^{d} (±0.54)	65.46^{a} (±0.98)	1.37^{a} (±0.19)	10.07^{a} (±0.59)	1.77^{a} (±0.86)	24.99^{a} (±0.98)	1.64^{a} (±0.45)	71.19^{a} (±0.28)

Values are means of triplicate samples (± SD). Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range tests.

Table (5): Effect of seed soaking and germination on the amino acids content (g/100g) of two fenugreek cultivars.

Amino acids	Cultivars									
	Saudi					Yemeni				
	Raw	Soaked	1-day sprouts	2-day sprouts	3-day sprouts	Raw	Soaked	1-day sprouts	2-day sprouts	3-day sprouts
Aspartic acid	1.37	1.33	1.15	3.51	1.52	2.41	1.90	2.51	3.30	2.63
Threonine	0.62	0.59	0.52	1.37	0.67	0.97	0.83	1.12	1.32	1.10
Serine	0.56	0.52	0.47	1.53	0.58	1.16	0.70	1.26	1.49	1.21
Glutamic acid	2.26	1.86	1.48	6.97	2.36	5.02	3.07	5.17	6.76	5.24
Glycine	0.40	0.23	0.20	1.24	0.37	0.79	0.52	0.87	1.08	0.88
Alanine	1.17	1.34	1.14	1.66	1.24	1.19	1.59	1.26	1.66	1.25
Cystine	0.11	0.09	0.07	0.28	0.12	0.16	0.15	0.19	0.24	0.18
Valine	0.96	1.02	0.90	1.76	1.05	1.26	1.31	1.39	1.71	1.36
Methionine	0.16	0.16	0.15	0.30	0.18	0.21	0.22	0.23	0.28	0.21
Isoleucine	1.12	1.22	1.07	2.05	1.26	1.50	1.58	1.60	2.04	1.58
Leucine	1.49	1.62	1.40	2.62	1.62	1.84	2.10	1.91	2.59	1.93
Tyrosine	0.45	0.45	0.36	0.83	0.48	0.62	0.63	0.64	0.80	0.66
Phenylalanine	0.84	0.85	0.76	1.77	0.94	1.16	1.18	1.31	1.63	1.39
Histidine	0.41	0.39	0.34	0.95	0.46	0.68	0.58	0.73	0.92	0.75
Lysine	0.82	0.65	0.56	2.12	0.88	1.39	1.12	1.46	1.95	1.48
Ammonia	0.81	1.07	0.92	1.26	0.92	0.85	1.21	0.90	1.26	0.89
Arginine	1.98	1.90	1.75	3.59	2.40	2.99	2.80	2.78	3.77	3.09
Proline	0.99	1.08	0.92	1.55	1.13	1.26	1.36	1.36	1.58	1.36

Values are means of duplicate samples.

The improvement in HCl extractability of all minerals after soaking and germination of fenugreek seeds could be attributed to the reduction in antinutrients that was reported to hinder the availability of both major and trace minerals. It has been reported that the phytase activity increases on germination causing catabolism of phytic acid by phytases, or myo-inositol hexaphosphate phosphohydrolases, to myo-inositol and inorganic phosphate and thereby increasing the *in vitro* availability of divalent minerals (Jood and Kapoor 1997). In a similar study, Sripriya *et al*. (1997) observed an increase in the HCl extractability of calcium of finger millet germinated for 24 h from 47.6% (unmalted grain) to 53.2%. Variations in extractable phosphorus at different times of germination were observed by Sripriya *et al.* (1997). A similar trend of trace minerals in millet as a result of germination has been reported by Obizoba and Atii (1994) and Sripriya *et al.* (1997).

3.4.Effect of soaking and germination on amino acid composition of fenugreek cultivars

The nutritive value of a dietary protein depends on its essential amino acid composition. The results of the quantitative determinations of the various amino acids of raw, soaked and germinated fenugreek seeds are shown in Table (5). Leucine, lysine, arginine, proline, alanine, aspartic and glutamic acids showed the highest amino acid contents in raw seeds of both cultivars. Yemeni fenugreek raw seeds had higher contents of most amino acids compared to Saudi fenugreek. The results show that soaking of seeds caused a slight decrease and increase in amino acid contents. It could be suggested that the loss in amino acids after soaking is related to the loss of some water-soluble protein such as albumin. After germination of Saudi fenugreek seeds for one day, all of the essential amino acids decreased, while those of Yemeni fenugreek seeds were increased. However, germination of the seeds of both cultivars for two days greatly increased the amino acids under investigation. Aspartic acid showed a greater increase, amounting 3.51 and 3.30 g/100g of the original content of 2-day sprout of Saudi and Yemeni cultivars, respectively. The increasing percentage of aspartic acid in the germinated seeds may be derived from the hydrolysis of its amide. The results obtained for the cultivars indicated that germination of the seeds for two days is an optimum time to get seedlings rich in amino acids. Similar results were reported by Hooda and Jood (2005) for Indian fenugreek.

