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Research Article

Multifaceted Properties of Fenugreek Seeds: Physicochemical, Functional, and Antioxidant Perspectives

Navjot Kaur,[1] **Hamid**,[1] **Rafeeya Shams**,[1] **Pintu Choudhary**,[2] **Kshirod Kumar Dash**,[3] **Ayaz Mukarram shaikh**,[4] **and Kovács Béla**[4]

[1]*Department of Food Technology and Nutrition, Lovely Professional University, Phagwara, Punjab, India*
[2]*Department of Food Technology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, India*
[3]*Department of Food Engineering and Technology, Ghani Khan Choudhury Institute of Engineering and Technology, Malda, West Bengal, India*
[4]*Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen, Hungary*

Correspondence should be addressed to Hamid; hamidfst6789@gmail.com, Kshirod Kumar Dash; kshiroddash14@gmail.com, and Kovács Béla; kovacsb@agr.unideb.hu

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Fenugreek seeds (*Trigonella foenum-graecum*) are widely recognized for their dual role, both as a culinary ingredient and a medicinal herb. Traditionally, fenugreek seeds have gained increasing scientific interest due to their rich nutrient profile and potential health benefits, making them valuable for food and nutraceutical applications. This study is aimed at comprehensively analyzing the physicochemical and fatty acid profile of fenugreek seeds. Moisture, ash, lipid, protein, and fiber content were determined, and various physical and functional properties were evaluated. The oil analysis investigated key physicochemical factors such as color, acid value, peroxide value, iodine value, and saponification value to provide a comprehensive evaluation of the oil's quality and stability. Proximate analysis revealed that fenugreek seeds are a source of protein (25.24%), dietary fiber (7.89%), and lipids (6.84%). The seeds demonstrated significant water and oil absorption capacities, indicating their potential as a food ingredient with good emulsifying and foaming properties. The research findings also indicated the presence of diverse fatty acids, including monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and negligible total trans-fatty acids. It was found that both the oil samples (cold-pressed and solvent-petroleum ether extracted) contained two primary components, namely, linolenic acid and linoleic acid, categorized as polyunsaturated Omega-6 fatty acids. These fatty acids are known for their notable therapeutic potential in managing cardiovascular diseases and anticancer properties.

Keywords: antioxidant properties; fatty acid composition; fenugreek; functional properties; physical properties

1. Introduction

Fenugreek (*Trigonella foenum-graecum*) is an aromatic legume from the Fabaceae family and has been used for centuries for its culinary and medicinal properties. Fenugreek has been recognized as a medicinal herb in Indian Ayurvedic and traditional Chinese medicines [1]. The fenugreek plant produces abundant long single pods ranging from 10- to 15-cm long. These pods typically contain an average of 18–20 seeds. It is common to find double pods occurring naturally in field conditions as well. Fenugreek seeds are small, yellowish-brown, and roughly rectangular [2]. They have a distinctive concavity on the outer surface that separates the radicle from the cotyledon. Typically, the size of fenugreek seeds ranges from 0.3 to 0.6 cm in length (L), 0.2 to 0.4 cm in width (W), and 0.2 cm in thickness (T) [3]. The size can vary slightly depending on the variety and growing conditions. Fenugreek seeds and leaves, whether whole, powdered, or in extract form, are extensively used for their nutritional and therapeutic benefits. The seeds of fenugreek are particularly well-known, often used as a spice, and have been widely studied for their medicinal properties [4]. They have a distinct, aromatic, characteristic odor due to the presence of volatile compounds [5, 6]. Fenugreek seeds can absorb water and form a gel-like substance. This property is attributed to the presence of galactomannans. The ratio of galactose to mannose (M/G) is widely recognized as a defining factor for the technofunctionality of galactomannans, which is approximately 1 in the case of fenugreek [7].

The composition of fenugreek seeds contributes to its potential nutraceutical value. They are responsible for various positive physiological effects such as anticancer, hypocholesterolemic, antimicrobial, antidiabetic, stomach stimulant, hepatoprotective, and anticancer properties [8, 9]. The fenugreek seed oil comprises linoleic acid, linoleic acid methyl ester, pinene, palmitic acid, and so forth. FTIR analysis indicates the presence of carboxyl groups, which were predominantly associated with unsaturated essential fatty acids. The oil also demonstrates potent antioxidant radical scavenging action [10]. Fenugreek seeds have been shown to have hypoglycemic effects and may help in managing blood sugar levels. Therefore, fenugreek flour has been utilized in several studies for the development of low GI food products [11]. Several studies suggest that fenugreek can improve insulin sensitivity, lower fasting blood sugar levels, and reduce postmeal glucose levels in individuals with diabetes [12, 13]. Fenugreek seeds have shown considerable potential in reducing total cholesterol and triglyceride levels in the body. Additionally, they have been effective in increasing levels of high-density lipoprotein (HDL) cholesterol, which are beneficial for cardiovascular health. The cholesterol-lowering effect of fenugreek seeds is attributed to their content of polyphenols, saponins, and alkaloids [14]. Similarly, fenugreek seeds possess anti-inflammatory and antioxidant properties attributed to the presence of flavonoids, polyphenols, and other bioactive compounds [15].

Understanding the physicochemical properties of fenugreek seed is important for various applications, including food processing, pharmaceutical formulations, and nutraceutical preparations. These properties influence factors such as product stability, sensory attributes, solubility, and functionality. However, it is important to note that specific physicochemical properties can vary depending on the seed source, processing techniques, and storage conditions. This research is an effort to highlight fenugreek seed as a functional food by exploring and studying its chemical composition and investigating the physical properties of fenugreek seed and its powder. The study further details the physicochemical characteristics and fatty acid profiles of fenugreek seed oil extracted by different methods.

