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Influence of growth stage and processing method on the phytochemical and nutraceutical attributes of Fenugreek (*Trigonella foenum-graecum* L.)

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is a widely utilized seed spice known for its nutritional, medicinal, and culinary applications. Both the leaves and seeds are rich in bioactive compounds, including proteins, fats, carbohydrates, minerals, steroidal sapogenins, and diosgenin, making the crop a valuable candidate for the nutraceutical industry. A study was undertaken at the Horticultural College and Research Institute, TNAU, Coimbatore, from August 2017 to June 2018, to assess the qualitative attributes of seven different forms of fenugreek: young seedlings (7-10 days post-germination), fresh and dry leaves at 30-40 and 60-70 days after sowing, dry seeds, and sprouted seeds.The findings indicated that both sprouted and dry seeds exhibited superior quality traits, including elevated levels of chlorophyll (a, b, and total), catalase activity, soluble proteins, carbohydrates, phenols, fats, crude fiber, polyphenol oxidase and peroxidase activity. Conversely, fresh leaves harvested at 60-70 days post-sowing showed the highest ascorbic acid content. Additionally, a separate experiment focused on value addition through different drying techniques for dry and sprouted seeds. Among the methods tested, freeze drying preserved the highest levels of key biochemical and mineral constituents, followed by spray drying. These results suggest that both the form of fenugreek and the processing method significantly influence its quality parameters, emphasizing their importance for nutraceutical applications.

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.), a member of the Fabaceae family, is a well-known annual herbaceous plant cultivated extensively in countries such as India, Egypt, Morocco, and other parts of Asia and the Mediterranean (Figure 1). Traditionally used as a culinary spice, medicinal herb, and forage crop, fenugreek has garnered significant attention in recent years due to its versatile health benefits and nutraceutical potential. The plant holds a prominent place in ancient medicine systems, including Ayurveda and Traditional Chinese Medicine, where it has been utilized to treat a variety of ailments such as digestive disorders, diabetes, inflammation, and infections (Mehrafarin *et al*., 2011). The renewed interest in natural remedies and plant-based health supplements has prompted increased research into the bioactive compounds found in fenugreek. Various parts of the plant, including seeds, leaves, and sprouts, are rich in phytochemicals like alkaloids, flavonoids, saponins, phenolic acids, and steroidal compounds such as diosgenin. These constituents are responsible for a wide range of pharmacological activities, including hypoglycemic, hypocholesterolemic, antioxidant, antimicrobial, and anti-inflammatory effects (Srinivasan, 2006). As the global demand for functional foods and nutraceuticals continues to rise, fenugreek emerges as a promising candidate for industrial applications. However, to fully harness its potential, it is essential to understand the variation in phytochemical and nutritional profiles across different plant parts and developmental stages. Moreover, processing methods, especially drying techniques, play a critical role in determining the retention or degradation of key nutrients and bioactive compounds (Petropoulos *et al*., 2022).

Fenugreek is widely recognized for its high nutritional value. Its seeds contain significant amounts of protein (20-30%), dietary fiber (50%), fats (6-8%), carbohydrates (58%), and essential minerals like calcium, iron, magnesium, and zinc. The seeds also provide a good source of vitamins, particularly thiamine, folic acid, riboflavin, niacin, and vitamins A and C (Yadav and Baquer, 2014). Equally noteworthy is the presence of steroidal sapogenins and alkaloids such as trigonelline, which have shown potential in regulating blood glucose and lipid profiles.In addition to seeds, fenugreek leaves and tender shoots are rich in chlorophyll, vitamins, and minerals. The green parts of the plant contain antioxidants such as ascorbic acid and flavonoids that contribute to their free radical-scavenging activity (Basch *et al*., 2003). Sprouted seeds, which undergo biochemical transformations during germination, often exhibit enhanced levels of bioavailable nutrients and enzymes, making them superior in terms of nutritional content compared to their non-sprouted counterparts. Diosgenin, along with other saponins and phenolic compounds, contributes significantly to the medicinal value of fenugreek, offering therapeutic benefits such as anticancer, anti-inflammatory, and hepatoprotective effects (Sharma *et al*., 2020). The presence of polyphenol oxidase and catalase enzymes in different parts of the plant further enhances its antioxidant profile.

