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Evaluation of Flours Produced from Debittered Fenugreek Seeds (*Trigonella foenum-graecum* L.) by Different Processing Methods.

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Abstract

Fenugreek seeds are valuable legumes known for their high protein content, dietary fiber, oils, minerals, and various functional components. However, their bitter taste limits their use in food applications. This study aimed to develop a simple method to reduce the saponin content responsible for the bitterness of fenugreek seeds and to compare this method with other debittering techniques. The developed method involved soaking fenugreek seeds for 12 hours in a 4% lactic acid solution, followed by a germination period of 72 hours. The soaking of fenugreek seeds in a 4% lactic acid solution led to a reduction of 77.65% in the initial saponin content for unsoaked seeds, and an 80.85% reduction when followed by germination. Additionally, significant reductions in saponin levels were observed after germinating the soaked seeds in yogurt (71.11%) and water (60.49%). In contrast, the roasting process only achieved a reduction of 40.63% in saponin content. Different germination processes of fenugreek seeds after soaking in the 4% lactic acid solution, water, or yogurt led to increases in non-reducing sugars, crude fiber, ash, phenolics, flavonoids, and antioxidants, while starch, fat, and phytic acid levels were decreased. The fortified Baton Sale', made from 90% wheat flour and 10% debittered fenugreek flour, exhibited the best sensory attributes when containing flour from fenugreek seeds soaked in a 4% lactic acid solution for 12 h and followed by germination for 72 h.

Keywords: Antioxidant, Flavonoids, Phenolics, Phytate, Saponin

Introduction

Fenugreek (*Trigonella foenum-graecum*) is a member of the legume family, also known as Greek hayseed and bird's foot (Naidu *et al.*, 2011). Its properties and benefits were documented in the Ebers Papyrus, one of the oldest known medicinal texts, dating back to 1500 B.C. in Egypt (Betty, 2008). Both the seeds and green leaves of fenugreek are used in food and various medicinal applications, a practice that has been part of human history for a long time (Wani and Kumar, 2018). Fenugreek originates from southeastern Europe and western Asia, but it is now cultivated in many regions worldwide, including Egypt, India, and the United

States (Altuntas *et al.*, 2005). This legume is distinctive for its high concentration of phytochemicals, which contribute to several pharmacological effects, including hypoglycemia (Tavakoly *et al.*, 2018), hypocholesterolemia (Belguith-Hadriche *et al.*, 2013), as well as antimicrobial, carminative, galactagogue, anticarcinogenic, and anti-inflammatory properties (Pandey and Awasthi, 2015; Wani and Kumar, 2018). Fenugreek is a nutritious food source, containing 25–35% protein, 5.7 grams of the amino acid lysine per 16 grams of nitrogen, and a combination of soluble and insoluble dietary fiber (20–25% and 25–30%, respectively). It also contains 5.0–7.5% fat, as well as significant amounts of calcium, iron, and beta-carotene (Naidu *et al.*, 2011). Recognized for its health benefits, fenugreek is rich in functional compounds such as phenolics, flavonoids, free amino acids, and polyunsaturated fatty acids (Dhull *et al.*, 2020). Additionally, fenugreek gum and proteins could interact with food ingredients, helping to stabilize and emulsify them, which indicates their potential use in various food products (Gadkari *et al.*, 2019; Wani and Kumar, 2018).

Fenugreek is recognized for its pleasantly bitter and slightly sweet seeds. These seeds, available in both whole and ground (flour) forms, are commonly used to flavor a variety of foods, including curry powders, spice blends, and teas (Dhull *et al.*, 2020). Despite gaining significant recognition in recent years for its excellent nutraceutical properties, the bitter taste of fenugreek limits its acceptability in food products. Addressing this key limitation presents a challenge for food researchers and culinologists. Previous research has explored different approaches to mitigate the bitterness of fenugreek, such as soaking, germination, and roasting (Ertas and Bilgiçli, 2012; Pandey and Awasthi, 2015). Additionally, incorporating small amounts of sugar (Sharafi *et al.*, 2013) or using curd (Dhull *et al.*, 2020) and yogurt (Srinivasan, 2010) has been suggested as effective methods to minimize the bitterness and pungent taste in traditional foods. Another method involves removing bitterness by defatting the seeds using various organic solvents; however, this approach has its drawbacks. It results in the loss of fenugreek lipids, which contain a favorable composition of fatty acids and functional compounds such as N-acyl ethanolamines and oleamide, both known for their strong pain-relieving and appetite-stimulating properties (Kaviarasan *et al.*, 2007). Furthermore, some amino acids may be washed away during defatting with solvents that contain 92–95% ethanol (Vigh *et al.*, 2017). Therefore, the goal of this study is to develop a solvent-free method for reducing the bitterness in fenugreek seeds.

One notable characteristic of fenugreek seeds is their bitter taste, which is due to the presence of saponin compounds. Since consumers generally prefer sweet or neutral flavors, this bitterness has led to significant restrictions on using fenugreek seeds in food product formulations. Therefore, this study was conducted to develop a simple method for reducing the saponin content in fenugreek seeds and to examine its effects on the chemical composition, bioactive, and functional components of the seeds. Additionally, this method was compared with other solvent-free debittering processes.