2. Materials and Methods

2.1. Preparation of Sample. The '*Pusa early bunching*' variety of fenugreek seeds was obtained from Pantnagar Agriculture University, Udham Singh Nagar, Uttarakhand. The seeds were manually cleaned and sieved to remove unwanted material such as stones, twigs, and dust. For powder formation, the seeds were washed, sun-dried, and ground using an electrical grinder. Sieving was done to obtain a fine powdered sample. All chemicals and reagents utilized in this study were of analytical grade.

2.2. Analysis of Dimensional, Gravimetric, and Frictional Properties of Fenugreek Seeds

2.2.1. Dimensional Properties

2.2.1.1. Size and Shape. The seed size was determined by randomly picking 20 seeds from the 500 g of sample and measuring their three principal dimensions using a digital vernier caliper: L, W, and T [16]. This was done in three replications. The seed shapes were examined visually with the naked eye.

2.2.1.2. Geometric Mean Diameter. The geometric mean diameter (D_g) provides insight into the overall geometry of the seed using the following expression [17]:

$$D_g = (LWT)^{1/3}. \quad (1)$$

2.2.1.3. Sphericity. Sphericity (φ) is defined as the ratio of the surface area of a sphere with the same volume as the seed to the surface area of the seed itself [18]. It was calculated using the following Equation (2):

$$\varphi = \frac{D_g}{L} \times 100. \quad (2)$$

2.2.1.4. Seed Surface Area. The surface area of the seed (A_s) was determined by analogy with a sphere of the same equivalent mean diameter using the following formula [19]:

$$A_s = \pi D_g{}^2. \quad (3)$$

2.2.2. Gravimetric Properties

2.2.2.1. Bulk Density. Bulk density (ρ_b) was determined based on the seed weight and the volume of the container [20]. An empty graduated cylinder was initially filled with 20 g of seeds, and the volume occupied was recorded. This procedure was repeated three times, and the bulk density for each trial was determined using the following formula:

$$\rho_b = \frac{W_s}{V_s},$$

where W_s is the weight of the sample (kilogram) and V_s is the volume occupied by the sample (cubic meter).

2.2.2.2. True Density. True density was determined using the toluene displacement method. Toluene was preferred instead of water due to its lesser absorption by seeds. It is defined as the ratio of seed weight to the true volume of the seeds. Then, 50 mL of toluene was added to a graduated measuring cylinder, and 20 g of seeds were immersed in it. The volume of toluene displaced by the seeds was recorded, and the seed weight ratio of the volume of displaced toluene was calculated [21].

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2.2.2.3. Porosity (%). The porosity (ε) of the seed represents the fraction of the total volume or space in the bulk that is not filled by the grain itself. It is determined using the bulk density and true density values [22]. The following formula is used to calculate the porosity of fenugreek seeds.

$$\varepsilon = \left(1 - \frac{\rho_b}{\rho_t}\right) \times 100 = \frac{\rho_t - \rho_b}{\rho_t} \times 100, \quad (4)$$

where ρ_b is the bulk density and ρ_t is the true density.

2.2.2.4. Specific Gravity. Specific gravity is the ratio of the true density of fenugreek seeds to the density of water at 25°C, as expressed in Equation (5) [23].

$$\text{specific gravity} = \frac{\rho_t}{\rho_w}, \quad (5)$$

where ρ_t and ρ_w are the true densities of fenugreek seeds and water at 25°C, respectively.

2.2.2.5. Thousand Seed Mass. Then, 100 randomly selected seeds were weighed with an electronic balance and then multiplied that weight by 10 to obtain the mass of 1000 seeds [24].

2.2.3. Frictional Properties

2.2.3.1. Angle of Repose. A hollow cylinder was used to determine the angle of repose [25]. The cylinder was placed on a circular plate and filled with 100 g of fenugreek seeds. The cylinder was then carefully lifted, letting the fenugreek seeds settle into a cone shape on the plate, as illustrated in Figure 1. The diameter and height of the resulting cone were recorded, and the angle of repose was determined using the given equation.

$$\theta = \tan^{-1}\left(\frac{h}{r}\right), \quad (6)$$

where h and r refer to the height and radius of the cone shape, respectively.

2.3. Analysis of Physicochemical and Functional Properties of Fenugreek Seed Powder

2.3.1. Physical Properties

2.3.1.1. Bulk Density. The bulk density (ρ_b) was determined by filling a measuring cylinder (100 mL) with fenugreek seed powder [26]. Then, the mass-to-volume ratio was calculated by Equation (7).

$$\rho_b = \frac{m}{v}, \quad (7)$$

where m is the mass of seed powder (g) and V is the volume occupied by a fixed mass of seed powder (mL).

2.3.1.2. Tapped Density. Tapped density (ρ_t) was measured by filling a 100-mL measuring cylinder with seed powder and then tapping it. Tapping was done using a bulk density

Figure 1: Pile of fenugreek seeds for determination of angle of repose. The lines labeled "h" and "r" represent the geometric dimensions of the cone-shaped seed pile. Specifically, "h" denotes the vertical height of the pile, while "r" indicates the base radius, which are used for calculating the value of angle of repose, i.e., θ.

meter until the volume no longer decreased. This volume was measured after leveling the surface [27].

$$\rho_t = , \quad (8)$$

where m is the mass of seed powder (g) and V is the volume occupied by seed powder after tapping until no further volume change is observed (mL).