It can be concluded that nutritional quality of fenugreek seeds can be improved through processing methods, especially soaking and germination. 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.

Acknowledgement to Center for Rresearch, Department of Science and Medical Studies-Malaz, King Saud University for supporting this research.

4. REFERENCES

Ahmed S. B., Mahgoub S. A. and Babiker E. E. (1996). Changes in tannin and cyanide contents and diastic activity during germination and the effect of traditional processing on cyanide content of sorghum cultivars. Food Chemistry, 56: 159-162.

Akeson W.E. and Stahmann M.A. (1964). A pepsin-pancreatin digestibility index of protein quality evaluation. J. Nutr., 83: 257-259.

AOAC (1995). Official Methods of Analysis. 15th Ed. Of the Association of Official Analytical Chemists, Arlington, Virginia, USA.

Arbab M.E. and EL Tinay A.H. (1997). Effect of cooking with sodium bisulphate or ascorbic acid on the *in vitro* protein digestibility of two sorghum cultivars. Food Chemistry, 59: 339-343.

Chapman H. D., and Pratt F. P. (1961). Ammonium vandate-molybdate method for determination of phosphorus. Methods of analysis for soils, Plants and Water,1st ed., Agriculture Division, California University, USA, p. 184–203.

Chapman H. D., and Pratt F. P. (1982). Determination of minerals by titration method. Methods of analysis for soils, Pplants and Water, 2nd ed., Agriculture Division, California University, , USA, p. 169–170.

Chauhan B. M., and Mahjan L. (1988). Effect of natural fermentation on the extractability of minerals from pearl millet flour. Journal of Food Science, 53: 1576–1577.

El-Maki H.B., Babiker E.E., and El Tinay A.H. (1999). Changes in chemical composition, grain malting, starch and tannin contents and protein digestibility during germination of sorghum cultivars. Food Chemistry, 66: 331-336.

Flammang A. M., Cifone M. A., Ereson G. L., and Stankowskci L. F. (2004). Genotoxicity

testing of fenugreek extract. Journal of Food and Chemical Toxicology, 42: 205–208.

Frias J., Dias-Pollan C., Hedley C. L. and Vival-Valverde C. (1995). Evaluation of trypsin inhibitor activity during germination of lentils. J. Agric. Food Chem., 43: 2231-2234.

Hooda S., and Jood S. (2005). Organoleptic and nutritional evaluation of wheat biscuits supplemented with untreated and treated fenugreek flour. Food Chemistry, 90: 427-435

Idris W. H., Abd El Rahman S. M., Elmaki H.B., Babiker E.E. and El Tinay A.H. (2005). Effect of germination, fermentation and cooking on phytic acid and tannin contents and HCl- extractability of minerals of sorghum (*Sorghum biocolor*) Cultivars. Food Technology, 3: 410-416.

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.

Kakade M.L., Rakis J.J., McGhee J.E., and Puski C., (1974). Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. Cereal Chemistry, 51: 376-382.

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.

Larrson M. and Sandberg A. (1995). Malting of oats in a pilot plant process. Effect of heat treatment, storage and soaking conditions on phytate reduction. Journal of Cereal Science, 21: 87- 95.

Moore S. and Stain W.H. (1963). Chromatographic amino acids determination by the use of automatic recording equipment methods. Enzymology, 63: 819–831.

Neeraja A. and Rajyalakshmi P. (1996). Hypoglycemic effect of processed fenugreek seeds in humans. J. Food. Sci. Technol. 33: 427-430.

Obizoba I.C. and Atii J.V. (1994). Evaluation of the effect of processing techniques on the nutrient and antinutrient contents of pearl millet (*Pennisetum glaucum*) grains. Plant Foods for Human Nutrition, 45: 23-34.

Osborne D.R. and Voogt P. (1978). Calculation of caloric value. In: Analysis of Nutrients in Foods. New York, Academic Press, p: 23-34.

Parrish F.W., Madacsi J.P., Phillipy B.Q.,Wilfred A.G. and Bucos M. (1990). Determination of phytic acid in cotton seed by near infrared reflectance spectroscopy. J. Agric. Food Chem., 38: 407 – 409.

