

Chemical, Functional and Nutritional Characteristics of raw, autoclaved and germinated fenugreek seeds

S. MABROUKI, B. OMRI AND H. ABDOULI *

Laboratory of Improvement & Integrated Development of Animal Productivity & Food Resources, Higher School of Agriculture of Mateur, University of Carthage, Tunisia.

* Corresponding author: abdoulihedi@gmail.com

Abstract - To evaluate fenugreek seeds potential as nutritional and functional feed, chemical compositions, functional properties and in vitro nutritional values of raw, autoclaved (121°C, 15min) and pre-germinated (emergence of the radical in 95% of the seeds) fenugreek seeds were compared. On dry matter basis, raw seeds had: 5.4, 23.8, 40.4, 16.8, 66.9 and 26.4% fat, crude protein, NDF, ADF, total carbohydrates and non-fibrous carbohydrates, respectively. Autoclaving increased ($P<0.05$) fat (8.5%) and ADF (17.6%) and reduced ($P<0.05$) carbohydrates (64.3%) and non-fibrous carbohydrates (19.2%). Pre-germinated increased ($P<0.05$) fat (7.7%), crude protein (24.1%) and ADF (17.3%) and reduced ($P<0.05$) carbohydrates (64.2%). Raw seeds total phenols, tannins, flavonoids and phytic acid contents were 7.1, 3.5, 2.6 and 6.6 mg /g dry matter, respectively. Autoclaving reduced ($P<0.05$) only flavonoids level and pre-germinated reduced ($P<0.05$) phenols, tannins and flavonoids levels. Among the distinctive functional properties of raw seeds were their bulk density (0.69 g DM/ml), water absorption capacity (4.72 g/g DM), foaming capacity (21.37%), swelling coefficient (305%) and extractable color value (8.3 ASTA units). Autoclaving decreased ($P<0.05$) bulk density by 13% and extractable color by 29%. Pre-germination reduced ($P<0.05$) water absorption capacity by 17.8% and extractable color by 73.3 to 74.2% and increased ($P<0.05$) foaming capacity by 37.1%. Autoclaving reduced ($P<0.05$) protein dispersibility index from 34.78 to 10.25%, had no effect ($P>0.05$) on digestibility by pepsin (74.12-75.84%) and increased ($P<0.05$) digestibility by pepsin-trypsin from 82.31 to 85.91%, digestible energy by 6.2% and metabolizable energy by 4 to 4.7%. Pre-germination enhanced ($P<0.05$) protein dispersibility index to 45.39% and digestible energy by 3.8% and metabolizable energy by 4.3 to 6.7%. It was concluded that fenugreek seeds can serve as a valuable nutritional and functional food. Autoclaving and pre-germination improved such potential and need to be further evaluated and implemented in In Vivo studies.

Keywords: Fenugreek seeds / autoclaving / pre-germination / functional properties / nutritional values.

1. Introduction

Fenugreek (*Trigonella foenum graecum*) has been used since ancient times as a spice, forage and medicinal crop and more recently as a functional food (food that provides benefit through the improvement of health or well-being in addition to its nutritional supply). In the domain of animal production, fenugreek seeds have been evaluated with the objective of improving the health status of the animals, the performance efficiency and the products quality (Abdouli et al. 2014a; Alloui et al. 2012). Such uses, which are examples of their wide spectrum of physiological and nutritional effects, are due to the many bioactive compounds they contain. In fact, in addition to being a rich source of protein, fenugreek seeds contain dietary fiber, soluble soluble fiber and many minor biologically active phytochemicals including saponins, tannins, flavonoids, carotenoids, alkaloids, minerals, vitamins etc. Among these bioactive compounds, some may have adverse effect when ingested by monogastric animals: tannins (Abdouli et al. 2014b) and trypsin and α -chymotrypsin inhibitors decrease protein digestibility. Phytic acid decreases phosphorus, iron, calcium and protein bioavailability (Cheryan 1980). Removal of these undesirable components may be essential for improving fenugreek seeds functional and



nutritional qualities. Several methods such as germination, soaking, cooking (El-Mahdy et al. 1982) have been investigated. However, such methods may cause losses in some nutrients.

Therefore, the objective of the present study was to evaluate the nutritional and functional potential of raw, autoclaved and pre-germinated fenugreek seeds. The same batches of fenugreek seeds were used in two subsequent studies to evaluate and implement the use of fenugreek seeds in attempts, on one hand, to control weaned rabbits health status and improve their productive performances and, on the other hand, to improve hens laying performance and to lower their egg nutritional and functional qualities.

