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Effect of Germination and Heat Treatment on Chemical Composition and Bioactive Components of Fenugreek Seeds

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Abstract: Fenugreek (*Trigonella foenum-graecum*) can be considered as a good source of many nutrients such as protein, dietary fibers, minerals and polyphenols along with high antioxidant activity but despite its promised nutritional value most people do not prefer it in their diets due to presence of alkaloids and some volatile compounds which are responsible for bitterness and bad odor sweat. So that present study aimed to estimate the effect of germination process and heat treatment on chemical composition, minerals, polyphenols, ascorbic acid, antioxidant activity, dietary fibers, alkaloids and volatile compounds in fenugreek seeds. Data confirmed that fenugreek is a good source of protein, minerals and polyphenols whereas the last is responsible for a high antioxidant activity of fenugreek. Germination of fenugreek seeds resulted in increment of protein, fibers, ash, P, Na, Ca, Zn, polyphenols and antioxidant activity but decreased each of fat, nitrogen free extract, K, Mg, Fe and alkaloids. Meanwhile, boiling of fenugreek seeds increased each of nitrogen free extract, alkaloids and decreased each of protein, ash, P, Ca, Zn, dietary fibers, polyphenols, ascorbic acid and antioxidant activity compared with raw fenugreek seeds. Boiled fenugreek seeds possessed the highest number of separated volatile compounds followed by raw and germinated fenugreek seeds respectively. Data in present study confirmed ability of germination process to increment of nutritional value of fenugreek seeds and decreasing each of alkaloids and volatile compounds to produce a fenugreek seeds more suitable for consumers.

Key words: Antioxidant activity · Alkaloids · Fenugreek seeds · Germination · Heat treatments · Nutritional value · Polyphenols · Volatile compounds

INTRODUCTION

Many traditional foods, especially plant foods, are reported to possess biological properties that can be of benefit to human health. An example of this is the leguminous plant fenugreek *(Trigonella foenum-graecum* L) [1]. Fenugreek is originally from southeastern Europe and western Asia, but today it is grown in many parts of the world, including India, northern Africa and the United States [2]. It is an annual plant belongs to the family *Leguminosae* and it is the famous spices in human food. Since ancient times the green leaves and seeds of fenugreek have been used in food for many medicinal and therapeutic purposes. It has been used to enhancement the color, flavor and texture of food materials [3]. Fenugreek has a beneficial effect on cleansing the blood and as a diaphoretic, it is able to bring a sweat and help in detox the body. The alkaloids and flavonoid content of fenugreek seeds can be responsible for antinociception and anti-inflammatory effects of the plant respectively [4]. Fenugreek is also known for its lymphatic cleansing activity due to its vital role in supplying the cells with many nutrients and removing toxic wastes, trapped proteins and dead cells from the body. Seeds of fenugreek spice have many other medicinal properties such as hypocholesterolemic, antibacterial, gastric stimulant, lactation help, antidiabetic agent, hepatoprotective effect and anticancer [5, 6]. These useful physiological effects including the antidiabetic and hypocholesterolemic effects of fenugreek are mainly attributable to the presence of dietary fiber which has promising nutraceutical value [3, 7, 8].

Fenugreek seeds are rich source of gum, fibers, alkaloids, flavonoids, volatile compounds, phenolic acids and polysaccharides [9]. Due to its high content of fiber, fenugreek could be used as food stabilizer, adhesive and

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emulsifying agent to change food texture to be more suitable for some special purposes [10]. Dietary fiber from fenugreek blunts glucose after a meal but the mechanisms for these effects have not been fully elucidated, also fenugreek seeds contain the gum is composed of galactose and mannose and these compounds are associated with reduced glycemic effect. The hypoglycemic effect of fenugreek has been especially documented in humans and animals with type 1 and type 2 diabetes mellitus [11]. Soluble fiber lowers serum cholesterol and helps to reduce the risk of heart attack and colon cancer. It dissolves in the gut to form a viscous gel that lowers the absorption of released glucose [12]. Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicine are important [13]. Plant drugs [14] and herbal formulation [15] are frequently considered to be more free from side effects and less toxic than synthetic one.