Materials and Methods

Plant Materials

Fenugreek seeds (*Trigonella foenum-graecum L*), cultivar Giza 2, were obtained from Agriculture Research Center; Shandawel, Sohag Governorate in season's year 2022. Wheat flour 72% extraction rate was purchased from market in Assiut city.

Chemicals

All chemicals used in this study were of analytical-reagent grade and sourced from the following suppliers: 3,5- Dinitrosalicylic acid (DSN), potassium dihydrogen phosphate, Folin-Ciocalton reagent, *p*-anisaldehyde and Quercetin were obtained from Merck, Darmstadt, Germany. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, and saponins were sourced from Sigma Chemical Company, Missouri, USA. Methyl and ethyl alcohol, ethyl acetate, lactic acid, aluminum trichloride and sodium nitrite (EL-Gamhoria trading chemicals and Drugs. Assiut city, Egypt.).

Methods

Soaking in lactic acid solution and followed by germination procedure

Our new method in this study depends on the efficiency of some concentrations of lactic acid solutions in removal of saponins compound. After being cleaned and cleared of dust, broken seeds, and other foreign objects, fenugreek seeds were surface sterilized by soaking in 95% ethanol for one minute and then rinsed with distilled water. To define the best concentration of lactic acid solution to take out the most saponins, the fenugreek seeds were washed and then steeped, separately, in different lactic acid solutions with concentrations between 2 to 7% for 12 h at room temperature (20-23 °C). Then the un-imbibed lactic acid solution was thrown away, and two rinses in distilled water were performed on the soaked seeds, followed by drying with an air fan for one hour then in an electric oven at 45°C for 24. Six dried samples were obtained by soaking in 2, 3, 4, 5, 6 and 7% lactic acid solutions were then fine ground and stored in plastic bags. The codes of these samples are shown in Table 1.

Table 1. Codes of samples of soaked fenugreek seeds in different concentrations of lactic acid solutions.

Code of sample	Treatments
A	Unsoaked fenugreek seeds (Control)
B	Fenugreek seeds soaked in 2% lactic acid solution
C	Fenugreek seeds soaked in 3% lactic acid solution
D	Fenugreek seeds soaked in 4% lactic acid solution
E	Fenugreek seeds soaked in 5% lactic acid solution
F	Fenugreek seeds soaked in 6% lactic acid solution
G	Fenugreek seeds soaked in 7% lactic acid solution

A part of soaked fenugreek seeds with the best results in removing most saponins and good bakery products were used for germination spending 72 hours in the dark on a screen. Distilled water was sprinkled on the seeds twice every twelve hours. The seedlings were kept in a fan to dry overnight and followed by

drying in electric oven at 45°C for 24 h. An electric grinder was used to grind the dried samples of germinating seeds into a fine powder, which was subsequently placed in plastic containers for future usage.

Other debittering processes

Previous research reported the application of several techniques, such as soaking in water (Ertas and Bilgiçli, 2012); soaking, germination and roasting (Pandey and Awasthi, 2015) as well as soaking in yoghurt (Srinivasan, 2010) or curd (Dhull, *et al.*, 2020) to address bitterness. These different processes were carried out in this study as described by abovementioned researchers as soaking in water for 12 h (Ertas and Bilgiçli, 2012) followed by germination for 72 h (Pandey and Awasthi, 2015). The processing of Srinivasan (2010) by soaking in yogurt was used. Seeds were soaked for 12 hours in a mixture of yogurt and water. The ratio of yogurt to water was 1:1, and the ratio of seeds to dilute yogurt was 1:4. After soaking, the seeds were thoroughly washed with water to remove any remaining traces of yogurt. A part of soaked seeds in diluted yoghurt were followed by germination as abovementioned described. Finally, the soaked and germinated seeds were dried in electric oven at 45 °C for 24 h.

The roasting process of raw seeds was achieved according to Pandey and Awasthi (2015); 50 g fenugreek seeds were roasted in an open pan at 130 ± 5 °C for 7 min. It is constantly stirred with a spoon to roast it properly and uniformly until it is slightly browned and leaves an acceptable aroma. Raw fenugreek seeds were used as control. Table 2 showed the codes of different processes to minimize the bitter taste of fenugreek seeds.

Table 2. Codes of different processes to minimize the bitter taste of fenugreek seeds.

Code of treatments	Processes
T0	Unsoaked fenugreek seeds.
T1	Soaking of fenugreek seeds in 4% lactic acid solution.
T2	Soaking of fenugreek seeds in 4% lactic acid solution and followed by germination.
T3	Soaking of fenugreek seeds in water.
T4	Soaking of fenugreek seeds in water and followed by germination.
T5	Soaking of fenugreek seeds in yogurt.
T6	Soaking of fenugreek seeds in yogurt and followed by germination.
T7	Roasting of fenugreek.

Preparation of Baton Sale'

Composite flour consists of 90% wheat flour (72% extraction rate) and 10% of each flour from unsoaked and soaked fenugreek seeds; separately, as shown in Table 1, were prepared. Seven samples of Baton Sale' were baked using composite flour. In addition, seven samples of Baton Sale' were baked from composite flours consisting of 90% wheat flour and 10% flour of each raw and debittered fenugreek seeds flour samples as shown in Table 2. The method described by El-Zainy *et al.* (2018) was used for preparation of Baton Sale'.