2. Materials and Methods

2.1. Material

About 45 kg of fenugreek seeds of bitter non identified genotype yet commonly cultivated and used for human and animal feeding were used. They were kindly offered by a farmer from Testour (80 km north Tunis, Tunisia). They were harvested in July 2013 and meanwhile stored indoors. At the start of the study, they were cleaned manually to remove foreign matter and damaged seeds, thoroughly mixed and divided into three batches: one was used unprocessed as a control, one was autoclaved at 121°C for 15 min and dried by cold air ventilation with fans for 26.5 hs; and the other one was pre-germinated. For this treatment, 23 kg of seeds were initially mixed with 46 liters of distilled water and divided into 6 plastic recipients. In each recipient, fenugreek seeds were spread in a layer of about 10mm between two layers of sheet paper and covered with a black plastic sheet to ensure darkness. During this process which lasted for 21 hs, distilled water was used to wet (sprinkle) the upper paper sheet so that the overall seeds to added water ratio was 1:2.5. When about 95% of the seeds germinated (at the emergence of the radical), the pre-germination was stopped first by cold air ventilation with fans for 48 hs and then by drying at 40 °C in a air forced oven for 20 hs.

2.2. Methods

2.2.1. Chemical composition

Two separate samples from each treatment were milled to pass through a 0.5 mm sieve and then crashed in a glass mortar to a fine powder. They were stored during the study in tight containers at room temperature. Dry matter (DM), ash, crude protein, crude fiber, ether extract, Acid detergent fiber (ADF) and neutral detergent fiber (NDF), total phenols, tannins and flavonoids were determined as reported in Abdouli et al. (2014b,d). Phytic acid content was determined using the procedure described by Chikpah and Demuyakor (2013). Finely ground seed samples (0.5 g) were extracted in 50 ml of 2 % concentrated HCl for 3 hs at room temperature. The samples were then centrifuged (2500 rpm, 15min) and filtered through Whatman filter paper. Twenty five ml of each filtrate were mixed with 50 ml of distilled water and 5 ml of 0.3 % ammonium thiocyanate solution and titrated with standard Iron (III) Chloride solution containing 1.97 mg iron per ml. The end point was signified by the appearance and persistence of a brownish-yellow coloration. The percentage phytic acid in the sample was calculated using the formula:

Phytic acid (mg/ g DM) = Titre Value \times 1.97 \times 1.19 / weight dry matter of the sample (g)

Carotenoids extraction and dosage were carried out as follows: 1 g ground sample was mixed with 10 ml of organic solvent mixture (hexane, acetone, ethanol; 50:25:25, v/v/v). The mixture was stirred for 90 min in the dark, centrifuged at 3000 rpm for 10min and filtered through dry sodium sulphate. The filtrate was used for the determination the absorbance values at 450 and 503 nm. β -carotene and lycopene concentrations were calculated using the formulas of Georgé et al. (2011)

$$\beta\text{-carotene } (\mu\text{g/g DM}) = (4.624 \times A_{450} - 3.091 \times A_{503}) \times \text{Vml/ dry weight}$$

$$\text{Lycopene } (\mu\text{g/g DM}) = (3.965 \times A_{450} - 0.806 \times A_{503}) \times \text{Vml/ dry weight}$$

where A_{450} and A_{503} represent the absorbance at 450 and 503nm, respectively.

2.2.2. Functional properties

The bulk density of ground fenugreek seeds was determined using the method of Narayana and Narasinga (1982). A calibrated tube was weighed and filled with a sample to 5 ml by constant tapping until there was no further change in volume. Bulk density was then calculated as the weight determined by difference per unit volume of the sample.

The water absorption capacity and oil absorption capacity were determined by the method of Sosulski et al. (1976). Sample (0.5 g) was mixed with 5ml distilled water or soybean oil and allowed to stand at ambient temperature for 30 min and then centrifuged for 30 min at 3000 rpm. The volume of the supernatant was recorded and water or oil absorbed was expressed as the weight (g) of water or oil bound per gram dry matter. Densities of water and oil were 1g/ml and 0.909 g/ml, respectively

The least gelation concentration was determined following the procedure of Coffman and Garcia (1977). Suspensions of the ground seed samples at 0.01 to 0.05% (w/v) were prepared in 10 ml distilled water in test tubes. The tubes containing these suspensions were heated in a water bath at 80°C for 60 min before rapidly cooled under running cold tap water and then at 4°C for another 2 hs. The least gelation concentration was determined as that concentration when the sample from inverted tube did not slip. The firmness of the gel was noted as low, moderate or high.