Germination starts when the dry seed begins to take up water and is completed when the embryonic axis elongates. At this point reserves within the storage tissues of the seed are mobilized to support seedling growth [16]. From the moment the seed breaks dormancy, protective responses emerge through the synthesis of phenolics and other compounds [17]. During germination stage the level of phenolic antioxidants is optimized and be attractive for the growth of edible sprouts with enhanced nutraceutical properties. Changes in phenolic synthesis and antioxidant activity would indicate seed preparation towards adverse conditions but variation in the level of phenolics, especially phenolic antioxidants throughout germination is not clear [18]. Germination process and heat treatments have an important effect on nutraceutical properties of all leguminous plants so that present study aimed to investigate the effect of boiling and germination processes on chemical composition, minerals, polyphenols, ascorbic acid, antioxidant activity, alkaloids, dietary fibers and identification of volatile compounds in boiled and germinated fenugreek seeds compared with raw one.

MATERIALS AND METHODS

Materials: Fenugreek (*Trigonella foenum-graecum* L.) seeds used in the present study were obtained from the local market in Alexandria, Egypt. The seeds were cleaned and directly used.

Methods:

Germination of Fenugreek Seeds: Fenugreek seeds were soaked in tap water (1:3 v/v seeds to water) at room temperature (25-28°C) for 6 hours. The soaked seeds were spread on wet cotton cloth in stainless steel baskets. A wooden cover was used to minimize moisture loss and also to protect the seeds from light during germination. Germinated seeds were collected at 2, 4 and 6 days of germination and prepared for organoleptic tests to estimate preferable germination time.

Heat Treatment of Fenugreek Seeds: Fenugreek seeds were put in a 2 L beaker with 1 L of distilled water and boiled for 15 min at 90 °C using a hot plate and directly cooled to room temperature.

Preparation of Fenugreek Seeds: The germinated and boiled fenugreek seeds were dried in an electric air drought oven at 40 °C for 24 hours. The obtained dried seeds were powdered by Braun grinder to obtain 40 mesh size and packaged in polyethylene bags, then kept in a deep freezer at -16 °C until used.

Proximate Chemical Composition: Moisture, ash, crude protein (N × 6.25) crude fat and crude fibers were determined as described in the AOAC [19]. Nitrogen free extract (NFE) was calculated by difference.

Minerals Content: P, Ca, Mg, Fe, Zn and Cu were determined according to the method described in AOAC [19] which were determined using Perkin-Elmer 2380 Atomic Absorption Spectrophotometer. The Na and K were estimated by flame photometer (Model PEP7, England) 410°C (Corning).

Alkaloids: Alkaloids were determined according to Harborne method [20]; 5 g of the minced fenugreek seeds were weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered then allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloids, which was dried and weighed.

Dietary Fibers: Contents of neutral detergent fibre (NDF) were determined according to Van Soest *et al*. [21] using heat-stable á-amylase and sodium sulphite. Acid detergent fibre (ADF) was determined according to

Goering and Van Soest [22]. Acid detergent lignin (ADL) was analysed by solubilisation of cellulose with sulphuric acid (720 ml/l) according to the method of Van Soest [23]. The fibre measurements were sequentially performed by using the ANKOM 220 Fibre Analyser unit (ANKOM Technology Corporation, Macedon, NY, USA) using the same sample in filter bags and expressed exclusive of residual ash.

Ascorbic Acid: Ascorbic acid was determined using 2,6 dichlorophenol indophenol dye (the dye from BDH company) according to AOAC method [19], except that 4% oxalic acid in 8% glacial acetic acid was used as an extraction media for samples [24]. Ascorbic acid was calculated as mg/100g dry weight.

Polyphenols Content: Polyphenols were determined colorimetrically by Folin-Denis reagent after extraction with methanol containing 0.1% HCl according to AOAC method [19]. Polyphenols in studied fenugreek seeds were calculated as mg gallic acid /100g dry weight.

Antioxidant Activity: Antioxidant activity was measured according to Fogliano *et al*. [25] by the N, N- Dimethyl-p-phenylenediamine dihydrochloride (DMPD). The half-inhibition concentration (IC_{50}) is the concentration of antioxidants required to reduce the absorbance of DMPD free radical to half of its initial value. The IC_{50} values were calculated according to Farhadi *et al.* [26].

Extraction and Identification of Volatile Compounds: Extraction and identification of volatile compounds in raw, boiled and germinated fenugreek seeds were carried out by gas chromatography coupled to ion-trap mass spectrometry (GC-IT-MS) according to Mebazaa *et al*. [27].