The sensory properties of Baton Sale'

Sensory evaluation of fenugreek fortified Baton Sale' samples were carried out according to Meilgaard *et al.* (1999) using a 9-point hedonic scale with 15 trained panelists to evaluate color, crispness, flavor/taste, and overall acceptability (9 = like extremely; 5 = neither like nor dislike; and 1 = detest extremely). After tasting each sample, water was administered to wash the oral cavity, and the evaluation was carried out under white light at room temperature (25–26 °C).

Analytical Methods

Gross Chemical composition

Moisture, fat, protein, starch, crude fiber and ash were determined according to the methods of A.O.A.C. (2005).

Reducing and non-reducing sugars

Were estimated by dinitro salicylic acid reagent_(DNS) as described by Miller (1959). The difference between total soluble sugars and reducing sugars was used to identify non-reducing sugars.

Extraction and Quantification of saponins

The extraction of saponins was carried out with chemical solvents according to the modified methodology described by Mora-Ocación *et al.* (2022). The extracted crude saponins was determined spectrophotometrically by the method described by Baccou *et al.* (1977) and modified by Wang and McAllister (2010). Standard saponins were used in a concentration of 4 to 40 µg as calibration curve.

Inorganic phosphorus (IP)

IP was determined after extracting the sample (0.5g) with 25 ml of hydrochloric acid (0.5 N) and mixed the mixture by a magnetic stirrer for 6 hours at 25°C. After centrifugation at 5000 rpm for 30 minutes an aliquot (10 ml) was diluted to 50 ml by distilled water in a measuring flask. The solution was then analyzed directly for its inorganic phosphorous content, using ammonium molybdate and stannous chloride as reported by Jackson (1973). Potassium dihydrogen phosphate (KH₂PO₄.2H₂O) was used as the standard calibration curve.

Total phosphorus (TP)

TP was determined by spectrophotometer (Jackson, 1973) after wet ashing as described in A.O.A.C. (2005) methods.

Phytic acid (PA)

PA was measured based on its phosphorus content, following the method described by Kent-Jones and Amos (1957). This approach involves separating phytic acid as ferric phytate, which is then converted into sodium phytate. Organic matter is eliminated by heating with concentrated sulfuric acid and perchloric acid. After neutralization, the resulting solution is used for the colorimetric determination of phytic acid phosphorus using the Jackson method (1973). The quantity of phytic acid is calculated from the phytate phosphorus, using the weight

ratio of phosphorus atoms per molecule of inositol hexaphosphate, which is 1:3.52 according to Abdel-Gawad (2016).

Free, bound and total phenolic compounds

The methods described by Abdel-Gawad (1982) for determination of free, bound and total phenolic compounds were used. Phenolic compounds were determined spectrophotometrically by Folin-Ciocalteu method (Pandey and Awasthi 2015). The gallic acid was used as standard and the results were expressed as mg gallic acid equivalents (GAE) per 100 grams of test materials. The bound phenolic compounds were calculated by subtracting the free phenolics from the total phenolics.

Flavonoid content

A portion of the total phenol extract was used to determine the flavonoid content using the method described by Kim *et al.*, (2003) with modifications based on those outlined by Subhasree *et al.*, (2009). Quercetin was used for the standard calibration curve at concentration between 0.1 to 0.5 mg/ml. The data were expressed as mg quercetin equivalents (QE)/g of dried sample.

DPPH free radical scavenging assay

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging experiment was performed in accordance with Yen and Duht's (1994) instructions. 2.9 ml of DPPH methanolic solution was mixed with 0.1 ml of each extract or standard antioxidant (butylated hydroxyanisole BHA, 25–250 mg/L). Absorbance values at 517 nm were measured after the mixture was agitated briskly and allowed to sit at room temperature for 45 minutes in the dark. Triplicate analyses are used to present values. The scavenging activity was determined using the formula $100 \times (A_{\text{Control}} - A_{\text{sample}}) / A_{\text{Control}}$, where A sample is the absorbance of the test materials and A control is the absorbance of the control reaction mixture without the test material.

Statistical analysis

Using CoStat6.303, the collected data were statistically analyzed using the entire randomized design as described by Gomez and Gomez (1984). Waller and Duncan (1969) used L.S.D. at a 5% probability level to compare the significant means of any attribute under study.

Results and Discussions

Effect of debittering process of fenugreek seeds by soaking in lactic acid solutions on saponins content.

The results in Table 3 showed a clear decrease in saponins content in flour of fenugreek seeds soaked; separately; in different lactic acid solutions with concentrations of 2 to 7%. The significant ($p \leq 0.05$) sharp diminish of saponins content was by soaking in 4% lactic acid solution with reduction of 77.65% from its original amount in unsoaked fenugreek seeds. Soaking in 5% and 6% lactic acid solutions showed removing saponins by 69.07% and 65.91%, respectively of its original content in unsoaked seeds. In addition, the increase of lactic acid

concentration to 7% removed only 12.87% of original saponins content in raw seeds. To ensure that increasing the concentration of lactic acid solution does not cause an increase in removal of saponins, the fenugreek seeds were soaked in a 10% lactic acid solution, under the same conditions as before. The result in Table 3 indicated that only 0.45% of original saponins content was reduced. Sharafi *et al.*, (2013) suggested the addition of small amounts of sweetener to reduce bitterness in vegetables. Alternatively, curd (Chauby *et al.*, 2018; Dhull *et al.*, 2020) or yogurt (Srinivasan, 2010) is widely used in Egyptian traditional foods to pacify pungency and bitterness. The use of curd and yogurt in reducing the bitterness in fenugreek seeds may be due to the effect of lactic acid present in them.