The foam capacity and foam stability were determined as described by Narayana and Narasinga (1982) with slight modification. Sample (0.5 g) was mixed with 10 ml distilled water at 30°C in a graduated tube and then shaken for 30 sec to foam. The volume of foam after shaking was noted for the determination of foaming capacity as follow:

$$\text{Foaming capacity (\%)} = ((V2-V1) / V1) \times 100$$

Where: V1 = initial volume of solution and V2 = volume of solution after shaking.

Foam stability was determined as the volume of foam that remained after 90 and 120 min and expressed as the percentage of initial foam volume.

The swelling capacity was determined by the method described by Okaka and Potter (1977). Ten ml distilled water were added to 0.8 g sample in a graduated tube and the suspension was mixed by inverting the tube. The tube was inverted again after 2 min and left to stand for a further 8 min. The volume occupied by the sample was noted at 8 and 120 min. The swelling coefficient was calculated as the percentage increase of the volume of the sample.

The extractable color was measured according to the ASTA method as described by José et al. (1999) with some modifications. Sample (0.1g) was mixed with 10 ml of 2-propanol in a tube wrapped in aluminum sheet. The tube was closed and then incubated in a water bath at 70°C for 3hs. After cooling to room temperature, the extraction was filtered and its absorbance was determined at 460nm using 2-propanol as a blank. Simultaneously, the absorbance of a standard color solution (0.5 mg potassium dichromate / ml of 1.8 M sulfuric acid) was determined using sulfuric acid as a blank. ASTA color units were calculated as:

$$\text{ASTA 20} = \text{Afs} \times 16.4 / \text{dry sample weight (g)}, \quad (\text{ASTA}, 1985).$$

The extractable color was also expressed as percentage of the color of the potassium dichromate standard solution. It was denoted hereafter as standardized extractable color and calculated as:

$$\text{Standardized extractable color} = \text{Afs} \times \text{Cstd} \times 100 / \text{Astd} \times \text{Cfs}$$

Where Afs = Fenugreek seed absorbance, Cstd = Concentration of the potassium dichromate standard color solution, mg/ml, Astd = potassium dichromate standard color solution absorbance and Cfs = fenugreek seed extract concentration, mg DM/ml.

2.2.3. Nutritional properties

Protein dispersibility index was determined by mixing 1 g sample with 30 ml of distilled water and stirring for 20 min. The mixture was centrifuged at 2500 rpm for 10 minutes and filtered. The protein content of the residue was determined by the Kjeldahl method and the protein dispersibility index was calculated as:

$$\text{Protein dispersibility index (\%)} = (\text{PIS} - \text{PIR}) \times 100 / \text{PIS}$$

Where PIS= protein in initial sample and PIR = protein in the residue

In vitro protein digestibility by pepsin was measured by mixing 0.5 g sample in 20 ml of 0.1 N HCl containing 50.4 mg pepsin and then incubating at 37°C for 20 hs. The mixture was manually shaken many times. After incubation, the sample was centrifuged at 2000 rpm for 30 min, the pellet was washed with distilled water on a filter paper and its nitrogen content was determined by the Kjeldahl method. For the determination of protein digestibility by pepsin followed by trypsin, the suspension obtained after the first step of digestion in pepsin was neutralized with 0.4 N NaOH and treated with 15 mg of trypsin in 15 ml of phosphate buffer (pH 8.0) containing 0.005 M sodium azide to prevent growth of microorganisms. The mixture was shaken and incubated at 37°C for 24 hs, centrifuged and filtered. Protein digestibility was calculated using the following formula;

$$\text{Protein digestibility (\%)} = (\text{PIS} - \text{PIR}) \times 100 / \text{PIS}$$

where PIS= protein in initial sample and PIR = protein in the residue.