2.3.1.3. Carr's Index and Hausner Ratio. The compressibility and flowability of the sample were determined by Carr's index and Hausner's ratio value [28]. The values were calculated using the following formulas (9) and (10), respectively.

$$\text{Carr index}(\%) = \frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \times 100, \quad (9)$$

$$\text{Hausner ratio} = \frac{\text{Tapped bulk density}}{\text{Loose bulk density}}. \quad (10)$$

2.3.2. Proximate Composition. The sample's moisture content was determined using the oven drying method (AOAC 930). Ash, fiber, and fat contents were assayed by the Association of the Official Analytical Chemists methods, AOAC 942.05, AOAC 978.10, and AOAC 2003.05, respectively. The protein content was measured using the Kjeldahl method and calculated with a factor of 6.25 N (AOAC 2001.11).

2.3.3. Functional Properties

2.3.3.1. Water Holding Capacity (WHC) and Oil Holding Capacity (OHC). The WHC and OHC were calculated using the method followed by Kaur and Singh [29], with slight modifications. Then, 1 g of powdered sample was added to a preweighed centrifuge tube to which 10 mL of distilled water was added and vortexed. The mixture was allowed to stand for 30 min before being centrifuged at 4000 g for 25 min. Excess water was removed by inverting the tube over absorbent paper, and the weight was recorded. For measuring oil absorption, 10 mL of refined sunflower oil was used in a similar process. The water and oil absorption capacities were reported as the quantity of water or oil absorbed per gram of the sample.

2.3.3.2. Least Gelation Concentration (LGC). The lowest gelation concentration was evaluated using the method followed

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by Paliwal et al. [30]. Suspensions of powder with various concentrations (5%, 10%, 15%, 20%, 25%, and 30% (w/v)) were prepared in 10 mL of water. The test tubes containing these suspensions were heated in a boiling water bath for 1 h and then cooled under running cold tap water. The test tubes were further cooled for 2 h at 4°C. The LGC was determined by identifying the concentration at which the sample remained intact and did not slide when the tubes were inverted.

2.3.3.3. Foaming Capacity (FC) and Foaming Stability (FS). Foaming capacity and stability were determined by the method followed by Sajib et al. with slight modification, using Equations (11) and (12), respectively [31]. Then, 1 g of sample was added to 50-mL distilled water in a measuring cylinder. The sample was shaken vigorously using a small blender for 2 min to foam. The volume of the foam formed after whipping was recorded as the foam capacity. A final observation was made after 1 h to record the stability of the foam.

$$\text{Foaming capacity (\%)} = \frac{\text{Volume after whipping} - \text{volume before whipping}}{\text{Volume before whipping}} \times 100, \tag{11}$$

$$\text{Foaming stability (\%)} = \frac{\text{Volume of foam after 1 hour}}{\text{Initial volume of foam}} \times 100. \tag{12}$$

2.3.3.4. Emulsion Capacity (EC). EC was determined according to the Adeleke and Odedeji method with slight modification [32]. Then, 2 g of fenugreek seed powder was added to 75 mL of distilled water. The mixture was then kept on a magnetic stirrer for 1 min at 800 rpm. Then, the vegetable oil was added continuously until the emulsion breakpoint was reached. The emulsifying capacity was expressed as the volume of oil (mL) emulsified by 1 g of flour.

2.4. FTIR. FTIR spectroscopy with potassium bromide (KBr) was used to identify the functional groups in fenugreek seed powder. The infrared spectra were recorded in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} at room temperature (25°C). The sample was prepared as a thin film on a diamond crystal surface for analysis [33].

2.5. Total Phenolic Content (TPC). TPC was determined in fenugreek extract using the Folin–Ciocalteu colorimetric method [34]. Then, 100-μL sample extract was taken and subsequently combined with 1 mL of FC-reagent (diluted 1:1). After 5 min, 2 mL of sodium carbonate (7.5%) was added. The mixture was then allowed to incubate for 30 min. The absorbance was measured at a wavelength of 765 nm. TPC was quantified in terms of milligram of gallic acid equivalents per gram of dry weight (mg GAE/gm d.w.) using the gallic acid standard curve.

2.6. Antioxidant Activity. Free-radical scavenging activity was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazil) method [35]. Various concentrations of fenugreek extracts were placed in separate test tubes, with the volume adjusted to 1000 mL by adding methanol (MeOH). To these test tubes, 2 mL of a 0.1 mM methanolic DPPH solution was added and mixed thoroughly. The tubes were then incubated in the dark at room temperature for 30 min. A control sample was prepared in the same manner but without the extract. Absorbance change in the samples was measured at 517 nm. The radical scavenging activity was expressed as a percentage inhibition, calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{sample OD}}{\text{Control OD}} \times 100. \tag{13}$$

2.7. Extraction and Analysis of Oil Using Different Methods

2.7.1. Extraction of Oil. Then, 10 g of fenugreek seed powder were extracted with n-hexane (200 mL) using a Soxhlet extractor for 6 h (65°C–70°C). The resulting extract was then transferred into a round flask, and the solvent was evaporated using a rotary evaporator (40°C). The cold-pressed oil and Soxhlet oil extract were stored at 4°C in amber-colored bottles to prevent compounds from degradation until further analysis.

2.7.2. Physiochemical Analysis. The extracted oil's acid value, peroxide value, iodine value, saponification value, and free fatty acid content were measured using the AOCS method.

2.7.3. FTIR. The functional groups in fenugreek seed powder were detected using FTIR spectroscopy with KBr. The infrared spectra were captured over a range of 4000–400 cm^{-1}, with a resolution of 4 cm^{-1} at room temperature (25°C).