Table 3. Effect of soaking of fenugreek seeds in different concentrations of lactic acid solutions on saponins content.

Samples	Saponins (mg/100g sample ^a)	Reduction of saponins
A	4.43 ^a ±0.04	-
B	2.66 ^c ±0.02	39.73 ^e ±0.02
C	2.06 ^d ±0.12	53.50 ^d ±0.40
D	0.99 ^f ±0.28	77.65 ^a ±0.40
E	1.37 ^e ±0.03	69.07 ^b ±0.12
F	1.50 ^e ±0.60	65.91 ^c ±0.02
G	3.86 ^b ±0.62	12.87 ^f ±0.56
Flour from fenugreek seeds soaked in 10% lactic acid	4.41 ^a ±0.10	0.45 ^g ±0.02
L.S.D. (0.05)	0.28	0.26
F Test	***	***

^aCalculated on dry weight basis; L.S.D. (0.05) = Least significant difference; values are the mean of triplicate determinations; the different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

Effect of debittering of fenugreek seeds by soaking in lactic acid solutions on sensory properties of fortified Baton Sale'

The result of Table 4 illustrated sensory evaluation of Baton Sale' baked from mixtures of 90% wheat flour and 10% flour from unsoaked and soaked fenugreek seeds in lactic acid solutions with different concentrations. Flavor, taste and overall acceptability were significantly ($p \leq 0.05$) improved with decreasing the saponins content. The sample (90%WF +10%D) in which the fenugreek seeds soaked in 4% lactic acid solution showed the highest scores in the abovementioned three sensory properties. Furthermore, the results in Table 4 were consistent with the diminishing of the saponins content in Table 3. Dhull *et al.* (2020) reported that sensory attributes of rusks were affected with debittered fenugreek seeds flour incorporation, and rusks with 15% debittered fenugreek seeds flour were found most desirable with enhanced sensory characteristics. Chaubey *et al.*, (2018) studies the effect of substitution of wheat flour with 10% debittered fenugreek flour on physical and sensory attributes of bread and found that organoleptic quality was acceptable.

Effect of different processes for debittering of fenugreek seeds on nutritional composition

The changes in gross chemical composition of flours from debittered fenugreek seeds by different methods are present in Table 5. The moisture content of flours from untreated (T0) and treated with other processes (T1 to T7) ranged from 4.00 to 6.88% except the roasting treatment (A7) showed 1.52%. The protein content showed a slight decrease by soaking in 4% lactic acid, water, or yogurt which may be due to the loss of water-soluble protein and other soluble nitrogenous compounds during soaking. While the germination caused significantly ($p \leq 0.05$) high increase in protein content. El-Shimi *et al.*, (1984) reported that protein content of fenugreek seeds decreased slightly after soaking while it increased after germination. According to Hooda and Jood (2003), a rise in protein content following germination may result from the conversion of seed nitrates into protein or ammonium compounds, or it may be the consequence of protein enzymatic synthesis (Mansour and EL-Adawy, 1994).

Table 5 showed decreases in fat, starch contents by soaking in most processes and high significant ($p \leq 0.05$) reduction by germination, whereas roasting process caused slight decrease of both contents. The reduction in fat and starch during soaking and germination probably, because of utilization of fat and sugar as energy source to start germination. These changes agree with those reported by El-Shimi *et al.* (1984); Mansour and EL-Adawy (1994) and Chaubey *et al.* (2018) for fenugreek seeds. The results in Table 5 indicated that the reducing sugars were decreased by soaking may be due to their diffusion into water during this process.

On the other hand, the germination process increased the content of reducing sugar as result of starch degradation by amylase enzymes. Non-reducing sugars content was significantly ($p \leq 0.05$) decreased by soaking process. Losses of reducing sugars during soaking could be from simple diffusion of sugars after being solubilized in soaked water. Soaking has been known to reduce the level of sugars in various pulses (Jood *et al.*, 1998); whereas germination caused a significant increase in content of total sugars. The increase in non-reducing sugar content of soaked seeds during germination may be because of mobilization and hydrolysis of oligosaccharides (raffinose, stachyose and verbascose), leading to more available sucrose (Abdel-Gawad 1993). The main non-reducing sugar in fenugreek seeds and sprouts is sucrose. Similar observations for increasing the non-reducing sugar of fenugreek and other legume seeds during germination had been reported earlier (El-Mahdy and EL-Sebaiy 1983; Hooda and Jood 2003). Crude fiber and ash contents of flour from debittered fenugreek seeds (Table 5) showed a raise in their percentage by soaking and more moderate increasing by germination processes compared to flour from untreated seeds. Such observation was mentioned by Dhull *et al.* (2020) when they minimized the bitter taste of fenugreek seeds by soaking in curd and by Chaubey *et al.* (2018) for debittering of fenugreek seeds by soaking in curd and followed by germination.

Table 4. Sensory evaluation of baton salé baked from mixtures of 90% wheat flour and 10% flour from unsoaked and soaked fenugreek seeds in different concentrations of lactic acid solutions.

