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Chemical composition, Elements and Phytochemical contents of *Trigonella foenum-graecum* L. (Seeds)

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Abstract: The objective of this study is to investigate the chemical composition, minerals and phytochemical contents of Fenugreek seeds which obtained from Albaha, KSA (Panda market). The analysis of chemical composition of fenugreek shows that the percentage of dry matter , Ash, protein, oils ,fiber and total carbohydrates were 89.77, 0.28, 29.7, 7.13, 6.28 and 46.32,respectively. Minerals contents were Ca (43.93mg/100g), Fe (11.17mg/100g) and P (698.21mg/100g).The contents of phytochemical (phytic acid, tannin and total polyphones) were 152.91, 1.32 and 452.12mg/100g, respectively.

Keywords: Fenugreek, Phytochemical, Elements, Chemical composition, phytic acid

INTRODUCTION

Fenugreek seeds (*Trigonella foenum-graecum L.*)is a legume commonly grown in many parts of the world for both culinary purposes and health benefits. The fenugreek seed is very bitter but does have interesting proximate composition. protein content ranges between 23 and 43% of the seed, carbohydrate represents up to 58% , moisture make up about 10 – 13% of the seed, lipid represent 5 – 6 % and minerals make up less than 1% fenugreek seed contains several bioactive compounds, including proteins, protease inhibitors. a unique amino acid known as 4- hydroxyl - isoleucine water soluble dietary fiber , steroidal saponins , flavonoids , iso-flavones, alkaloids, polyunsaturated oil, and phytic acid[1].

MATERIALS AND METHODS

Materials: Fenugreek seeds (*Trigonella foenum - graecum* L.) were brought from Jeddah local marker, KSA then cleaned and freed from extraneous matter, and milled in to fine flour using house blender and mortar to passed through 0.4mm screen and stored in polyethylene bags at 4℃ for further analysis.

Proximate analysis:

Moisture content: Moisture was determined by the AOAC method[2]. Two grams of well-mixed samples were weighed accurately using sensitive balance in clean dry and pre-weighed crucible and then placed in an oven at 105^0C. The crucible was transferred to a desiccators and allowed to cool and then weighed. Additional placements in the oven were carried out until a constant weight was obtained. Moisture was calculated using the following formula:

$$\mathbf{MC\ (\%)} = \frac{(\mathbf{W2-W1})-(\mathbf{W1-W3})}{(\mathbf{W2-W1})} \times \mathbf{100}$$

Where:

MC = Moisture content; W_1 = Weight of empty crucible

W_2 = Weight of crucible with sample; W_3 = Weight of crucible with dry sample

Ash content determination: Ash content of the sample was determined according to the method of AOAC[2]. Two grams of sample were placed in a clean dry pre- weighed crucible, and then the crucible with it is content ignited in a muffle furnace at 550℃ for 3 hours or more until light grey ash was obtained. The crucible removed from the furnace to desiccators to cool and then weighed. Ash content was calculated using the following equation:

$$AC = \frac{W2-W1}{W3} \times 100$$

Where:

AC = Ash content; W1 = Weight of empty crucible

W2 = Weight of crucible with ash; W3 = Weight of sample

Crude protein determination: The crude protein was determined by using the micro- kjeldahl method according to AAOC[2]as follows:

1. Digestion: About 0.2 gram of the sample was weighed and placed in small digestion flask (250ml). Two catalysts tablets (anhydrous sodium sulphate + copper sulphate) were added to the sample, 3.5 ml of approximately 98%H_2SO_4wasadded. The content of the flask was then heated on electrical heater for two hour till the color changed to blue – green. The tubes were then removed from digester and allowed to cool.

2. Distillation: The digested sample was transferred to the distillation unit and 15 ml of 40% NaOH were added. The ammonia was received in 100ml conical flask containing 10 ml of boric acid plus 3-4 drops of methyl red indicator. The distillation was continued until the volume reached 50ml.

3. Titration: The content of the flask were titrated against 0.02N HCl. The titration reading was recorded. The crude protein was calculated using the following equation:

$$CP\ \% = \frac{(T\text{-}B) \times N \times 14 \times 100 \times 6.25}{WS \times 1000}$$

Where:

CP = Crude protein; T = Titration reading; B = Blank titration reading;

N = Normality of HCl ; WS = Weight of sample; 1000 = to convert to mg

6.25 = Protein factor

Fat content determination: Fat content was determined according to the method of AOAC[2] using Soxhlet apparatus. An empty clean and dry extraction round bottomed flask was weighed. About two gram of sample was weighed and placed in a clean extraction thimble and covered with cotton wool. The thimble was placed in extractor. Extraction was carried out for 8hours with petroleum either. The heat was regulated to obtain at least 15 siphoning per hour. The residual ether was dried by evaporation. The flask was placed in an oven at 105℃ till it dried completely and then cooled in a dedicator and weighed. The fat content was calculated using the following equation:

$$CF = \frac{W2 - W1}{W3} \times 100$$

Where:

CF = Fat content; W1= Weight of extraction flask;

W2= Weight of extraction flask with fat

W3= Weight of sample

Crude fiber determination: Crude fiber was determined according to AOAC[2]. Two grams of defatted sample were treated successively with boiling solution of $H_2SO_4$of 0.26 N and KOH of 0.23 N. The residue was then separated by filtration, washed and transferred into a crucible then placed into an oven adjusted to 105℃ for 18-24 hours. The crucible with the sample was weighed and ashed in a muffle furnace at 500℃ and weighed. The crude fiber was calculated using the following equation:

$$FC = \frac{W2 - W1}{W3} \times 100$$

Where:

CF= Crude fiber; W1= Weight of crucible with sample before ashing

W2= Weight of crucible with sample after ashing; W3= Weight of sample

Determination of Carbohydrates: Carbohydrates were determined by difference. The sum of moisture, fat, protein and ash contents was subtracted from 100 to obtain the total carbohydrates by difference[3].

Carbohydrates = 100- (Ash % + moisture % + CP % + oil % + fiber %).

Phytochemical analysis:

Determination of tannin content: Quantitative estimation of tannins was carried out using the modified vanillin – HCl methanol, according to Price *et al*,[4] . The vanillin HCl reagent was prepared by mixing equal volumes of 8% concentrated HCl in methanol and 1% vanillin in methanol, the two solvents of the reagent were mixed just prior to use, it was discarded if a trace of color appeared. Catching was used as reference standard.

Determination of phytic acid: The physic acid content was determined according to the method of Wheeler and Ferrell[5] .Tow grams of finely ground samples were weighed in 50 ml tube. The sample was extracted with 50 ml of 3% trichloroacetic acid (TCA) for 3 hr with shaking. The suspension was centrifuged for 5 min. at 2500 rpm. Ten milliliters aliquot of the supernatant were transferred to 50 ml tube, 4 ml $FeCl_3$ (Solution containing 2 mg Fe^{+3} iron/ml 3% TCA were added to the aliquot by blowing rapidly from the pipette. The tube and contents was heated in a boiling water bath for 45 min. One or two drops of 3% Na_2SO_4 in 3% TCA were added to develop a precipitate. Then tube was cooled and centrifuged for 10-15 min at 2500 rpm.

The clear supernatant was decanted and the precipitate was washed twice by dispersing well in20- 25 ml 3% TCA, and heated for 10-15 min in boiling water bath, then cooled and centrifuged. The precipitate was washed one or two times with distilled water, and was dispersed in a few ml of distilled water. Three milliliters of 1.5N NaOH were then added and the volume completed to 30 ml with distilled water. The tube was heated in a boiling water bath for 30 min, and hot filtered using What man No. 1 filter paper.

The precipitate was washed with hot 60-70 ml of distilled water and the washings were decanted. The precipitate was dissolved from the filter paper with 40 ml hot 3.2N HNO_3 into 100 ml volumetric flask and the paper was washed again with a hot distilled water in the same flask and completed to the volume with distilled water. A volume of the above suspension was transferred into 10ml volumetric flask. Two milliliters of 1.5NKSCN (potassium thiocyanate) were added and completed the volume with distilled water, then immediately (within one minute) the absorbance was read using spectrophotometer (JENWAY 6305 UV) at 480 nm.

A standard curve of different Fe $(NO_3)_3$ concentrations was plotted to calculate the ferric iron concentration. The phytate phosphorus was calculated from the ferric iron concentration assuming 4:6 irons: phosphorus molar ratio.

Calculation:

$$\text{Phytate (mg/ 100g)} = \frac{6}{4} \times \frac{A \times C \times 20 \times 10 \times 50 \times 100}{1000 \times 2}$$

A= Optical density: C = concentration corresponding to the optical density

Total Polyphenols determination: Total Polyphenols were determined by use of Prussian blue spectrophotometric method[6]. Sixty mg of sample were shaken manually for sixty second with 3 ml of absolute methanol in a test tube. The mixture was filtered, then the tube quickly rinsed with 3 ml of methanol and the contents poured at once into the funnel. The filtrate was mixed with 50 ml of distilled water and analyzed within one hour. Three ml of 0.1M $FeCl_3$ in 0.1N HCl were added to 1 ml of filtrate, followed immediately by timed addition of 3ml of0.008M $K_3Fe(CN)_6$. The absorbance was read on spectrophotometer (Jenway6306 Uv/Vis spectrophotometer) at 720nm after 10 min. Tannic acid was used to make the standard curve following the same steps in the procedure above. The polyphenol content was calculated as follows:

$$\text{Total polyphenol \% (Tannic equivalent)} = \frac{\mathbf{C \times 56 \times 100}}{\mathbf{60}}$$

C= Concentration corresponding to optical density

56= Volume of extract.;60= Weight of sample

Minerals determination: Minerals of samples were extracted according to the dry-ashing method as described byPearson[7]. Two grams of each sample were placed in Porcelain dish and burnt in muffle furnace at 550°Cand placed in the sand bath for 10 minutes after addition of 5 N HCl. Then the solution was carefully filtered in a 100ml volumetric flask and finally distilled water was added up to mark. From this extract, the elements calcium and iron were determined using Perkin Elmer Atomic Absorption Spectroscopy.