Organoleptic Properties: Color, taste, odor, texture and overall acceptability of germinated fenugreek samples were evaluated by fifteen panelists of Food Science and Technology Department, Faculty of Agriculture, Alexandria University, Egypt, using a hedonic scale rating of 1-9 (1=dislike very much, 9=like very much) as described by Kramer and Twigg [28]. Sensory evaluation was carried on fenugreek samples germinated for 2, 4 and 6 days to choose the more prefer germinated time.

Statistical Analysis: Data were statistically analyzed using Randomized Complete Block Design (R.C.B.D). Comparisons between means were carried out using least significant difference at 0.05 probability level (LSD 0.05) according to Steel and Torrie [29].

Table 1: Sensory evaluation of germinated fenugreek seeds

	Germination period		
	2days	4days	6days
Color	$7.60^{a}\pm0.69$	$7.90^{a}\pm0.73$	$6.10^{b}\pm1.37$
Taste	$7.60^{ab}\pm0.51$	$8.00^{a}\pm1.05$	$7.10^{b}\pm1.10$
Odor	$7.60^{ab}\pm0.84$	$8.20^{a}\pm0.63$	$7.00^{b}\pm0.66$
Texture	$7.60^{b}\pm0.51$	$8.20^{a}\pm0.63$	$7.20^{b}\pm0.63$
Overall acceptability	$7.80^{a}\pm0.63$	$8.20^{a}\pm0.78$	$6.40^{b}\pm0.51$

Means in a row followed by the same letter are not significantly different at ($p\leq 0.05$).

RESULTS AND DISCUSSION

Table (1) shows the sensory evaluation of germinated fenugreek seeds for various time (2, 4 and 6 days) which occurred to select the most appropriate germination time for panelists, the results of sensory evaluation reflected that germinated fenugreek seeds for 4 days were more preferred by panelists in most of sensory properties so fenugreek seeds germinated for 4 days were chosen to complete the study along with raw and boiled fenugreek seeds to evaluate the effect of germination and boiling processes on chemical composition, mineral content, dietary fibers, bioactive and volatile compounds in fenugreek.

Effect of Heat Treatment and Germination on Proximate Chemical Composition of Fenugreek Seeds: The results shown in Table (2) reflect the effect of heat treatment (boiling for 15 min at 90 °C) and germination for 4 days on chemical composition of fenugreek seeds. Present data illustrate that all studied fenugreek seeds contained relatively high amount of protein ranged from 22.85±0.18 to 32.02±0.05 % (on dry weight) whereas germinated fenugreek contained the highest amount of protein followed by raw and boiled ones respectively. Fats were found in a considerable but not highly amount in all studied fenugreek seeds whereas the percentages of fats were 8.19±0.16, 5.32±0.14 and 4.72±0.09% (on dry weight basis) in raw, boiled and germinated fenugreek seeds respectively. Germinated fenugreek contained the highest amount of fibers followed by raw and boiled ones respectively. Nitrogen free extract were found in the highest concentration in boiled fenugreek seeds reached 59.86±0.57% followed by raw fenugreek (53.83±0.44%) whereas germinated fenugreek possessed the lowest amount (48.32±0.23% on dry weight basis). From obtained results it could be concluded that germination of fenugreek seeds led to increase each of protein, fibers and ash but decreased fat and nitrogen free extract. Meanwhile boiling process decreased each of protein and

Table 2: Gross chemical composition of raw, boiled and germinated fenugreek seeds (on dry weight basis)

Component (%)	RF	BF	GF
Protein	27.11^{b}±0.16	22.85^{c}±0.18	32.02^{a}±0.05
Fat	8.19^{a}±0.16	5.32^{b}±0.14	4.72^{c}±0.09
Fibers	7.06^{c}±0.16	8.70^{b}±0.26	10.55^{a}±0.12
Ash	3.81^{b}±0.33	3.27^{c}±0.10	4.39^{a}± 0.06
Nitrogen free extract	53.83^{b}±0.44	59.86^{a}±0.57	48.32^{c}± 0.23

Means in a row followed by the same letter are not significantly different at ($p \leq 0.05$).