Samples	General appearance	Color	Flavor	Taste	Crispy	Overall acceptability
90%WF+10% A	8.52 ^a ± 0.13	9.00 ^a ± 0.20	4.00 ^c ± 0.30	4.50 ^f ± 0.40	8.5 ^a ± 0.40	4.00 ^g ± 0.40
90%WF+10% B	7.51 ^b ± 0.04	8.00 ^c ± 0.24	5.50 ^c ± 0.08	5.50 ^d ± 0.15	7.52 ^b ± 0.38	5.50 ^c ± 0.20
90%WF+10% C	7.55 ^c ± 0.34	8.09 ^c ± 0.18	6.32 ^b ± 0.40	6.50 ^b ± 0.40	7.02 ^c ± 0.04	6.50 ^b ± 0.26
90%WF+10% D	8.00 ^b ± 0.30	8.52 ^b ± 0.14	7.00 ^a ± 0.04	7.00 ^a ± 0.30	7.06 ^c ± 0.12	7.00 ^a ± 0.20
90%WF+10% E	8.00 ^b ± 0.14	8.55 ^b ± 0.60	6.88 ^a ± 0.66	6.00 ^c ± 0.40	7.07 ^c ± 0.30	6.00 ^c ± 0.02
90%WF+10% F	7.80 ^b ± 0.34	8.60 ^b ± 0.60	6.20 ^b ± 0.10	6.46 ^b ± 0.11	7.00 ^c ± 0.14	5.75 ^d ± 0.50
90%WF+10% G	7.50 ^c ± 0.40	8.00 ^c ± 0.46	5.00 ^d ± 0.50	5.00 ^e ± 0.50	6.52 ^d ± 0.16	5.00 ^f ± 0.66
L.S.D. (0.05)	0.23	0.34	0.32	0.30	0.22	0.32
F Test	***	***	***	***	***	***

WF= Wheat flour; L.S.D. (0.05) = Least significant difference; values are the mean of triplicate determinations; the different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

Table 5. Changes in gross chemical composition of flours from debittered fenugreek seeds by different processes (g/100g sample on dry weight basis).

Treatments	Moisture %	Protein* %	Fat* %	Starch* %	Reducing sugar* %	Non Reducing Sugar* %	Crude fiber* %	Ash* %
T0	5.00 ^g ±0.12	31.22 ^g ±0.04	6.15 ^g ±0.13	41.26 ^g ±0.06	1.17 ^d ±0.12	1.37 ^e ±0.12	6.09 ^g ±0.15	3.61 ^e ±0.03
T1	4.13 ^f ±0.16	29.12 ^h ±0.11	5.97 ^b ±0.08	39.84 ^c ±0.22	1.02 ^e ±0.04	1.43 ^{de} ±0.08	10.36 ^d ±0.08	3.35 ^{cd} ±0.17
T2	5.75 ^b ±0.17	32.55 ^c ±0.08	5.00 ^f ±0.05	35.84 ^f ±0.08	3.11 ^c ±0.22	1.53 ^c ±0.04	11.25 ^c ±0.07	4.13 ^{ab} ±0.09
T3	4.00 ^g ±0.11	29.44 ⁱ ±0.02	5.90 ^d ±0.11	37.35 ^e ±0.11	0.98 ^e ±0.10	1.46 ^{cd} ±0.06	7.35 ^f ±0.13	3.45 ^d ±0.11
T4	5.46 ^e ±0.15	33.70 ^b ±0.05	3.93 ^h ±0.06	33.01 ^g ±0.02	3.71 ^a ±0.06	1.79 ^a ±0.10	15.24 ^a ±0.05	4.32 ^a ±0.05
T5	6.88 ^a ±0.22	29.18 ^g ±0.03	5.92 ^d ±0.02	38.08 ^d ±0.06	1.03 ^e ±0.06	1.42 ^{de} ±0.08	10.02 ^e ±0.11	3.32 ^d ±0.04
T6	5.14 ^d ±0.07	35.41 ^a ±0.09	4.04 ^g ±0.13	32.01 ^h ±0.04	3.57 ^b ±0.20	1.68 ^b ±0.06	12.20 ^b ±0.09	3.98 ^b ±0.13
T7	1.52 ^h ±0.09	30.68 ^h ±0.12	5.71 ^e ±0.09	41.03 ^b ±0.02	1.09 ^{de} ±0.08	1.39 ^{de} ±0.08	5.82 ^h ±0.07	3.5 ^{cd} ±0.21

*Calculated on dry weight basis; values are the mean of triplicate determinations with standard division; the different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