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Numerous scientific investigations have highlighted the pharmacological benefits of fenugreek, which are largely attributed to its complex phytochemical composition. One of the most studied properties is its antidiabetic potential. Fenugreek seeds contain galactomannan, a soluble fiber that slows down glucose absorption, and trigonelline, which is known to have insulinotropic properties. These factors collectively contribute to better glycemic control in diabetic individuals (Yadav *et al*., 2011). Fenugreek also exhibits lipid-lowering effects, making it beneficial for individuals with hypercholesterolemia. The saponins in fenugreek interfere with cholesterol absorption in the intestines, while the dietary fiber binds bile acids, promoting their excretion and thereby reducing serum cholesterol levels. Moreover, its anti-inflammatory and antioxidant properties render fenugreek effective in managing chronic inflammatory conditions. The polyphenolic content helps scavenge free radicals and reduce oxidative stress, which is implicated in the pathogenesis of diseases such as cancer, cardiovascular disorders, and neurodegenerative diseases. Additionally, fenugreek demonstrates antimicrobial activity against various bacterial and fungal pathogens, further supporting its role in traditional medicine (Basu *et al*., 2010).

Although fenugreek's health-promoting attributes are well documented, variations in phytochemical content and efficacy depending on the developmental stage of the plant are less explored. The physiological and biochemical composition of fenugreek undergoes significant changes from the seedling phase to full maturity. These transformations impact not only the nutrient density but also the concentration and efficacy of bioactive compounds.Young seedlings, for instance, may possess higher levels of enzymes and certain secondary metabolites that play a role in early plant defense mechanisms. Mature leaves may have accumulated more chlorophyll and minerals, while sprouted seeds often demonstrate enhanced bioavailability of nutrients due to enzymatic activation during germination. Therefore, identifying the optimal stage of harvest is crucial for maximizing the therapeutic benefits of the plant. The study conducted at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, between August 2017 and June 2018, was designed to investigate this variation. Seven different forms of fenugreek were examined: young seedlings (7-10 days after germination), fresh and dry leaves at two maturity stages (30-40 and 60-70 days after sowing), dry seeds, and sprouted seeds. The research aimed to evaluate and compare various quality parameters, including enzymatic activity, and phytochemical concentrations.

Figure 1: Fenugreek plant with flowers.

2. Materials and Methods

2.1 Authentication of plant material

Dr. R. Ramasubbu, Associate Professor, Department of Biology, Gandhigram Rural Institute, Gandhigram, Dindigul, conducted the entire Botanical Authentication and Identification of the Plant Specimen. The Plant Specimen is Catalogued under Collection 396 and stored at the GUD Herbarium.

2.2 Study location

The experimental study was conducted at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The research work was carried out over a ten-month period, from August 2017 to June 2018, under controlled environmental and field conditions suitable for fenugreek cultivation.

2.3 Plant material

The plant species used in the study was *T.foenum-graecum* (fenugreek), a well-known medicinal and culinary herb. The investigation included seven distinct forms of fenugreek collected at different developmental stages. The experimental forms selected for qualitative analysis were:Young seedlings (7-10 days after germination), fresh mature leaves (30-40 days after sowing), dried mature leaves (30-40 days after sowing), fresh mature leaves (60-70 days after sowing), dried mature leaves (60-70 days after sowing), dry seeds and sprouted seeds All samples were collected at the respective growth intervals. Mature leaves were dried in shade for 5-7 days to prepare dry leaf samples. Seeds were soaked in water and allowed to germinate in a dark, humid chamber to obtain sprouted seed samples.

2.4 Preparation of samples for analysis

Collected plant materials were thoroughly washed with distilled water to remove dust and debris. The fresh and sprouted forms were used immediately after harvest, while the dried samples were powdered using a mechanical grinder and stored in airtight containers at 4°C until analysis.