Chemical composition data were used to calculate apparent digestible energy (DE) for rabbits and nitrogen-corrected apparent metabolizable energy (AMEn) for poultry using the equations:

$$\text{DE (Kcal/kg DM)} = [12.912 - 0.0236\text{CF} + 0.010\text{CP} + 0.020\text{EE}] / 0.004187; \text{ (Wiseman et al.1992):}$$

Specific equation for protein feedstuffs (1); (Nascimento, 2007):

$$\text{AMEn (Kcal/kg DM)} = 2707.71 + 5.863\text{EE} - 1.606\text{NDF}$$

General equation for energetic and protein feedstuffs (2); (Nascimento et al., 2009):

$$\text{AMEn(Kcal/kgDM)} = 4101.33 + 5.628\text{EE} - 23.297\text{Ash} - 2.486\text{NDF} + 1.042\text{ADF}$$

General equation for energetic and protein feedstuffs (3); (Nascimento et al., 2009):

$$\text{AMEn(Kcal/kg DM)} = 4095.41 + 5.684\text{EE} - 22.526\text{Ash} - 2.224\text{NDF}$$

Where: Ash, CF(crude fiber), CP (crude protein) and EE(ether extract), ADF(acid detergent fiber), ADL(acid detergent lignin) in g /kg DM (dry matter)

2.2.4. Statistical analysis

Collected data were subjected to analysis of variance using the GLM procedure (SAS, 1989). Comparisons between treatments were made with the DUNCAN test. Values of $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Chemical composition

3.1.1. Gross chemical composition

The effect of fenugreek seeds processing on their gross chemical composition are shown in table 1. The raw seeds had low ash content (3.96% DM) and thereby were rich in organic matter which is the source of energy. As legume, they had moderate ether extract (5.36%DM) and crude protein (23.79% DM) contents. They were rich in total carbohydrates (66.86% DM) and NDF (40.40% DM). The non- fibrous carbohydrates accounted for 26.45% of the dry matter. Such composition was comparable to many reported ones (Hooda and Jood 2003; Kochhar et al. 2006; Abdouli et al. 2014c).

Autoclaving the seeds at 121°C for 15 min increased levels of ether extract by 58.6% and of NDF by 11.5% and reduced levels of total carbohydrates by 3.8% and of non-fibrous carbohydrates by 27.2 %. Such changes suggested that losses of solids made mainly of non fibrous carbohydrates occurred during the autoclaving process despite the fact that fenugreek seeds were not mixed with water in the autoclave. To the best knowledge of the authors, data are lacking on autoclaving effect on fenugreek seeds constituents. Fenugreek seeds soaking, however, showed variable results. Abdouli et al. (2014 c) reported that soaking fenugreek seeds in distilled water (1:10, w/v) for 24 hs and then in hexane for 24 hs resulted

in 7.04% dry matter loss and that the solubilized matter was made up mostly of cytoplasmic constituents (cell content) and of little fat. Consequently, there was an increase of NDF and CP levels and a low decrease of fat level. Hooda and Jood (2003) found that soaking fenugreek seeds for 12 hs reduced total dietary fiber but not total carbohydrates and crude fiber contents. Mansour and El-Adawy (1994) reported that boiling increased crude protein and carbohydrate and reduced fat and reducing sugars levels. They also reported that toasting followed by boiling reduced fat, carbohydrates and reducing sugars levels. Pre-germination increased levels of ether extract by 43.6%, crude protein by 1.5% and of ADF by 2.9% and reduced total carbohydrates level by 4.0%. The large increase of ether extract was not expected and was not in agreement with the finding of Leila and Ahmed (1983) and of Mansour and El-Adawy (1994) who reported a decrease of total lipid in germinated fenugreek seeds in the dark for 4 and 3 days, respectively. Conflicting results have been reported on the effect of fenugreek seeds germination on their chemical composition. Hooda and Jood (2003) found that germination decreased crude fat, dietary fiber and total carbohydrates thereby raising the level of crude fiber, sugars and crude protein. Nabila et al. (2012) found that fenugreek seed germination tended to decrease its fat, had no effect on crude fiber, decreased total carbohydrates and increased crude protein levels. Consequently, the increase of crude protein and the decrease of carbohydrates levels of fenugreek seeds appear to be common results of their germination. The increased protein content during germination could be attributed to a net synthesis of protein from nitrogen sources not determined as Kjeldahl nitrogen in raw fenugreek seeds. The decreased carbohydrates levels of the germinated seeds might be attributed to an increase of amylase activity which breaks down complex carbohydrates to absorbable sugars needed for the growing seedlings during the early stage of germination.