Effect of different debittering processes of fenugreek seeds on saponins content

The results in Table 6 showed the saponins contents and their reduction by different processes. It can be seen that soaking fenugreek seeds in 4% lactic acid solution (sample T1) recorded significant ($p \leq 0.05$) less value of saponins content (0.99 mg/100g sample) and sharp reduction amounted to 77.6% of its original value in untreated samples T0 (4.43 mg/100g sample) compared to other soaking processes used in this work. It is worth noting that soaking in yogurt (T5) caused a reduction in saponins by 57.33%, whereas the soaking in water (T3) recorded only a reduction of 12.18% of the original content in the untreated sample. In addition, the germination after soaking by different processes showed more reduction in saponins content. A significant ($p \leq 0.05$) sharp drop in saponins content (0.86 mg/100g sample) was found in the sample T2 that germinated after soaking in 4% lactic acid solution with a reduction by 80.58% of its original content in untreated sample (T0). The germination after soaking in yogurt (T6) and in water (T4) recorded reduction in saponins by 71.11% and 60.49% of its original content in untreated sample; respectively. Roasting process sample T7 showed less reduction value of saponins content (40.36%). Abdel salam *et al.* (2023) reported that the presence of bitter saponins in fenugreek seeds limited their acceptability in foods and it is possible to decrease the bitter taste of fenugreek seeds by using diverse household processes, such as soaking, germination, boiling, fermentation and roasting. Chaubey *et al.*, (2018) used the soaking in dilute curd and Dhull *et al.* (2020) used the same procedure of Chaubey *et al.* (2018) followed by germination to reduce the bitterness of fenugreek seeds.

Table 6. Effect of different debittering processes of fenugreek seeds on saponins content.

Treatments	Saponins (mg/100g sample*)	Reduction (%)
T0	4.43 ^a ± 0.13	-
T1	0.99 ^g ± 0.07	77.65 ^b ±0.03
T2	0.86 ^h ± 0.11	80.58 ^a ±0.05
T3	3.89 ^b ± 0.09	12.18 ^g ±0.07
T4	1.75 ^d ± 0.12	60.49 ^d ±0.09
T5	1.89 ^e ± 0.05	57.33 ^e ±0.02
T6	1.28 ^f ± 0.07	71.11 ^c ±0.13
T7	2.63 ^c ± 0.06	40.63 ^f ±0.06

* Calculated on dry weight basis; values are the mean of triplicate determinations with standard division; the different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

Effect of debittering processes of fenugreek seeds on phenolics, flavonoids and antioxidant activity

In this study the phenolic compounds are divided into free and bound phenolics based on their extractability and interaction with other cell components, particularly cell wall components. The free phenolics of untreated fenugreek seeds (Table 7) amounted to 921.70 and this value increased to 1868.45, 1725.42, and

1562.93 mg/100g sample after soaking in 4% lactic acid solution, water, and yogurt, respectively. In addition, more increase in free phenolics content was observed after all germination processes; the highest value 2397.05 mg/100g sample was recorded for the germination after soaking in water. The roasting process liberates the lesser value of free phenolics compared with other treatments. Acosta-Estrada *et al.* (2014) reported that several food processes enhanced the liberation of free phenolics. These include malting and fermentation as well as thermo mechanical processes such as extrusion cooking and alkaline hydrolysis. The bound phenolics of untreated fenugreek seeds represented 72.6% of total phenolics. Adom and Liu (2002) stated that about 85, 75, and 62% of the total phenolics present in corn, wheat and rice; respectively, are in bound form. The germination after soaking in water (Table 7) showed significant ($p \leq 0.05$) highest value of total phenolics (4730.02 mg/100g sample). Germinating fenugreek seeds rich in bioactive antioxidant substances are used extensively as an important ingredient in daily food preparations and herbal formulations (Kholeam *et al.*, 2014).

The results in Table 7 showed the flavonoids content significantly ($p \leq 0.05$) increased after soaking and germination. The significant ($p \leq 0.05$) highest value of flavonoids content was recorded for the sample T6 that soaked in yogurt and followed by germination (291.68 mg/100g sample). DPPH scavenging (Table 7) also exhibited significant enhancing after soaking and germination in all samples because of the increase in phenolics and flavonoids contents. Dhull *et al.* (2020), Chaubey *et al.* (2018), and Cevallos-Casals and Cisneros-Zevallos (2010) found an increase in phenolics and flavonoids contents after germination of fenugreek seeds accompanied with high raising in antioxidants. The roasting process had less effect on liberating free phenolics and increased the total phenolics by 22.7%; flavonoids 7.20% and antioxidant 4.4% of their original contents in untreated seeds.

Table 7. Effect of different debittering processes of fenugreek seeds on phenolic compounds, flavonoids, and Scavenges activity.

Treatments	Phenolic compounds (mg/100g sample*)			Flavonoids (mg/100g sample*)	DPPH Scavenging (%)
	Free	Bound	Total		
T0	921.70 ^h ±0.60	2438.47 ^b ±0.12	3360.17 ^h ±0.10	264.50 ^h ±0.60	79.71 ^h ±0.06
T1	1868.45 ^d ±0.18	1753.66 ^h ±0.22	3622.11 ^g ±0.06	278.09 ^c ±0.16	89.00 ^d ±0.18
T2	2067.41 ^c ±0.20	2221.68 ^d ±0.18	4289.09 ^b ±0.18	288.33 ^c ±0.16	93.23 ^c ±0.10
T3	1725.42 ^c ±0.14	1982.79 ^f ±0.12	3708.21 ^c ±0.12	272.55 ^g ±0.22	82.57 ^g ±0.16
T4	2397.05 ^a ±0.10	2332.97 ^c ±0.10	4730.02 ^a ±0.04	290.45 ^b ±0.14	96.25 ^a ±0.08
T5	1562.93 ^f ±0.22	2121.09 ^e ±0.08	3684.02 ^f ±0.04	275.05 ^f ±0.10	85.10 ^e ±0.16
T6	2245.16 ^b ±0.14	1974.94 ^g ±0.06	4220.10 ^c ±0.20	291.68 ^a ±0.12	94.42 ^b ±0.10
T7	1361.71 ^g ±0.10	2743.56 ^a ±0.10	4105.27 ^d ±0.16	283.50 ^d ±0.40	83.20 ^f ±0.14