2.7.4. Fatty Acid Analysis. Methyl esters of fatty acids (FAMEs) were prepared. Then, the diluted FAME were separated on an Agilent 8890 series GC ($58 \times 51 \times 49$ cm) equipped with an SP-2560 fused silica capillary column (100 m, 0.25 mm, and 0.25 μm) and a flame ionization detector (FID). Then, 1 μL sample was injected into the GC injector operated in split mode with a split ratio of 100:1 and a run time of 65 min. Helium was used as the carrier gas at a 1.0 mL/min flow rate. The temperature was programmed from 100°C to 240°C at 3°C/min, held for 4 min. Detector and injector temperatures were set at 260°C and 250°C, respectively. For obtaining the weight percentage of various fatty acids, their retention time was compared with authentic standards.

2.8. Statistical Analysis. All the data was measured in triplicates and expressed as mean ± standard deviation using MS Excel. One-way analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) were performed to determine the significance of data using Minitab (Version 19.1).

3. Results and Discussion

3.1. Dimensional, Gravimetric, and Frictional Properties of Fenugreek Seeds

3.1.1. Dimensional Properties. Fenugreek seeds were compact and dense, typically oblong, with a somewhat rectangular outline. Some had a slightly curved shape, while others were straight-edged. The L, W, and T of the fenugreek seeds were

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recorded as 3.49 ± 0.06 mm, 2.30 ± 0.10 mm, and 1.27 ± 0.04 mm, respectively. Altuntaş et al. reported the *L*, *W*, and *T* of fenugreek seed in the range from 4.01 to 4.19 mm, 2.35 to 2.61 mm, and 1.49 to 1.74 mm, respectively [36]. The geometric mean diameter (D_g) and sphericity (ϕ) were calculated as 2.19 ± 0.09 mm and 62.82% ± 1.88%, respectively, which is similar to their results (60.79%–64.06%).

3.1.2. Gravimetric Properties. Values of true density in our study were observed to be 1360 kg/m^3, which was slightly higher than results reported by Altuntaş et al. [36] for fenugreek, which may be due to varietal differences. The studied fenugreek's true density was higher than that reported for coriander seeds [37]. This variation could be attributed to the disparity in seed types. The fenugreek seeds had a true density above 1000 kg/m^3, higher than the density of water. This suggests that the seeds are denser than water, allowing them to effortlessly sink in water and enabling their easy separation from lighter foreign materials. Bulk density of fenugreek seeds was recorded as 740 kg/m^3. Altuntaş et al. reported the bulk density of fenugreek seeds in the range from 701.16 to 645.81 kg/m^3 [36]. The value of bulk density observed in our study was lower than that of cumin seeds [38]. The value of specific gravity was recorded to be 1.36 ± 0.04. As the value is more than 1, this indicates that the seeds will easily sink in the water [23]. The porosity (ε) of the seeds was calculated to be 44.98 ± 2.41. The porosity of fenugreek seeds was lower than that of cumin seeds [39] and tung seeds [40]. The mass of 1000 seeds is another important characteristic of all seeds and grains. It was measured to be 7.63 g ± 0.16, which was lower than that reported by Altuntaş et al. of 15.48 g for fenugreek. This suggests that the fenugreek under examination is lighter. Parameters such as true density, bulk density, and porosity can be useful in designing kernel hoppers and storage facilities. During the aeration and drying process, the porosity of the seeds also determines and affects the resistance of seeds to airflow [41].

3.1.3. Frictional Properties. The angle of repose of fenugreek seed was observed to be 24.88° ± 0.24. Altuntaş et al. [36] reported 14.34° for fenugreek, which is less than our observed value [39]. This might be due to a difference in seed variety. It was also observed that the value noted in this study was lower than coriander seed [37], sesame seed [42], and cumin seed [38] and higher than flaxseed [43]. The observed values for various dimensional, gravimetric, and frictional properties of fenugreek seed are represented in Table 1.

TABLE 1: Dimensional, gravimetric, and frictional properties of fenugreek seeds.

Parameters	Mean values ± SD
Dimensional properties	
Length (mm)	3.49 ± 0.06
Width (mm)	2.30 ± 0.10
Thickness (mm)	1.27 ± 0.04
Geometric mean diameter (mm)	2.19 ± 0.09
Sphericity (%)	62.82 ± 1.88
Seed surface area (mm)	15.13 ± 1.27
Gravimetric properties	
Bulk density (g/mL)	0.74 ± 0.013
True density (g/mL)	1.36 ± 0.04
Porosity (%)	44.98 ± 2.41
Specific gravity	1.36 ± 0.04
1000 seed mass (g)	7.63 ± 0.16
Frictional properties	
Angle of repose (°)	24.88 ± 0.24

3.2. Physicochemical and Functional Properties of Fenugreek Seed Powder

3.2.1. Physical Properties. Bulk density refers to the mass of the powder in its unaltered or natural state. The bulk density was recorded as 0.59 ± 0.006 g/mL. On the other hand, tapped density is the maximum packing density of the powder. It is achieved when the powder is tapped or vibrated to reduce void spaces between particles. Here, the tapped density for fenugreek seed powder was recorded as 0.81 ± 0.01 g/mL, respectively. Carr's index is a measure of the compressibility of powder. Carr's index value was observed as 26.85 % ± 0.25%, indicating moderate flow properties. Hausner's ratio, which is the ratio of tapped density to bulk density, was recorded as 1.36 ± 0.00. The slight difference between bulk and tapped density suggests that the powder particles were not highly cohesive. However, the tapped density is significantly higher than the bulk density, indicating the powder can be compacted to a high extent [44].