RF: Raw fenugreek seeds

BF: Boiled fenugreek seeds

GF: Germinated fenugreek seeds

Table 3: Minerals composition of raw, boiled and germinated fenugreek seeds (mg/100 g on dry weight basis)

	RF	BF	GF
P	261.83^{b}±1.75	120.46^{c}±1.68	446.76^{a}±1.43
K	816.50^{a}±2.40	730.80^{b}±2.4	511.80^{c}±2.80
Na	115.16^{b}±1.15	116.76^{b}±2.5	135.10^{a}±1.80
Ca	132.93^{b}±2.8	93.50^{c}±1.37	147.90^{a}±1.35
Mg	222.76^{a}±2.13	183.16^{b}±2.85	161.80^{c}±1.87
Fe	17.86^{a}±1.59	14.93^{b}±1.42	9.20^{c}± 0.65
Zn	4.06^{b}±0.88	2.17^{c}±0.40	5.27^{a}± 0.31
Cu	1.86^{b}±0.05	2.06^{a}±0.25	2.21^{a}±0.27

Means in a row followed by the same letter are not significantly different at ($p \leq 0.05$).

RF: Raw fenugreek seeds

BF: Boiled fenugreek seeds

GF: Germinated fenugreek seeds

ash but increased nitrogen free extract. Obtained results are in agreement with Abd El Aal and Rahma [30] they found that germination of fenugreek seeds for 3 and 5 days increased crude protein, crude fibers and ash contents, but total lipids and carbohydrates were decreased. Also results in present study are in accordance with those reported by US Department of Agriculture [31] where it reported that fenugreek contains 6–7% fat, 23–26% protein, 58% carbohydrates of which about 25% is dietary fiber. Also Isikli and Karababa [32] reported that fenugreek has a high proportion of protein ranging from 20 to 30%. In addition Naidu *et al*. [33] stated that chemical composition of fenugreek (g/100g) was total ash 3.9, protein 27.57 and fat 6.71% (on dry weight). Norziah *et al*. [34] found that during the process of germination, increase in protein occurred.

Effect of Heat Treatment and Germination on Minerals Composition of Fenugreek Seeds: Data presented in Table (3) show the minerals composition of boiled and germinated fenugreek seeds compared with the raw seeds. From presented data it could be concluded that there were a significant differences between studied fenugreek samples in minerals contents which confirmed the influence of boiling and germination processes on minerals composition of fenugreek. As is evident from the Table all studied fenugreek seeds contained a high amounts of K ranged from 511.80±2.8 to 816.50±2.4 and a considerable amount of P ranged from 120.46±1.68 to 446.76±1.43, Na from 115.16±1.15 to 135.10±1.8 and Ca from 93.50±1.37 to 147.90±1.35 (mg/100g dry weight) but they contained a little amounts of Fe, Zn and Cu. Germination of fenugreek seeds resulted in a significant increment in P, Na, Ca, Zn and Cu and a significant reduction in K, Mg and Fe compared with the raw fenugreek seeds, meanwhile boiling of fenugreek seeds resulted in a significant decrease in most of minerals specially P,Ca and Zn. Results obtained for minerals composition are in agreement with Winton and Winton [35] who stated that fenugreek seeds are found to contain high levels of minerals, e.g. 270 mg/100g total phosphorus, 160 mg/100g calcium, 530 mg/100 g potassium and 14.1 mg/100g iron but my determination of sodium was higher than them where they found 19 mg sodium in 100 g dry seeds.

Effect of Heat Treatment and Germination on Alkaloids and Dietary Fibers Contents of Fenugreek Seeds: The effect of boiling and germination processes on each of alkaloids and dietary fibers contents of fenugreek seeds is given in Table (4). Data in this Table reflect that all studied fenugreek seeds contained a considerable amount of alkaloids (which responsible for bitter taste of fenugreek) the alkaloids content in studied seeds possessed the highest concentration in boiled fenugreek seeds reached 3.18±0.11% (on dry weight basis) meanwhile the lowest concentration of alkaloids was found in germinated seeds (1.16±0.11 % on dry weight) which reflected the ability of germination process to decrease alkaloids and consequently bitterness in fenugreek seeds by 52%. In contrast of germination seeds a significant increase in alkaloids concentration was noted in boiled fenugreek seeds whereas the alkaloids content was increased by 31.4%, this increment perhaps accrued due to destruction of alkaloids within boiling and repolymerization again. In regards of my results Hooda and Jood [36] concluded that nutritional quality can be improved and bitterness reduced in fenugreek seeds by processing. Meanwhile Chauhan *et al*. [37] showed that the fenugreek seeds contain a variety of alkaloids and saponins.