* Calculated on dry weight basis; values are the mean of triplicate determinations with standard division; the different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

Effect of different debittering processes of fenugreek seeds on phosphorus compounds

The phosphorus compounds in raw, soaked and germinating fenugreek seeds are shown in Table 8. Total phosphorus content in most soaking processes showed slight diminishment, while it increased after 72 h by all germination processes. This apparent increase is mainly due to a decrease in dry weight of seeds by germination. El-Mahdy and El-Sebaiy (1982) reported that after 96 h germination of fenugreek seeds, the dry weight decreased, and the ash content increased with raising of phosphorus contents. Abd El-Aal and Rahma (1986) found a marked increase in phosphorus after germination of fenugreek seeds; they recorded 384, 402 and 540 mg/100g dry matter for un-germinating, 3-, and 5-day germination; respectively. Inositol hexaphosphate (phytic acid) content determined as phytate phosphorus in un-germinating seeds amounted to 71.85 mg/100g sample and represented 38.32% of total phosphorus. Gad (1976) found that the phytic acid content of two Egyptian varieties of fenugreek seeds represented 41 - 46% of the total phosphorus. Phytate phosphorus slightly decreased after 12 h soaking and sharply diminished after 72 h in all used germination processes used. Reduction of phytic acid amounted to 57.88, 58.87, and 59.10 % in germinating fenugreek seeds after soaking in water, 4% lactic acid solution and yogurt, respectively. The loss of phytic acid after germination is accompanied by liberation of inorganic phosphorus which increased dramatically after germination. Finally, the roasting process showed a slight increase in total phosphorus and less effect in decreasing of phytic acid.

Effect of debittering of fenugreek seed flours on the sensory properties of fortified Baton Sale'

The sensory properties of Baton Sale' baked from composite flours consisted of 90% wheat flour and 10% untreated and debittered fenugreek seed flours are shown in Table 9. The sample that contained untreated fenugreek seeds flour (90%WF+10%T0) exhibited significantly lesser scores for flavor, taste and overall acceptability whereas the sample 90%WF+10%T2 significantly the highest. This indicated that the Baton Sale' baked from composite flour which contained debittered fenugreek seeds by soaking in 4% lactic acid solution and followed by germination was the best than that of other treatments. These results are consistent with that reported by Chaubey *et al.* (2018) and Dhull *et al.* (2020) for baked fortified bread and rusk from flour mixture of wheat and debittered fenugreek seeds; respectively.

Table 8. Effect of debittering of fenugreek seeds by different processes on phosphorus compounds (mg/100g flour sample on a dry weight basis).

Treatments	Total phosphorus*	Phytate phosphorus*	Phytate phosphorus as % of total phosphorus	Phytic acid*	% Reduction of phytic acid	Inorganic phosphorus*	Inorganic phosphorus as % of total phosphorus
T0	187.50 ^e ±0.22	71.85 ^a ±0.05	38.32 ^a ±0.14	254.34 ^a ±0.50	-	54.25 [±] 0.52	28.93 [±] 0.30
T1	179.34 ^h ±0.10	60.00 [±] 0.90	33.45 ^b ±0.16	212.40 [±] 0.43	16.48 ^d ±0.28	55.40 [±] 0.35	30.89 [±] 0.40
T2	235.90 ^b ±0.26	29.55 [±] 0.22	12.52 [±] 0.18	104.60 [±] 0.06	58.87 ^b ±0.12	116.94 ^a ±0.10	49.57 ^a ±0.26
T3	185.48 ^f ±0.20	61.66 [±] 0.66	33.24 [±] 0.10	218.27 [±] 0.33	14.18 [±] 0.34	79.48 ^d ±0.66	42.85 ^d ±0.14
T4	246.03 ^a ±1.00	30.26 ^f ±0.20	12.29 [±] 0.22	107.12 [±] 0.25	57.88 [±] 0.20	108.76 ^b ±0.37	44.20 [±] 0.10
T5	182.35 [±] 0.14	61.10 ^d ±0.01	33.50 ^b ±0.16	216.29 ^d ±0.03	14.96 [±] 0.26	71.91 [±] 0.17	39.43 [±] 0.20
T6	227.24 [±] 0.10	29.38 ^h ±0.08	12.92 [±] 0.08	104.00 [±] 2.00	59.10 ^a ±0.36	106.63 [±] 0.41	46.92 ^b ±0.20
T7	196.88 ^d ±0.24	64.00 ^b ±0.05	32.50 ^d ±0.18	226.56 [±] 0.17	10.92 [±] 0.20	53.45 [±] 0.20	27.14 ^b ±0.28

* Calculated on dry weight basis; values are the mean of triplicate determinations with standard deviation; the different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

Table 9. Effect of debittering of fenugreek seed flours by different processes on sensory characteristics of baton salé baked from 90% wheat-10% fenugreek flour Mixtures.