Table 1. Effect of fenugreek seed processing on its chemical composition (in % dry matter)

Treatments	Control	Autoclaving	Pre-germination	MSE	P-value
Ash	3.96 a	3.80 a	3.92 a	0.076	0.429
Ether extract	5.39 a	8.55 b	7.74 b	0.229	0.005
Crude protein	23.79 a	23.34 a	24.15 b	0.130	0.049
Crude fiber	13.42 a	12.47 a	13.44 a	0.288	0.154
NDF	40.40 a	45.06 b	39.07 a	0.440	0.005
ADF	16.83 a	17.61 b	17.33 b	0.097	0.025
ADL	3.71 a	3.91 a	3.73 a	0.175	0.70
Total carbohydrate [€]	66.86 a	64.30 b	64.18 b	0.260	0.009
Non fiber carbohydrate [£]	26.45 a	19.24 b	26.11 a	0.513	0.004

Total carbohydrate[€] = 100 - (% ash + % Ether extract + % crude protein)
 Non fiber carbohydrate[£] = 100 - (% NDF + % crude protein + % Ether extract + % Ash)
 a,b : means of the same row having the same letter are not different (P>0.05)

3.1.2. Secondary metabolites and carotenoids

The effect of fenugreek seeds processing on their secondary metabolites and carotenoids determined in the present study are shown in table 2. The phenols level in raw fenugreek seeds was higher than those reported by Kochhar et al. (2006) and Hooda and Jood (2003) which were 1.05 and 1.48 mg/g, respectively. It was in agreement with those Abdouli et al. (2014b) who found levels of 5.92 and 6.16 mg/g DM in two fenugreek seeds genotypes. Much higher levels varying from 9.42 to 16.22 mg/g have been reported by Singh et al. (1994). In the current study, tannins level in raw fenugreek seeds was close to those reported by Abdouli et al. (2014b). Total flavonoids level was within the range (1.36 to 2.74 mg/g) found by Singh et al. (1994) and higher than that (0.31 mg/g DM) reported by Abdouli et al. (2014b). Phytic acid in raw seeds was in agreement with value (5.88 mg/g) reported by Hooda and Jood (2003).

The determined secondary metabolites, namely, tannins and phytic acid are considered anti-nutritional factors particularly for non ruminants. They form digestion-resistant complexes with carbohydrate, protein and minerals in the feed and make them unavailable. They also inhibit some digestive enzymes like α -amylase, pepsin and pancreatin. Consequently, several processes, such as soaking, cooking and germination have been used to eliminate or minimize these adverse effects. In the present study, autoclaving did not affect phenols and tannins levels and resulted in a slight decrease of flavonoids level. Phytic acid tended to increase which might be attributed to the increase of the NDF fraction provided that phytic acid was present in the outer husk like in beans, seeds and nuts (Abdoulaye et al.2011). In addition, autoclaving the seeds without direct contact with water in the autoclave and without prior soaking might have prevented phytic acid from leaching out. In this respect, soaking followed by autoclave cooking of common bean reduced its phytic acid content (Nilgun 2011). Mansour and El-Adawy (1994) reported that while boiling had no effect on phytic acid level, toasting followed by boiling reduced it. Pre- germination did not also affect phytic acid level in fenugreek seeds and decreased levels of phenols by 31.5%, tannins by 58.6% and flavonoids by 27.2%. Such finding was partially in agreement with that of Hooda and Jood (2003) who found a decrease in both polyphenols and phytic acid levels. Generally, germination is preceded or combined with soaking in water which decreases phytic acid level due its leaching out into soaking water under the concentration gradient and to its hydrolysis following the activation of the endogenous phytase during the germinating process. Therefore, the lack of phytic acid loss observed in our study remained inexplicable unless the used titration method employing standard Iron (III) Chloride and ammonium thiocyanate solutions was inappropriate.

Table 2. Effect of fenugreek seed processing on its secondary metabolites and carotenoids contents

Treatments	Control	Autoclaving	Pre-germination	MSE	P-value
Total phenols, mg/g DM ^y	7.13 a	7.03 a	4.88 b	0.263	0.015
Total tannins, mg/g DM	3.53 a	3.60 a	1.46 b	0.101	0.001
Total flavonoids, mg/g DM	2.61 a	1.98 b	1.90 b	0.010	0.03
Phytic acid mg/g DM	6.57 a	8.39 a	7.12 a	0.455	0.134
β -carotenes, μ g/g DM	12.12 a	13.08 b	10.72 c	0.177	0.006
Lycopene, μ g/g DM	11.32 a	12.08 b	10.04 c	0.115	0.002

DM^y: dry matter
 a,b ,c : means of the same row having the same letter are not different (P>0.05)