3.2.2. Proximate Composition. The proximate composition of fenugreek seed is presented in Table 2. The moisture content of fenugreek was calculated as 12.08% ± 0.94%. It showed a significantly high crude protein content (25.24% ± 0.48%), similar to other studies. The protein content of 27.57% and 25.90% has been reported by Naidu et al. [35] and Hooda and Jood [45], respectively. Crude fat content was determined as 6.84% ± 0.59%. Naidu et al. and Hooda and Jood reported 6.71% and 6.90% crude fat content in fenugreek seed flour. It showed an ash content of 3.46% ± 0.36% and crude fiber content of 7.89% ± 0.27%, near the results obtained by Hooda and Jood for fenugreek. The minor differences observed in the proximate composition could be attributed to different cultural practices, soil, and environmental conditions.

3.2.3. Functional Properties. WHC represents the ability of a product to associate with water. The primary components that improve the WHC of flour are proteins and carbohydrates. These elements possess hydrophilic segments, including polar or charged side chains, which contribute to their ability to retain water [46]. The WHC and OHC of fenugreek seed powder were recorded as 2.79 g/g ± 0.16 and 0.93 g/g ± 0.02, respectively. The results of the water absorption capacity tests indicate that ground fenugreek seeds can retain water due to the presence of carbohydrates

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TABLE 2: Physicochemical and functional properties of fenugreek seed powder.

Parameters	Mean values ± SD
Physical properties	
Bulk density (g/mL)	0.59 ± 0.01
Tapped density (g/mL)	0.81 ± 0.01
Carr's index (%)	26.85 ± 0.25
Hausner's ratio	1.36 ± 0.00
Functional properties	
Water holding capacity (g/g)	2.79 ± 0.16
Oil holding capacity (g/g)	0.93 ± 0.02
Least gelation concentration (%)	15 ± 0.00
Foaming capacity (%)	24.66 ± 0.94
Foaming stability (%)	25.00 ± 0.82
Emulsion capacity (%)	51.37 ± 0.41
Proximate composition	
Moisture content (%)	12.08 ± 0.94
Ash content (%)	3.46 ± 0.36
Fat content (%)	6.84 ± 0.59
Protein content (%)	25.24 ± 0.48
Fiber content (%)	6.84 ± 0.59

and fibers. Mabrouki et al. reported WHC and OHC of fenugreek flour as 4.72 g/g and 1.50 g/g, respectively [47].

EC is defined as the ability of the flour to emulsify oil. It is linked to the soluble protein content of a substance [48]. Fenugreek powder showed an emulsion activity of 51.33%. The foaming activity (FA) and FS are used as indices of whipping properties and were found to be 25% and 24%, respectively. The stability of foam relies on the capacity of trapped air bubbles to stay intact without collapsing. Therefore, stable foams are created using highly surface-active substances [49, 50]. The LGC serves as a measure of a substance's ability to form a gel. A lower LGC indicates an enhanced gelling ability. In this study, the fenugreek flour had the lowest gelation concentration of 15%.

3.3. FTIR. Infrared spectrum of the fenugreek seed powder is shown in Figure 2. The absorption frequency at 3282.89 cm^{-1} indicates O–H stretching vibrations, while the sharp peaks at about 2924.99 and 2854.71 cm^{-1} indicate the presence of asymmetric and symmetric C–H stretching vibrations. The presence of the band of relatively medium intensity at 1743.96 cm^{-1} indicates the presence of C=O stretching vibration. The absorption bands may characterize the amines due to N–H and C=O stretching vibrations. The spectrum of amines is especially important due to its relation to proteins. The presence of protein is indicated by prominent peaks at 1639.35 cm^{-1}, corresponding to N-H bending [51]. Additionally, within the range of 1200–900 cm^{-1}, the spectrum shows absorption bands at 1039.76 cm^{-1}, which are associated with amines.

3.4. TPC. Phenolic compounds are recognized as primary contributors to the overall antioxidant activity of spices [52, 53]. The TPC of fenugreek aqueous ethanol extracts was calculated as 74.85 ± 2.29 mg GAE/g. These results were in line with the previous studies for *Trigonella spruneriana* extracts ranging from 18.59 to 113.59 mg GAE/g extract [54]. The results were, however, higher than those obtained by Pandey and Awasthi [55]. Phenolic acids and flavonoids possess hydrophilic characteristics, which predominantly contribute to the TPC analysis, explaining the higher amounts of TPC in more aqueous ethanolic solvent [56]. The significant phenolic content in fenugreek aligns with the findings of Marathe et al. [57]. They classified fenugreek as part of the high-phenolics category among a selection of 30 legume varieties commonly consumed in India.

3.5. Antioxidant Activity. The free radical scavenging activity of the phenolic extract was expressed in terms of percent inhibition against DPPH radical. The extracts exhibited a DPPH radical scavenging activity of 29.06% ± 0.27%. Pandey and Awasthi recorded antioxidant activity as 18.10% in fenugreek seed flour [55], whereas Dhull et al. [58] found a much higher antioxidant activity of 90.6% in raw fenugreek seeds [53]. The variations in results could be due to different cultivars, diverse extraction techniques, sample preparation, and solvents utilized. Research findings indicated a strong correlation between radical scavenging activity and TPC [59, 60]. Marathe et al. classified fenugreek among crops with high DPPH free radical scavenging activity [57].