Baton Salé samples	General Appearance	Color	Flavor	Taste	Crispy	Overall acceptability
90%WF+10% T0	7.45 ^b ± 0.16	7.27 ^{cd} ± 0.54	4.84 [±] 0.12	4.72 ^d ± 0.08	7.54 ^a ± 0.02	4.68 [±] 0.10
90%WF+10% T1	7.09 ^d ± 0.18	7.54 ^b ± 0.18	6.11 ^a ± 0.06	6.14 ^a ± 0.10	7.17 ^d ± 0.02	6.00 ^b ± 0.06
90%WF+10% T2	7.18 ^{cd} ± 0.36	7.45 ^{bc} ± 0.26	6.16 ^a ± 0.10	6.22 ^a ± 0.10	6.18 [±] 0.14	6.13 ^a ± 0.08
90%WF+10% T3	7.36 ^{bc} ± 0.20	7.36 ^{bcd} ± 0.14	5.36 ^d ± 0.04	5.44 [±] 0.20	7.39 [±] 0.08	5.58 [±] 0.20
90%WF+10% T4	7.63 ^a ± 0.16	7.36 ^{bcd} ± 0.34	5.83 ^{bc} ± 0.12	5.86 ^b ± 0.18	7.25 [±] 0.12	5.93 [±] 0.02
90%WF+10% T5	7.36 ^{bc} ± 0.10	7.00 [±] 0.14	5.30 [±] 0.12	5.54 [±] 0.16	7.41 ^{bc} ± 0.08	5.57 [±] 0.18
90%WF+10% T6	7.09 ^d ± 0.16	7.17 ^{de} ± 0.02	5.89 ^b ± 0.14	5.90 ^b ± 0.18	7.21 ^d ± 0.14	5.92 ^{bc} ± 0.14
90%WF+10% T7	7.72 ^a ± 0.22	7.81 ^a ± 0.04	5.78 [±] 0.11	5.81 ^b ± 0.14	7.49 ^{ab} ± 0.16	5.72 ^d ± 0.12
L.S.D. (0.05)	0.17	0.22	0.09	0.12	0.09	0.10
F Test	***	***	***	***	***	***

WF= Wheat flour; L.S.D. (0.05) = Least significant difference; values are the mean of triplicate determinations; the different letters at the column means significant differences at ($p \leq 0.05$) and the same letters means no significant differences.

Conclusion

Fenugreek is a unique legume crop known for its bitter taste and pharmacological properties, along with various health benefits. Soaking fenugreek seeds in a 4% lactic acid solution followed by germination reduces their bitter taste and phytic acid content. In contrast, this process increases levels of crude fiber, ash, inorganic phosphorus, phenolic compounds, flavonoids, and antioxidants. The results showed that baking of Baton Sale', from a composite flour consisting of 90% wheat flour and 10% flour from germinated fenugreek seeds soaked in the 4% lactic acid solution, exhibits a moderate taste and acceptable sensory properties for consumer

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تقييم دقيق بذور الحلبة المنزوعة المرارة بطرق معالجة مختلفة

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الملخص

تعتبر بذور الحلبة من البقوليات ذات قيمة لاحتوائها على محتويات عالية من البروتين والألياف الغذائية والزيت والمعادن والمكونات الوظيفية المختلفة. ومع ذلك، فإن مذاقها المر يحد من استخدامها في التطبيقات الغذائية. لذلك تم إجراء هذا البحث لتطوير طريقة بسيطة لتقليل محتوى السابونين المسؤول عن الطعم المر لبذور الحلبة ومقارنة هذه الطريقة مع الطرق الأخرى لعمليات نزع المرارة. واعتمدت الطريقة المطورة على نقع بذور الحلبة لمدة 12 ساعة في محلول حامض اللاكتيك 4% ثم إنباتها لمدة 72 ساعة. وظهرت النتائج ان النقع في محلول حمض اللاكتيك 4% أدى إلى إزالة 77.65% من محتوى السابونين في بذور الحلبة غير المنقوعة وكذلك إزالة 80.85% من المحتوى الاصلي للسابونين في البذور غير المعاملة عندما تم أنباتها بعد عملية النقع. بالإضافة إلى ذلك، تم تسجيل انخفاض ملحوظ في مادة السابونين بنسبة 71.11% وكذلك 60.49% بعد إنبات البذور المنقوعة في الزبادي والماء؛ على التوالي. كما سجلت عملية التخميص أقل قيمة (40.63%) في خفض نسبة السابونين. وأظهرت عمليات إنبات بذور الحلبة المختلفة بعد نقعها في محلول حامض اللاكتيك 4% أو الماء أو الزبادي زيادة في نسبة السكر غير المختزل والألياف الخام والرماد والمركبات الفينولية والفلافونويدات ومضادات الأكسدة، في حين انخفضت نسبة النشا والدهون وحامض الفيتيك. كما أستبان من نتائج الباتون ساليه المخبوزة من مخلوط دقيق مكون من 90% دقيق قمح و10% دقيق الحلبة منزوع المرارة ان أفضل الصفات الحسية المسجلة كانت للعينة التي تحتوي علي دقيق من بذور الحلبة المنقوعة في محلول 4% حامض لاكتيك لمدة 12 ساعة متبوعا بعملية الانبات لمدة 72 ساعة.

الكلمات الدالة: السابونين، الفلافونويدات، الفيتات، الفينولات، مضادات الاكسدة.