Carotenoids are a group of compounds synthesized by plants and many microorganisms whereas animals have to obtain them from food. They are responsible for different beneficial effects and for the colors. Therefore, it was essential to minimize their loss or structural changes due the fenugreek seeds autoclaving and pre-germination. To determine the sensitivity of such compounds, β -carotenes and lycopene levels were determined and shown in table 2. Levels of both of them were increased with autoclaving by 7.9% and 6.7% and decreased by germination by 11.5% and 11.3% for β -carotenes and lycopene, respectively. Since these levels were estimated using the differences between absorbencies at 450 and 503nm (see materials and methods), it was suggested that autoclaving increased the fraction of substances absorbing at 450 nm at the expense of those absorbing at 503 nm. In contrast to autoclaving, pre-germination decreased the level of substances absorbing at 450 nm. To the best of our knowledge, information in the literature regarding these aspects is scanty. On this subject, Aruna and Baskaran (2010) reported that α -carotene and β -carotene were not detected in fenugreek seed and that their absence might have been related to their complete conversion to lutein which was 0.79 mg/100 g of dry weight.

3.2. Functional properties

Functional properties of a food are those physico-chemical parameters that reflect the interaction between the structure, the molecular conformation and the level of its components. They provide useful information that help to predict how the food may behave in a specific systems. For instance, when measured in conditions resembling animal digestive tract, they may give more light on the food nutritional value and its incorporation rates with other foods.

The effects of autoclaving and pre-germination on some functional properties of ground fenugreek seeds were presented in Table 3. Autoclaving decreased bulk density by 13% and extractable color by 29%. Pre-germination reduced water absorption capacity by 17.8% and extractable color by 73.3 to 74.2% and increased foaming capacity by 37.1%.

The effect of autoclaving on bulk density might be linked to its effect on the NDF fraction which increased from 40.4 to 45.06 % on dry matter basis. Thus, based on bulk density values, occupied space in digestive tract and ingested amounts would be in favor of raw and pre-germinated fenugreek seeds. However, independently of the treatment, bulk density values were within the range of values (0.69 to 0.80 g/ml) for cowpea flour (Appiah et al. 2011). Water absorption capacity values demonstrated that ground fenugreek seeds could bind water at a ratio of about 1:4 to 1:5. This high ability could be attributed to the high carbohydrates and fiber fractions (table 1). The somewhat decrease in water absorption capacity due to autoclaving and pre-germination might be due to the increase in the fat contents as these create lipophilic environment that blocks the water binding sites on other compounds. Regardless of the applied treatment, our water absorption capacity values were lower than those for raw (5.59 g/g), boiled (7.83 g/g), toasted and boiled (6.28 g/g) fenugreek seed (Mansour and El-Adawy 1994), for potato flour (7.52 g/g) and higher than those for germinated fenugreek seed (2.53 g/g) reported by Mansour and El-Adawy (1994), for green gram flour (1.96 g/g), rice flour (1.92g/g) and wheat flour (1.40g/g) reported by Chandra and Samsher (2013). They were also lower than values for cowpea (4.6 - 5.1 g/g) and higher than those for soybean (2.9 - 3 g/g) flour reported by Okaka et al (1979). The high water absorption capacity of the ground fenugreek seeds suggested that these would be highly exposed to digestive agents in the animal. Oil absorption capacity values which ranged from 1.3 to 1.5 g/g in pre-germinated and raw fenugreek seeds, were higher than the 0.73 to 0.85 g/g reported for raw, boiled, toasted and boiled or germinated fenugreek seed (Mansour and El-Adawy 1994). Our values were within the ranges of values (1.46 to 1.68 g/g) for wheat, rice, green gram and potato flour (Chandra and Samsher 2013). The high oil absorption capacity makes the fenugreek seeds suitable carriers of fat soluble vitamins and for flavor enhancement when they are incorporated in food preparations. It also makes them able to absorb/bind bile acids and increase their excretion and, thereby, contributes to reduction of plasma cholesterol level.

With regards to foaming capacity values, which ranged from 15.54% in autoclaved to 29.30% in pre-germinated fenugreek seeds, they were higher than values reported by Chandra and Samsher (2013) for wheat flour (12.92%), rice flour (3.52%) and potato flour (6.84%) as well as for pigeon pea seed flour (9.81%) reported by Oloyo and Akoja (2005). Ours values appeared to vary with their corresponding crude protein dispersibility indexes (table 5). Protein in the dispersion may reduce surface tension at the interface between air bubbles and the surrounding liquid and thereby increase foam formation. The foaming capacity values might have been also influenced by saponins levels in fenugreek seeds. Observed foams were stable since 27.6 to 39.6% remained at 120 min (table 3).