3.6. Physiochemical Analysis of Oil. Understanding the physicochemical properties is crucial to optimize and simplify different formulations and processes. Other physical and chemical parameters of cold-pressed and solvent-extracted oil were analyzed to monitor the compositional quality of oils [61, 62]. These parameters include acid, peroxide, saponification, and iodine values. The results of the physicochemical properties are presented in Table 3. The acid value signifies the overall acidity of the oil, reflecting the combined impact of all fatty acids present in the glyceride molecule and the degree of oil hydrolysis [63]. In this study, cold-pressed oil exhibited an acid value of 0.13 ± 0.02 mg KOH/g. Fenugreek oil's free fatty acid content (0.06%) indicated its suitability for edible purposes, as it contained a significantly lower percentage of free fatty acids. This result has comparatively less value than the findings of Ali et al. [64], who reported a comparable value of 1.3%.

On the other hand, solvent (petroleum ether) extracted oil had an acid value of 6.38 ± 0.31 mg KOH/g. This might be due to the heat provided during the extraction process. The saponification value serves as an indicator of the average molecular weight of fatty acids within lipid glycerides. A lower saponification value indicates the presence of fatty acids with larger molecular weights in the glycerides, whereas a higher value indicates smaller molecular weight fatty acids. The saponification value was calculated to be 193.87 ± 0.08 and 170.89 ± 0.08 mg KOH/g for cold-pressed and Soxhlet-extracted seed oil, respectively. These results were similar to those reported previously (195 mg

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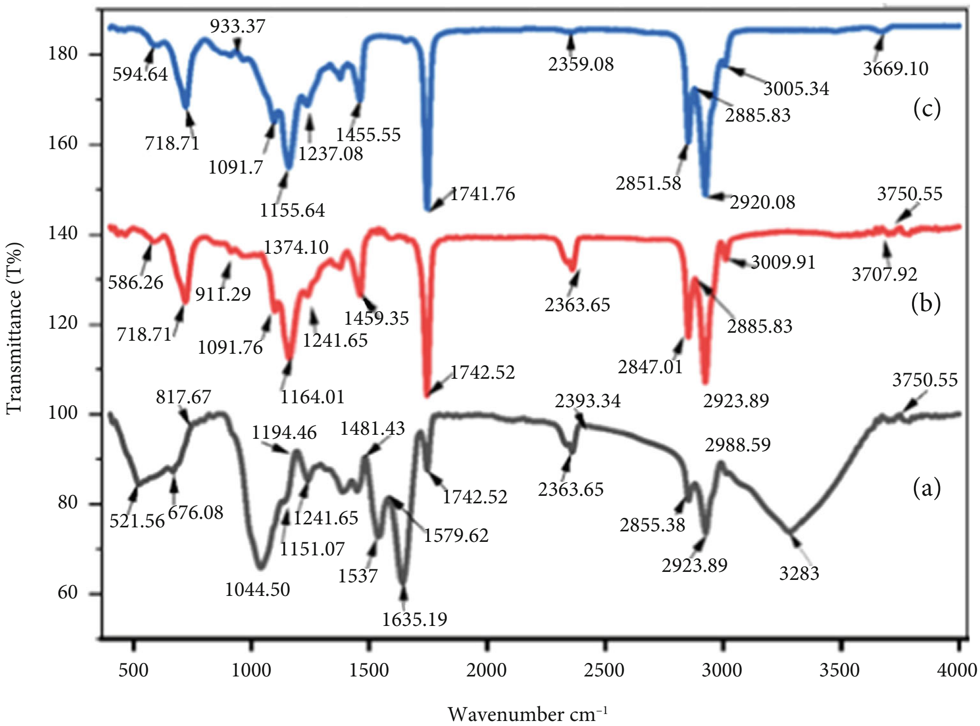

Figure 2: FTIR spectrum of (a) fenugreek seed powder, (b) Soxhlet oil extract–petroleum ether, and (c) cold-pressed oil of fenugreek seed.

TABLE 3: Physicochemical characteristics of cold-pressed and solvent-extracted fenugreek seed oil.

Characteristics	Mean values	
	Cold pressed	Soxhlet (petroleum ether)
Color	Greenish yellow	Reddish yellow
Acid value (mg KOH/g of oil)	0.13 ± 0.02^b	6.38 ± 0.31^a
Free fatty acid (%)	0.06 ± 0.00^b	3.36 ± 0.08^a
Iodine value (g/100 g of oil)	135.49 ± 0.88^a	128.65 ± 0.47^b
Peroxide value (meq/kg)	0.09 ± 0.00^b	3.36 ± 0.08^a
Saponification value (mg KOH/g of oil)	193.87 ± 0.08^a	170.89 ± 0.08^b

Note: Results are presented as Mean ± standard deviation. Different superscripted letters show significant difference ($p \leq 0.05$).

KOH/g) by Sulieman et al. [65] and those observed by Ali et al. (177.0 mg KOH/g) [66]. The oil's high saponification value suggested a high content of low molecular weight triacylglycerols in oil. Oil's saponification values are crucial in assessing its suitability for soap making [67]. The peroxide value of cold pressed oil was observed as 0.09 ± 0.00 meq/kg oil. The results were lower than those of Gu et al. and Ali et al. for fenugreek seed oil. However, the Soxhlet-extracted oil reported a higher peroxide value of 3.36 ± 0.08 meq/kg. High iodine value was observed for cold-pressed (135.49 ± 0.88 g/100 g) oil and Soxhlet-extracted oil (128.65 ± 0.47 g/100 g). Marathe et al. [57] also reported a high iodine value for fenugreek oil, which is attributed to its low content of saturated fatty acids. The iodine value of seed oil was less than that of soybean oil and sunflower oil. But it was found to be very high in comparison with other fruit seed oils like avocado pear (62.8) and native pear (55.6) [68]. However, the elevated iodine value suggested that the oil contained more double bonds.