Table 4: Alkaloids and dietary fibers of raw, boiled and germinated fenugreek seeds (on dry weight basis)

Component (%)	RF	BF	GF
Alkaloids	2.42^{b}±0.12	3.18^{a}±0.11	1.16^{c}±0.11
Dietary fibers:-			
Neutral detergent fibers	17.12^{a}±0.11	7.61^{c}±0.50	14.81^{b}±0.70
Acid detergent fibers	17.20^{a}±0.18	6.5^{c}±0.09	8.29^{b}±0.34
Acid detergent lignin	15.12^{a}±0.07	2.33^{c}±0.12	3.32^{b}±0.20

Means in a row followed by the same letter are not significantly different at ($p \leq 0.05$).

RF: Raw fenugreek seeds

BF: Boiled fenugreek seeds

GF: Germinated fenugreek seeds

Table 5: Polyphenols, Ascorbic acid and antioxidant activity of raw, boiled and germinated fenugreek seeds

Component	RF	BF	GF
Polyphenols (mg gallic acid/100g)	1186.00^{b}±3.2	849.00^{c}±2.4	1815.00^{a}±3.5
Ascorbic acid (mg/100g)	19.55^{a}±0.40	3.44^{c}±0.14	11.52^{b}±0.79
Antioxidant activity			
a-Inhibition of DMPD (%)	56.30^{b}±0.92	53.30^{c}±1.17	68.30^{a}±1.08
b- IC_{50} (mg/ml)	0.24^{b}±0.02	0.44^{a}±0.04	0.09^{c}±0.01

Means in a row followed by the same letter are not significantly different at ($p \leq 0.05$).

RF: Raw fenugreek seeds

BF: Boiled fenugreek seeds

GF: Germinated fenugreek seeds

Dietary fibers consisting of non-digestible carbohydrates and lignin that are intrinsic and intact in traditional plants, has received much attention due to its health benefits [38]. Present study concerned with presence of dietary fibers in fenugreek seeds and how they affected by studied treatments. Data in Table (4) confirm that fenugreek seeds can be considered as a good source of dietary fibers. Raw fenugreek seeds contained the highest amount of neutral detergent fibers (17.12±0.11%), also the highest content of acid detergent fibers (17.20 ±0.18%) and acid detergent lignin (15.12±0.07%) followed by germinated and boiled fenugreek seeds respectively. In accordance of my results, Srinivasan [3] stated that fenugreek seeds are an excellent source of dietary fibers and have been shown to lower blood glucose in various human and animal model studies and exert beneficial effects on serum lipid profile. About the importance of dietary fibers Abou El-Soud *et al*. [39] reported that these days' fenugreek seeds are used as food stabilizer, adhesive and emulsifying agent due to its high fibers, protein and gum content. Moreover, Roberts [11] also reported that fenugreek seeds contain 45.4% dietary fiber (32% insoluble and 13.3% soluble). Meanwhile Meghwal and Goswami [10] found that dietary fibers of fenugreek seeds is about 25% which changes the texture of food.

Effect of Heat Treatment and Germination on Ascorbic Acid, Polyphenols and Antioxidant Activity of Fenugreek Seeds: Ascorbic acid and polyphenols contents along with antioxidant activity of raw, boiled and germinated fenugreek seeds are presented in Table (5). Data presented in this Table illustrat that all studied fenugreek seeds contained a significantly high content of polyphenols ranged from 849.00±2.4 to 1815.00±3.2 mg gallic acid /100g dry weight. Germinated fenugreek seeds possessed the highest concentration of polyphenols followed by raw and boiled fenugreek seeds respectively. Data obtained from present study confirmed the effect of germination process on polyphenols increment in fenugreek which indicates that a new polyphenols synthesis may be occurred within germination of fenugreek seeds. In contrary of polyphenols a significant decrease was noted in ascorbic acid due to boiling and germination of fenugreek seeds compared to the raw ones, whereas the raw fenugreek seeds showed the highest content of ascorbic acid followed by germinated and boiled fenugreek seeds respectively. All studied fenugreek seeds showed a high antioxidant activity whether germinated or raw and boiled seeds, whereas germinated fenugreek samples showed the highest value of DMPD inhibition (68.30±1.08%) and lowest value of IC_{50} (0.09±0.01 mg/ml) which reflected high antioxidant activity of germinated seeds followed by raw seeds (56.30 ±0.92%), (0.24±0.02 mg/ml) and boiled fenugreek seeds (53.30±1.17%), (0.44±0.04mg/ml) respectively. Obtained results confirmed the highly significant amount of polyphenols in fenugreek seeds which responsible for a high antioxidant activity of it, germination process increased polyphenols and antioxidant activity but decreased ascorbic acid, meanwhile heat treatment represented in boiling process decreased each of polyphenols, antioxidant activity and ascorbic acid compared with the raw seeds. Results of present study in regards to ascorbic acids, polyphenols and antioxidant activity are in agreement with other researches whereas El-Mahdy and El- Sebaiy [40] found that fenugreek seeds contain 12.00 mg vitamin C /100g dry weight. Jani *et al*. [41] stated that there are nearly 7.4 and 10.8% loss of the vitamins by steaming and frying or boiling in water of fenugreek, respectively. Meanwhile Meghwal and Goswami [10] found that boiling in water of fenugreek loses 10.8% of the vitamin C content. Also Buba *et al*. [42]