The swelling capacity (table 3) and the least gelation concentration (table 4) determined in the present study were the most striking functional properties of fenugreek seeds irrespective of the treatment applied. The swelling coefficients, denoted by the percentage increase of ground fenugreek seeds volume after soaking in distilled water and standing for 8 or 120min, approached the 300 and 350%, respectively. The least gelation concentrations were less than 0.01 % (w/v) regardless of the treatment. Even at this low concentration, the firmness of the formed gel was noted as low, moderate or high for raw, autoclaved and pre-germinated ground fenugreek seed, respectively. The high swelling capacity and low gelation concentration of ground fenugreek seeds demonstrated their ability to absorb water to swell and to form gel. The extensive gel formation and the low viscosity of the resulting gels inside the intestine have been suggested to delay gastric emptying, to decrease intestinal transit time of the food mass and to slow

leaching of glucose trapped inside the gel and, thereby, prevent the sudden raise of blood-glucose level and beneficially influence certain blood lipids (Krishna Kumar and Maliakel 2008). Such functional properties ought to be due mostly to the known fenugreek seeds gum content and quality. Gum (galactomannan or mucilage) which represents the major polysaccharide found in fenugreek seeds and accounts for 17 to 30 % of the seed weight (Kochhar et al.2006) acts as a thickener or stabilizer in foods. It constitutes the major soluble fiber fraction which is fermentable in the large intestine by the action of colonic bacteria to produce short chain fatty acids and, thereby, serves as a prebiotic and contributes toward a variety of health benefits (Wong et al. 2006).

In regard to the effect of processing fenugreek seeds on their extractable color, data in table 3 showed that extractable color values expressed in ASTA units were higher than those expressed as percentage of the color of a potassium dichromate standard solution. They also showed that both autoclaving and pre-germination reduced the color intensity and the reductions due to pre-germination were more substantial. These effects did not match those on β -carotene and lycopene levels which increased by autoclaving and decreased by pre-germination (table 2). Although carotenoids profiles can be used to evaluate extractable color, other compounds like saponins and polyphenols may largely contribute to fenugreek seeds color. On the other hand, the difference in extraction solvents used for carotenoids determination (hexane-ethanol-acetone) and for color determination (2-propanol) may explain the mismatch of carotenoids levels to extractable color results. According to the extractable color results, it appeared that fenugreek seeds, particularly pre-germinated, would not be suited source for enriching other products with pigments.

Table 3. Effect of fenugreek seed processing on its functional properties

Treatments	Control	Autoclaving	Pre-germination	MSE	P-value
Bulk density, g DM [§] /ml	0.69 a	0.60 b	0.67 a	0.007	0.007
Water absorption capacity, g/g DM	4.72 a	4.19 ab	3.88 b	0.122	0.037
Oil absorption capacity, g/g DM	1.50 a	1.42 a	1.30 a	0.083	0.377
Foaming capacity, % volume	21.37 a	15.54 a	29.30 b	1.354	0.012
Foaming stability at 90min,%	45.97 a	36.38 a	47.68 a	2.731	0.111
Foaming stability at 120min,%	39.63 a	27.56 a	33.31 a	2.109	0.061
Swelling coefficient at 8 min,%	305.0 a	295.0 a	305.0 a	5.00	0.385
Swelling coefficient at 120 min, %	350.0 a	355.0 a	350.0 a	2.886	0.465
Extractable color, ASTA20	8.33 a	5.91 b	2.15 c	0.36	0.003
Extractable color, standardized [£]	0.45 a	0.32 b	0.12 c	0.020	0.003

DM[§]: dry matter
 Extractable color, stanardized [£] : percentage of the color of the potassium dichromate standard solution (0.5 mg / ml of 1.8 M sulfuric acid)
 a,b ,c : means of the same row having the same letter are not different (P>0.05)

Table 4. Effect of fenugreek seed processing on its least gelation concentration

Treatments	Ground fenugreek seed concentrations (w/v,%)				
	0.01	0.02	0.03	0.04	0.05
Control	H [£]	H	H	H	H
Autoclaving	L-M	H	H	H	H
Pre-germination	H	H	H	H	H

L, M, H[£] = The firmness of the gel was noted as low, moderate or high, respectively.