3.7. FTIR Analysis of Fenugreek Seed Oil. FTIR spectroscopy was used to analyze the functional groups in both fenugreek oil samples, as illustrated in Figure 2. The notable absorption peaks at 2853.84–2923.58 cm^{-1} correspond to the symmetric and asymmetric stretching of methyl (-CH_3) and methylene (-CH_2) groups, suggesting the presence of fatty acids and their methyl esters within the carboxyl groups (COOH). The peaks at around 1743 cm^{-1} correspond to aldehyde. The distinct bands at 1460.12 and 1461.18 cm^{-1} correspond to the alcoholic (C-OH) group. Additionally, the aromatic acid esters (C-O-C) and the stretching vibrations of phenolic compounds (C-OH) were observed at 1097.26 and 1099.95 cm^{-1}, and these peaks may also be associated with amide groups [69]. The peaks at 720.51 and 721.11 cm^{-1} indicate the presence of a benzene ring. The FTIR spectrum results reveal that the cold-pressed and Soxhlet-extracted fenugreek seed oils have a range of functional groups, including carboxyl, hydroxyl, fats, alcohols, amides, and phenolic compounds.

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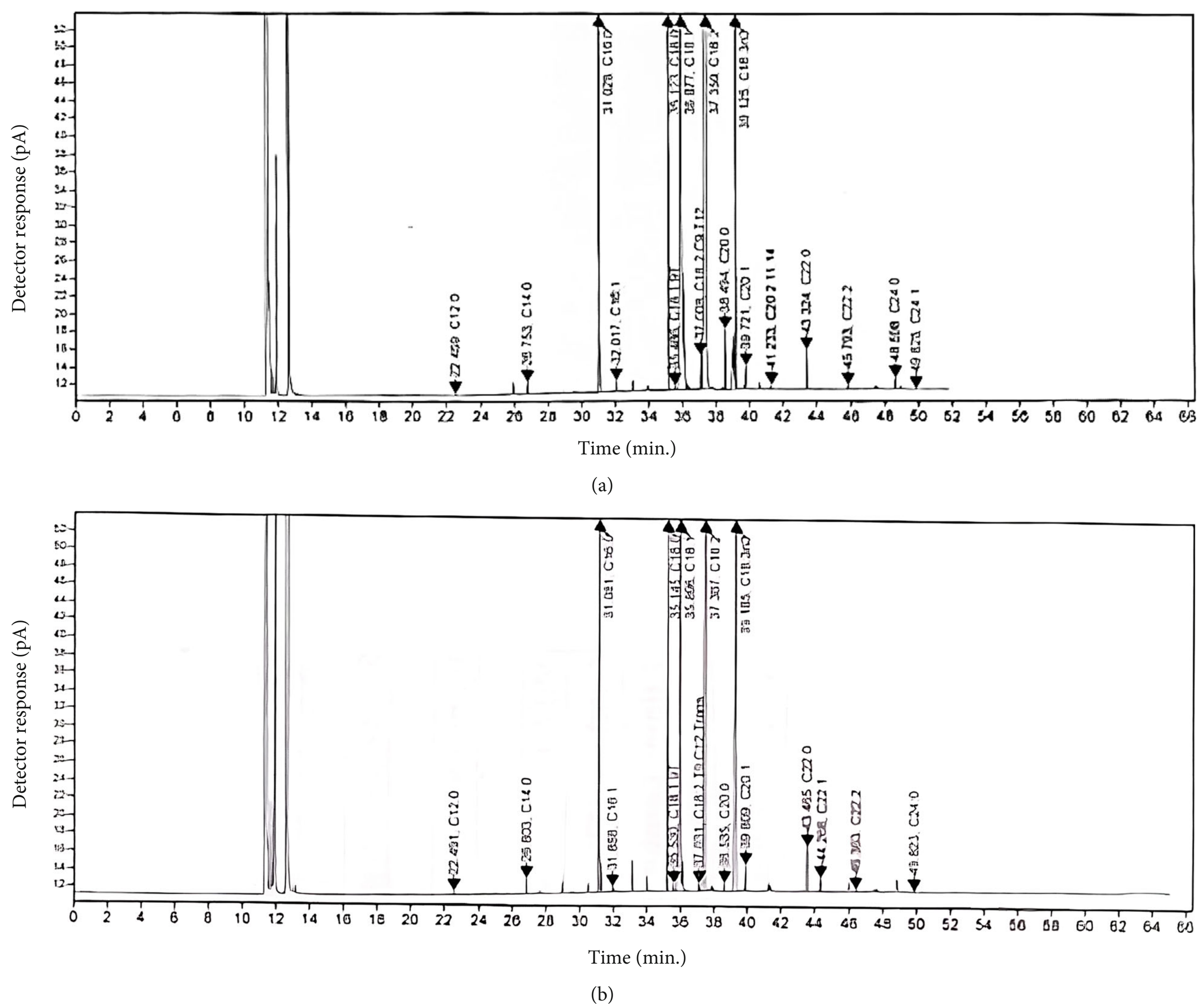

FIGURE 3: GC-Chromatogram of (a) cold-pressed fenugreek seed oil and (b) Soxhlet-extracted fenugreek seed oil.