2.5 Drying methods for value addition study

A separate experiment was conducted using dry seeds and sprouted seeds to assess the impact of post-harvest drying on quality retention. Two drying techniques were employed. Freeze drying (Lyophilization): Samples were frozen at – 20°C and then subjected to freeze drying under vacuum to remove moisture.Spray drying: Samples were mixed with a carrier material and subjected to atomization using a laboratory spray dryer under controlled inlet and outlet temperatures. These processed samples were then analyzed to determine the retention of nutritional and phytochemical constituents.

2.6 Biochemical analysis

A comprehensive analysis of biochemical and nutritional parameters was carried out to evaluate the quality and nutraceutical potential of fenugreek in its various developmental forms. The following methods were employed to quantify enzymatic activities, macronutrient levels, phytochemical contents, and mineral composition. All tests were performed in triplicate to ensure data accuracy and reproducibility.

2.6.1 Estimation of chlorophyll content (a, b, and total)

Chlorophyll content was measured using a spectrophotometric method following the procedure described by Arnon (1949). Approximately 0.5 g of fresh leaf tissue was ground in 80% acetone and centrifuged at 5000 rpm for 10 min. The supernatant was collected, and the absorbance was read at 645 nm and 663 nm using a UV-Visible spectrophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll content were calculated using Arnon's formula and expressed in mg/g of fresh weight.

2.6.2 Catalase activity

Catalase activity was assessed by measuring the rate of decomposition of hydrogen peroxide (H_2O_2) as described by Aebi (1984). A reaction mixture containing phosphate buffer (pH 7.0), 0.1 M H_2O_2, and enzyme extract was prepared. The reduction in absorbance at 240 nm was recorded at 30 sec intervals for 2 min. Enzyme activity was expressed in units per mg of protein, where one unit corresponds to the amount of enzyme required to decompose 1 µmol of H_2O_2 per min.

2.6.3 Peroxidase activity

Peroxidase activity was determined using the guaiacol oxidation method as per Hammerschmidt *et al*. (1982). The reaction mixture consisted of phosphate buffer (pH 6.0), guaiacol, hydrogen peroxide, and the enzyme extract. The increase in absorbance at 470 nm, due to the oxidation of guaiacol, was monitored for 2 min. Enzyme activity was expressed in units per minute per mg of protein.

2.6.4 Polyphenol oxidase activity

Polyphenol oxidase (PPO) activity was analyzed by using catechol as a substrate following the protocol of Mayer *et al*. (1965). The assay mixture included phosphate buffer (pH 6.5), catechol solution, and crude enzyme extract. The reaction was initiated by adding catechol, and the increase in absorbance was recorded at 495 nm over 2 min. The results were expressed as units of activity per mg protein.

2.6.5 Protein content

Protein content was estimated using the Lowry method, a widely accepted colorimetric technique (Lowry *et al*., 1951). A known quantity of plant extract was reacted with an alkaline copper solution followed by Folin-Ciocalteu reagent. The resulting blue color was measured spectrophotometrically at 660 nm. A standard curve was prepared using bovine serum albumin (BSA), and protein content was expressed in mg/g fresh weight.

2.6.6 Carbohydrate content

The Anthrone method was used for the determination of total carbohydrates as outlined by Hedge and Hofreiter (1962). Plant samples were hydrolyzed with concentrated sulfuric acid and reacted with anthrone reagent. The reaction mixture developed a green color, which was measured at 620 nm. Glucose was used as the standard for calibration, and carbohydrate content was expressed in mg/g dry weight.

2.6.7 Total phenolic content

Phenolic compounds were quantified using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). A known volume of methanolic plant extract was mixed with Folin-Ciocalteu reagent and sodium carbonate. After incubation, the absorbance was recorded at 765 nm. Gallic acid served as the standard, and results were expressed in mg gallic acid equivalents (GAE) per gram of sample.

2.6.8 Total fat content

Total lipid content was measured using Soxhlet extraction with petroleum ether as the solvent, following AOAC (2005) guidelines. Dried, ground plant samples were weighed and placed in a thimble, then extracted for 6-8 h. After solvent evaporation, the remaining fat was weighed, and total fat content was expressed as a percentage of dry weight.