3.3. Nutritional properties

3.3.1. Protein quality

The main objective of processing fenugreek seeds was to inactivate and/or minimize the anti-nutritional factors they might contain. Since process using excessive heat may result in lowering protein availability and despite the fact that we avoided drying at above 50 °c, it was necessary to evaluate protein quality. The approach was to use an indirect test, namely the protein dispersibility index, as well as in vitro digestibility by pepsin alone and by pepsin- trypsin. The results of such measurements (table 5) showed that autoclaving reduced protein dispersibility index, had no effect on digestibility by pepsin and increased digestibility by pepsin-trypsin. The reduction of protein dispersibility index suggested that the autoclaving conditions (121 °C for 15 min) might have been excessive causing dissociation of protein quaternary structures, denaturation of their subunits, and promoting the formation of protein aggregates via electrostatic, hydrophobic and disulfide interchange mechanisms like in the case of soy protein heated at above 80°C (Caprita et al. 2010). However, protein digestibility values were high and increased by pepsin-trypsin suggesting a reduction in trypsin inhibitor activity due to autoclaving. Pre-germination enhanced protein dispersibility index and did not affect protein digestibility. Digestibility coefficients in the present study were much higher than those reported by Hooda and Jooda (2003) for raw, soaked and germinated fenugreek seeds which varied from 58.50 to 65.60%. However, in their study, digestibility assessed by employing pepsin and pancreatin, was increased after 48 hs germination. Likewise, Mansour and El-Adawy (1994) reported that boiling, toasting followed by boiling and germination of fenugreek seeds improved protein digestibility by trypsin, pepsin, pancreatin and trypsin-pancreatin.

3.3.2. Energy values

In view of the observed effects of processing fenugreek seeds on their gross chemical compositions, it appeared opportune to evaluate the impact of such process on calculated digestible energy for rabbits and metabolizable energy for poultry using published equations and various determined chemical components contents as predictive variables. Data shown in table 5 indicated that raw fenugreek seeds digestible energy was quite high (3152.91 Kcal/kg DM) and increased by 6.2% due to increased ether extract level for autoclaving and by 3.8% due to increased crude protein and ether extract levels for pre-germination. Likewise, raw fenugreek seeds metabolizable energy values were quite high varying from 2374.91 to 2652.89 Kcal/ kg DM and increased by 4 to 4.7% for autoclaving and by 4.3 to 6.7% for pre-germination.

Table 5. Effect of fenugreek seed processing on its nutritional properties					
Treatments	Control	Autoclaving	Pre-germination	MSE	P-value
Protein dispersibility index	34.78 a	10.25 b	45.39 c	1.394	0.001
Protein digestibility-Pepsin,%	75.84	74.12	77.48	0.609	0.066
Protein digestibility-Pepsin & trypsin,%	82.31 a	85.91 b	83.41 a	0.334	0.010
Digestible energy [¥] , kcal/kg DM	3152.91 a	3347.16 b	3273.08 c	8.411	0.001
Metabolizable energy (1) [£] , kcal/kg DM	2374.91 a	2485.55 b	2534.17 b	17.677	0.017
Metabolizable energy (2) [§] , kcal/kg DM	2652.89 a	2760.30 b	2831.78 c	11.607	0.004
Metabolizable energy (3) [€] , kcal/kg DM	2610.99 a	2723.04 b	2782.18 c	11.232	0.004

a,b ,c : means of the same row having the same letter are not different (P>0.05)
 Digestible energy [¥] = [12.912 – 0.0236CF + 0.010CP + 0.020EE]/0.004187; (Wiseman et al.1992);
 Metabolizable energy (1)[£] = 2707.71 + 5.863EE– 1.606NDF ; (Nascimento et al.2007)
 Metabolizable energy (2)[§] =4101.33+5.628EE–23.297ASH–2.486NDF+1.042ADF; (Nascimento et al.2009)
 Metabolizable energy (3)[€] =4095.41 + 5.684EE–22.526ASH–2.224NDF; (Nascimento et al.2009)

4. Conclusion

Considering the data obtained herein and the above discussion, it can be concluded that fenugreek seeds can serve as a valuable nutritional and functional food. Autoclaving and pre-germination improved such potential and need to be further evaluated and implemented in In Vivo studies.

Acknowledgements. The authors would like thank **Dr Zouar Kamel** for kindly providing fenugreek seeds as the working material . The also thank **Mr. Lassaad Tayachi** for the help in some analyses.

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