3.8. Fatty Acid Composition. The fatty acid composition of fenugreek seed oil, extracted using cold pressing and Soxhlet methods, was analyzed and presented as GC chromatograms in Figure 3a,b. The results obtained are presented in Table 4. The data show the presence of various monounsaturated fatty acids (MUFAs) (24.28%), polyunsaturated fatty acids (PUFAs) (58.98%), and total trans-fatty acids (0.55%) in cold-pressed oil. The percentage of MUFA (17.01%) was comparably less, and PUFA (66.27%) was higher in soxhlet extracted oil. Cold-pressed fenugreek seed oil was observed to have two main constituents: linoleic acid (52.81%) and oleic acid (23.97%) in the majority.

In contrast, Soxhlet-extracted oil comprised linoleic (43.24%) and linolenic (23.00%) acids in higher percentages. Linoleic acid, classified as a polyunsaturated Omega-6 fatty acid, consists of an 18-carbon chain with double bonds (cis-configuration). Research has highlighted its significance in the human diet, particularly its potent anticancer properties [70]. Linolenic acid is known for the treatment of cardiovascular diseases [71]. Oleic acid is also known for treating coronary heart disease and diabetes [72]. Palmitic acid is present in oil extracted using different methods. It is recognized as one of the most prevalent saturated fatty acids, featuring a 16-carbon backbone, and is found in plants, animals, and microorganisms. It is also used as a food additive and for its anti-inflammatory properties. These compounds could account for the antioxidant activity observed in fenugreek seed oil. Hence, it can be proposed that the oil obtained from this study possesses potential applications in the pharmaceutical sector for various medicinal purposes. Linoleic acid was found to be 52.81%, which is much higher than that of various almond oil cultivars (12.9% and 26.1%) [73] but was comparable with that of sesame oil (45.7%) [74]. The difference in fatty acid composition from other studies can be attributed to the differences among varieties, growing conditions, and the extraction conditions applied, which may result in incomplete separation of some fatty acids [75]. However, all oil extracts were rich in unsaturated fatty acids such as linoleic acid, linolenic acid, oleic acid, and palmitic acid. The abundance of unsaturated

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TABLE 4: Percentage fatty acid composition of fenugreek seed oil.

Components	Percentage Cold-pressed	Percentage Soxhlet (petroleum ether)
Monounsaturated fatty acid	**24.28**	**17.01**
Oleic acid (C18:1)	23.97	16.40
Palmitoleic acid (C16:1)	0.08	0.06
Nervonic acid (C24:1)	0.01	—
CIS-10 heptadecanoic acid (C17:1)	—	—
Eicosenoic acid (C20:1)	0.20	0.34
Erucic acid (C22:1)	—	0.20
Polyunsaturated fatty acid	**58.98**	**66.27**
Linolenic acid (C18:3)	6.07	23.00
Linoleic acid (C18:2)	52.81	43.24
Docosadienoic acid (C22:2)	0.04	0.02
Eicosadienoic acid (C20:2)	0.03	—
Saturated fatty acids	**16.20**	**16.52**
Arachidic acid (C20:0)	0.49	0.10
Caproic acid (C6:0)	—	—
Caprylic acid (C8:0)	—	—
Capric acid (C10:0)	—	—
Lauric acid (C12:0)	0.01	0.04
Palmitic acid (C16:0)	10.84	11.55
Myristic acid (C14:0)	0.08	0.18
Heptadecanoic acid (C17:0)	—	—
Stearic acid (C18:0)	4.19	3.90
Behenic acid (C22:0)	0.41	0.71
Lignoceric acid (C24:0)	0.15	0.01
Total trans-fatty acids	**0.55**	**0.2**
Elaidic (trans, oleic C18:1)	0.03	0.08
T-9 C-12 (trans, linoleic C18:2)	0.22	0.06
C-9 T-12 (trans, linoleic C18:2)	0.28	0.04

Note: Monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, and total trans-fatty acids represent the principal groups of fatty acids present in the seed oil. Each of these categories encompasses specific individual fatty acids, such as oleic acid under monounsaturated and linolenic acid under polyunsaturated fatty acids. The bold categories indicate the aggregated percentage composition of all fatty acids belonging to that group.

fatty acids, especially PUFAs, in the fenugreek oil's fatty acid composition makes it suitable for functional food applications.

4. Conclusion

The comprehensive analysis presented in this study enhances the understanding of fenugreek seeds, providing valuable information for various industrial applications and highlighting their potential health benefits. Analyzing fenugreek seeds' dimensional, gravimetric, and frictional properties provided insights into their physical characteristics. The fenugreek seeds exhibited dimensions comparable to those of previous studies, indicating consistency in seed morphology. Gravimetric and frictional properties contribute to understanding the handling characteristics of fenugreek seeds in various processes. Its interaction with water and oil makes it a valuable ingredient in various food applications. These physicochemical and functional properties are important in the pharmaceutical and food industries for formulating powders with desired flow characteristics. The proximate composition revealed its nutritional content, with significant protein, fat, and fiber levels. FTIR analysis verified the presence of these compounds, which aligns with the observed physicochemical and functional properties. The evaluation of TPC and antioxidant activity demonstrated the potential health-promoting properties of fenugreek seed powder. The findings revealed the presence of several types of fatty acids, including MUFAs and PUFAs, along with negligible levels of total trans fatty acids. Fenugreek seed oil consisted primarily of key constituents, namely, linolenic acid and linoleic acid, classified as polyunsaturated Omega-6 fatty acids. The presence of these compounds may contribute to the antioxidant activity of fenugreek seed oil. Consequently, it can be suggested that the fenugreek seed oil holds value for pharmaceutical industries, with potential applications in various medicinal uses. The abundance of unsaturated fatty acids, particularly PUFAs, makes it a promising ingredient for functional food formulations.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

The authors have nothing to report.

Consent

Informed consent was obtained from all individual participants included in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

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