found that vitamin C content in fenugreek had values of 10.54 mg/100 g dry weight. There are many of scientists studied polyphenols and antioxidant activity of fenugreek seeds, Balch [43] suggested that fenugreek has powerful antioxidant property, since antioxidant properties have been linked to health benefits of natural products, such properties are studied with germinated fenugreek seeds which are observed to be more beneficial than dried seeds because germination process can increase the bioavailability of different constituents of fenugreek [2,44]. Meanwhile Dixit *et al*. [45] showed that germinated fenugreek seeds exhibit high antioxidant activity. Also Cevallos-Casals and Cisneros-Zevallos [18] reported that there were a noticeable increase in antioxidant activity of fenugreek seed with germination due to increase in phenolic synthesis. Moreover Chatterjee *et al*. [46] and Benayad *et al*. [47] found that germinated fenugreek seeds could be considered as a rich source of bioactive phenolic compounds. In regards of amount of polyphenols. Kratchanova *et al*. [48] found that polyphenols in fenugreek seeds were 1692 and 1445 mg/100 g in acetone and water extraction respectively. Also Pandey and Awasthi [49] have shown that phenolic content of soaked, germinated and roasted fenugreek seed flours was significantly higher than raw fenugreek seed flour. Also Khole *et al*. [50] reported that germination of fenugreek seeds increased its nutritive value due to significant increase in the bioavailability of its constituents. Hence the change in the phytochemical composition could be the reason for the increased antioxidant activity throughout germination, caffeic acid and its derivatives detected in germinated fenugreek seeds, which have not been reported in ungerminated fenugreek seeds. Norziah *et al*. [34] found that % inhibition of DPPH for fenugreek methanolic extract were 64.04 and 67.95% for raw and germinated seeds with IC_{50} 0.361 and 0.04 mg/ml respectively.

Effect of Heat Treatment and Germination on Volatile Compounds in Fenugreek Seeds: Presence of volatile substances in the fenugreek seeds is responsible for bad odor of the sweat which prevents a large number of people from using fenugreek in their meals despite its many benefits. So that this part of study concerned with separation and identification of the volatile compounds from boiled and germinated fenugreek seeds along with raw ones. Data in Table (6) show the separated volatile compounds from raw, boiled and germinated fenugreek seeds by gas chromatography-ion trap mass spectrometry analysis. From present data it could be concluded that there were many of volatile compounds separated from raw, boiled and germinated fenugreek seeds. Ten chemical volatile compounds were separated from raw fenugreek seeds at various retention times. The dominant volatile compounds found in raw fenugreek seeds were á-Sitosterol (31.75%); Dextroamphetamine (19.05%); 9,19-Cyclolanost-24-en-3-ol,acetate (15.69 %); Hydrocortisone Acetate (9.41%); Bufa-20, 22-dienolide14, 15-epoxy-3, 16-