2.6.9 Crude fibre content

Crude fibre was analyzed by sequential acid and alkali digestion, as described in AOAC (2005) official methods. The dried plant material was boiled in 1.25% sulfuric acid, followed by treatment with 1.25% sodium hydroxide. The residue was filtered, dried, and incinerated in a muffle furnace at 550°C. Fibre content was calculated by subtracting ash from the dry residue and was expressed as a percentage of dry weight.

2.6.10 Diosgenin content

Diosgenin, a steroidal sapogenin, was quantified using High-Performance Liquid Chromatography (HPLC) based on the method described by Shishodia *et al*. (2008). Methanolic extracts were filtered and injected into an HPLC system fitted with a C18 column. A mobile phase of acetonitrile: water (70:30) was used, and detection was carried out at 210 nm. The concentration was calculated using a diosgenin standard curve and expressed as mg/g dry weight.

2.7 Statistical analysis

All biochemical parameters were measured in triplicates. The data were statistically analyzed using analysis of variance (ANOVA) to evaluate the significance of differences among treatments. Mean separation was performed using the Least Significant Difference (LSD) test at a 5% probability level.

3. Results

3.1 Effect of different growth stages on biochemical properties of fenugreek

3.1.1 Chlorophyll content

The chlorophyll 'a' content in fenugreek exhibited significant variation across different growth stages. Young seedlings (T1) demonstrated the highest concentration of chlorophyll 'a' at 2.19 mg/g, followed closely by the fresh leaves at 30-40 days after sowing (T2) with 2.13 mg/g. Conversely, the lowest value was observed in dried leaves harvested at 60-70 days (T5), which recorded 0.643 mg/g.Similarly, the chlorophyll 'b' content was highest in T1 (1.16 mg/g), followed by T2 (1.09 mg/g), and least in T5 (0.216 mg/g). The total chlorophyll content was maximum in T1 (3.35 mg/g), while the lowest was noted in T5 (0.983 mg/g), indicating a clear decline in chlorophyll content as the plant matured and was subjected to drying.

Table 1: Effect of different growth stages on biochemical properties of fenugreek on chlorophyll content

Treatment	Chlorophyll 'a' content (mg/g)	Chlorophyll 'b' content (mg/g)	Total chlorophyll content (mg/g)
T1 (Young seedlings at 7-10 days after germination)	2.19	1.16	3.35
T2 (Fresh leaf at matured stage at 30-40 days after sowing)	2.13	1.09	3.22
T3 (Dry leaf at matured stage at 30-40 days after sowing)	0.964	0.341	1.18
T4 (Fresh leaf at matured stage at 60-70 days after sowing)	2.07	1.02	3.09
T5 (Dry leaf at matured stage at 60-70 days after sowing)	0.643	0.216	0.983
Mean	1.60	0.765	2.36
SED	0.037	0.014	0.052
CD (*p*=0.05)	0.081**	0.032**	0.115**

3.1.2 Catalase and peroxidase activities

Catalase activity varied significantly among the treatments. The dry seed (T6) recorded the highest catalase activity (11.27 µg of H_2 O_2 g^{-1} min^{-1}, followed by the sprouted seed (T7) with 10.21 µg of H_2 O_2 g^{-1} min^{-1}. The lowest activity was recorded in dried leaves at 30-40 days (T3) with 8.67 µg of H_2 O_2 g^{-1} min^{-1}. Peroxidase activity followed a different trend, being highest in sprouted seeds (T7) at 2.324 $min^{-1}g^{-1}$ and lowest in dry leaves at 60-70 days (T5), which showed only 0.146 min^{-1} g^{-1}.

3.1.3 Protein and carbohydrate content

The soluble protein content was highest in sprouted seeds (T7) with 10.98 g/100 g, followed by dry seeds (T6) with 10.52 g/100 g. Dried leaves at 60-70 days (T5) showed the lowest protein content (6.25 g/100 g). In terms of carbohydrates, dry seeds (T6) again had the highest value (21.24 g/100 g), while dried leaves at 30-40 days (T3) recorded the least (5.09 g/100 g).