Phosphorus determination: The phosphorus for both total and HCl-extractable were carried out according to the method of Chapman and Pratt[8]. Five ml of the ash extract were pipette in to a 50 ml volumetric flask. Ten ml of the ammonium molybdate ammonium vanadate reagent (22.5g) of (NH_4)$Mo_7O_{24}.4H_2O$ in 400ml distilled water + 1.25 gram ammonium vanadate in 300 ml boiling water + 250 ml concentrated HNO_3 then diluted to liter) were added. The content in the flask were mixed and diluted to the volume. The density of the color was read after 30 minutes at 470 nm suing spectrophotometer (JENWAY 6305 UV/ Vis). Phosphorus was determined from the standard curve.

Calculation:

$$\text{P (mg/ 100 g)} = \frac{\frac{\text{mg}}{\text{L}} \times \text{volume} \times 100}{1000 \times \text{Wt}}$$

Where:

mg/ L = ppm (curve reading); Volume used = volume of extract

1000 = conversion form mg/ L to mg /m; Wt. of sample = 2 grams

Statistical Analysis: All data were subjected to statistical analysis; each determination was carried out and analysed in triplicate then averaged. Data was assessed by the analysis of Variance (ANOVA) Gomez and Gomez[9].

Table (1): Proximate composition of defatted Fenugreekseeds

Parameter (%)	Dry matter	Ash content	Crude protein	Oil content	Crude fibre	Carbohydrate
Fenugreek seeds	89.77 (±0.25)	0.28 (±0.00)	29.7 (±0.17)	7.13 (±0.14)	6.28 (±0.26)	46.38 (±0.23)

Values are means (±SD) of three replicates

The proximate compositions of fenugreek seeds are shown in Table 1. The dry matter of fenugreek seeds (FS) was 89.77% which was slightly lower than that study reported by Elnasri[10] who reported 93.13%. The ash content of (FS) was 0.28% which was significantly lower than the range 3.00-5.9% reported by El Hassan[11]. The protein content of

(FS) was 29.7% which within the range of 22-38% reported by El Hassan[11]. The oil content of FSF was 7.13% which was in agreement with the value 7.5% reported by Hemavathy and Prabhakar [12]. The crude fiber content of FS was 6.28% which was lower than the range 9.5 – 11.00% reported by El Hassan[11]. The carbohydrate content of FS was 46.38% which was lower than value reported by Elnasri[10] who reported 47.1%.

Table (2): Total (mg /100g) minerals contents of defatted Fenugreekseeds:

Samples	Calcium	Phosphorus	Iron
Fenugreek seeds	43.93 (±0.04)	698.21 (±3.27)	11.17 (±0.05)

Values are means (±SD) of three replicates

The total minerals contents of fenugreek seeds flour are shown in **Table 2.** Calcium content fenugreek seeds was found to be 43.93 mg/100g and Phosphorus content was 698.21 mg/100g, while iron content was 11.17 mg/100g.

Table (3): Phytochemical (Phytic acid, Tannin and Total Polyphenols of Fenugreek seeds

Sample	Physic acid	Tannin %	Totalpolyphenols
Fenugreekseeds	152.91 (±3.16)	1.32 (±0.006)	452.12 (±3.45)

Values are means (±SD) of three replicates

The physiochemical content of fenugreek seeds are shown in Table 3. The phytic acid content of fenugreek seeds (FS) was found to be 152.91 mg/100g which was very lower than the value 549.omg/100g., tannin content was 1.32% and total polyphenols was 452.12 mg/100g.

CONCLUSION

We have analyzed the chemical composition of fenugreek seeds. Testified that fenugreek seeds contained Dry matter 89.77 %, Ash 0.28%, %, Crude protein 29.7%, oils 7.13%, fiber 6.28% and total carbohydrates 46.32%. Fenugreek seeds contained minerals (in 100 gram) of seed nearly: Calcium 43.93mg, Fe 11.17mg, and Phosphorus 698.21mg. In 100 gram of Fenugreek seed contained nearly Phytic acid 152.91, Tannin 1.32 and total Polyphenols 452.12.

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