Table 6: Volatile compounds in raw, boiled and germinated fenugreek seeds

	Raw fenugreek seeds	
Compounds	Concentration (%)	RT(min)
Dextroamphetamine	19.05	2.31
Benzenemethanol, à-(chloromethyl)	2.46	3.02
2-Methylpyrrolidine	5.72	19.44
Bufa-20,22-dienolide, 14,15-epoxy-3,16-dihydroxy-,(3á,5á,15á,16á)	6.05	73.01
Astaxanthin	4.93	73.30
á-Sitosterol	31.75	74.12
4a-Phorbol 12,13-didecanoate	2.19	74.34
psi.,.psi.-Carotene, 1,1, 2,2 -tetrahydro-1,1'-dimethoxy	2.75	74.46
9,19-Cyclolanost-24-en-3-ol, acetate	15.69	74.99
Hydrocortisone Acetate	9.41	75.73
Boiled fenugreek seeds		
Dextroamphetamine	12.19	2.28
Ethyl 2-hydroxybenzyl sulfone	2.13	2.99
(2,2-Dimethylcyclobutyl)methyl amine	6.58	17.37
2-Propen-1-amine, N-ethyl	4.77	19.42
9-Octadecenamide, N-propyl-	1.66	23.75
Estragole	8.87	28.53
Phenol,2-methoxy-6-(1-propenyl)-	1.89	51.31
Butanoic acid, 2-methyl-, 4-methoxy-2-(3-methyloxiranyl)phenyl ester	1.51	52.45
Octadecanoic acid, 4-hydroxy-, methyl ester	1.54	56.45
Astaxanthin	1.42	71.46
[1,3]Benzodioxolo[5,6-e][2]benz azecin-14(6H)-one,5,7,8,15-tetrahydro-3,4-dimethoxy-6-methyl-	1.47	71.91
Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	2.56	72.48
Ethyl iso-allocholate	4.04	73.01
Bis(trimethylsilyl) lorazepam	2.50	73.30
á-Sitosterol	23.40	74.12
Lycoxanthin	1.62	74.67
Betulin	10.43	75.03
Rhodopin	11.78	75.73
Germinated fenugreek seeds		
Dextroamphetamine	10.21	2.31
2-Butanone, 3-hydroxy-	4.15	2.56
Azafrin	5.77	73.01
á-Sitosterol	42.71	74.12
Betulin	23.67	75.04
á-Sitosterol acetate	13.50	75.73

dihydroxy-, (3á,5á,15á,16á) (6.06%); 2-Methylpyrrolidine (5.72%); Astaxanthin (4.93 %) and slightly little amount of Benzenemethanol à-(chloromethyl); 4a- Phorbol12, 13-didecanoate and psi., psi-carotene, 1,1,2,2-terrahydro-1 1'-dimethoxy. Meanwhile a total of eighteen volatile compounds were separated and identified in boiled fenugreek seeds whereas the dominant compounds were á-Sitosterol (23.40%); Dextroamphetamine (12.19); Rhodopin (11.78%); Betulin (10.43%); Estragole (8.87%) and 2,2-(Dimethylcyclobutyl) methyl amine (6.56%). In regards to germinated fenugreek seeds there were only six volatile compounds were separated from seeds extract whereas germinated fenugreek possessed the lowest number of separated volatile compounds. Chemical compounds separated from germinated seeds were á-Sitosterol; Betulin; á-Sitosterol acetate; Dextroamphetamine; Azafrin and 2-Butanone, 3-hydroxy-. Present data could be shown that á-Sitosterol and dextroamphetamine compounds were found in three investigated fenugreek seeds in high concentration but there is a difference in other separated components whereas boiled fenugreek possessed the highest number of separated compounds and the germinated fenugreek had the lowest number this means that germination process can reduce the presence of volatile compounds in fenugreek seeds which represent the big problem in fenugreek consumption meanwhile heat treatment increase the number of volatile compounds may be due to decomposition of the high molecular compounds to small ones or reconfiguration of the last to new compounds. In accordance of my data Girardon *et al*. [51] identified 39 different compounds including *n*-alkanes, sesquiterpenes and some oxygenated compounds in the volatile oil of fenugreek seeds. Mazza *et al*. [52] reported that despite fenugreek interesting properties, it use remains limited because, after ingestion, this plant leads to bad taste in cattle's meat and milk, as well as a strong odor in human's sweat and urine (odor of ''maple syrup" urine disease). Meanwhile Faeste *et al.* [53] stated that alkaloids and volatile compounds are the two major components, which cause bad odor and bitter taste of fenugreek seeds.

CONCLUSIONS

Fenugreek seeds have an important nutritional and medicinal values known since ancient times, but presence of some components such as alkaloids and some volatile compounds may represents the main problem in its uses in human and animal meals due to undesirable effects of two components on taste and sweat odor. Present study investigated the effect of some traditional treatments on desirable and undesirable substances in fenugreek seeds. Data confirmed the good effect of germination process in increment of some important nutrients such protein, polyphenols and antioxidant activity of fenugreek seeds with a significant decrease in alkaloids and volatile compounds in fenugreek seeds compared with raw and boiled seeds.

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