Price M. L. and Butler L. G. (1977). Rapid visual estimation and spectrophotometric determination of tannin in sorghum grain. J. Agric. Food Chem., 25: 1268–1273.

Price M. L., Socoyoc S. V. and Butler L. G. (1978). A certical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem., 26: 121418.

Salini H., and Sudesh J. (2004). Organoleptic and nutritional evaluation of wheat biscuits supplemented with untreated and treated fenugreek flour. Journal of Food Chemistry, 90: 427–435.

Snedecor G. W. and Cochran W. G. (1987). Statistical Methods (p. 221– 222) 17th ed. The Iowa State University Press. Ames, IA, USA.

Sripriya G., Anthony U., and Chandra T S. (1997). Changes in carbohydrate, free amino acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). Food Chemistry, 58: 345-350.

Sulieman M. Ahmed., Elfadil E. Babiker and abullahi H. El Tinay (2008). Effect of sprouting on chemical composition and amino acid content of Sudanese Lentil cultivars. Journal of Applied Sciences 8(12): 2337-2340.

Traoré T., Mouquet C., Icard-Verni C., Traor A.S. and Treche S. (2004). Changes in nutrient composition, phytate and cyanide contents and α-amylase activity during cereal malting in small production units in Ouagadougou (Burkina Faso). Food Chemistry, 88: 105–114.

Vidal-Valverde C., Frias J., Estrella I., Gorospe MJ., Ruiz R. and Bacon J. (1994). Effect of processing on some antinutritional factors of lentils. J Agric Food Chem., 42: 2291–2295.

Weder JKP and Link I. (1993). Effect of treatments on legume inhibitor activity against human proteinases, in Recent Advances of Research in Antinutritional Factors in Legume Seeds, Ed. by van der Poel AFB, Huisman J. and Saini HS. Wageningen University Press, Wageningen, p. 481–485.

Wheeler E. L. and Ferrel R. E. (1971). A method for phytic acid determination in wheat and wheat fractions. Cereal Chemistry, 28: 313–320.

تأثير نقع وإنبات بذور الحلبة (*Trigonella foenum- graecum* L.) **على التركيب الكيميائي وتركيب الأحماض الأمينية والعوامل المضادة للتغذية وقابلية البروتين للهضم والمعادن للإستخلاص**

أمل عبدالله سعد الحسين

قسم علوم الأغذية والتغذية – كلية علوم الأغذية والزراعة – جامعة الملك سعود -الرياض – المملكة العربية السعودية

ملخص

تم تقدير التركيب الكيميائي، مستويات مضادات التغذية وقابلية البروتين للهضم والمعادن الكلية والقابلية للإستخلاص وتركيب الأحماض الأمينية في بذور الحلبة (الخام والمغمورة في الماء والمنبتة) من مصدرين مختلفين (صنف سعودي وآخر يمني).

أظهرت النتائج احتواء بذور الحلبة الخام على كميات كبيرة من المكونات الغذائية لكل من النوعين من الحلبة خاصة البروتين، كذلك أوضحت النتائج أن النقع لكل من النوعين من البذور ليس له تأثير على التركيب الكيميائي، بينما أدى الإنبات إلى زيادة معنوية ($p\geq 0.05$) في نسبة البروتين الكلي والألياف ولكن أدى الإنبات إلى إنخفاض في نسبة المادة الجافة والكربوهيدرات في حين لم تتغير نسبة الرماد والدهون. كما حدث انخفاض طفيف في الطاقة الكلية نتيجة النقع ولوحظ انخفاض معنوي ($P\geq 0.05$) بعد الإنبات لبذور الحلبة من النوعين. وقد أدى النقع إلى خفض مستويات مضادات التغذية في النوعين وبدرجة معنوية ($p\geq 0.05$) مقارنة بمعاملة الإنبات.

وقد ازدادت قابلية الهضم للبروتين خارج الجسم، وكذلك قابلية الاستخلاص للمعادن، بدرجات ملحوظة نتيجة للغمر في الماء والإنبات وقد يرجع ذلك للإنخفاض في مستويات مضادات التغذية نتيجة لهذه المعاملات. وجد أن بذور الحلبة الخام، من النوعين غنية بحمض الجلوتاميك وحمض الأسبارتيك. وكان للنقع تأثير طفيف على نسب بعض الأحماض الأمينية ولكن أدى الإنبات لمدة يومين إلى زيادة نسب معظم الأحماض في بذور الحلبة في النوعين (عند مستوى معنوية $p\geq 0.05$).

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (61) العدد الثالث (يوليو 2010):263-273.