3.1.4 Phenol, fatand fibre content

Total phenol content peaked in dry seeds (T6) with 4.86 mg/g and was lowest in dried leaves at 30-40 days (T3) at 1.08 mg/g. The fat content was similarly higher in T6 (5.57%) and T7 (4.38%), whereas the lowest fat percentage (0.903%) was noted in T3. Crude fibre content followed the same trend, with the highest value observed in T6 (14.67%) and the lowest in T1 (8.43%).

3.1.5 Polyphenol oxidase activity

Among treatments, the highest polyphenol oxidase activity was seen in dry seeds (T6) with 1.357 min^{-1} g^{-1}, while young seedlings (T1) recorded the lowest at 0.324 min^{-1} g^{-1}. Fresh leaves at 60-70 days (T4) also had a relatively high value of 1.245 min^{-1} g^{-1}.

Table 2: Effect of different growth stages of fenugreek on catalase, peroxidase, protein and carbohydrate content

Treatment	Catalase activity (µg of H_2O_2 g^{-1} min^{-1})	Peroxidase activity (min/g)	Protein content (g/100 g)	Carbohydrate content (mg/g)
T1 (Young seedlings at 7-10 days after germination)	9.24	0.963	8.93	11.88
T2 (Fresh leaf at matured stage at 30-40 days after sowing)	10.33	0.876	8.75	6.51
T3 (Dry leaf at matured stage at 30-40 days after sowing)	8.67	0.176	6.74	5.09
T4 (Fresh leaf at matured stage at 60-70 days after sowing)	10.51	0.823	8.27	6.49
T5 (Dry leaf at matured stage at 60-70 days after sowing)	8.93	0.146	6.25	5.25
Mean	11.27	0.447	10.52	21.24
SED	10.21	2.324	10.98	18.07
CD (*p*=0.05)	9.88**	0.822**	8.63**	10.65**

Table 3: Effect of different growth stages of fenugreek on phenol, fat, fibre and polyphenol oxidase activity

Treatment	Phenol content	Fat content (%)	Fiber content (%)	Polyphenol oxidase activity (min/g)
T1 (Young seedlings at 7-10 days after germination)	(mg/g)	1.01	8.43	0.324
T2 (Fresh leaf at matured stage at 30-40 days after sowing)	1.42	1.16	10.62	1.013
T3 (Dry leaf at matured stage at 30-40 days after sowing)	2.64	0.903	8.57	0.937
T4 (Fresh leaf at matured stage at 60-70 days after sowing)	1.08	1.24	11.47	1.245
T5 (Dry leaf at matured stage at 60-70 days after sowing)	3.49	0.918	8.92	0.987
Mean	1.26	5.57	14.67	1.357
SED	4.86	4.38	12.58	0.973
CD (*p*=0.05)	4.12**	2.17**	10.75**	0.977**

3.2 Influence of drying methods on biochemical parameters

3.2.1 Catalase and protein content

Different drying methods had a pronounced effect on catalase activity. Freeze drying (T2) showed the highest catalase activity in both dry seeds (10.43 µg of H_2O_2 g^{-1} min^{-1}) and sprouted seeds (9.51 µg of H_2O_2 g^{-1} min^{-1}). The lowest activity was observed in hot air oven drying at 40°C (T4). Freeze drying also resulted in the highest soluble protein levels, with dry seeds showing 9.28 g/100 g and sprouted seeds 10.34 g/100 g. Solar tunnel drying yielded the lowest protein content in both seed forms.

3.2.2 Phenol and fat content

Carbohydrate content was highest in freeze-dried dry seeds (20.76 mg/g) and sprouted seeds (17.54 mg/g). Spray drying followed closely, while hot air oven drying had the lowest values. Total phenol content also favored freeze drying, recording the highest values in both dry and sprouted seeds (4.73 mg/g and 3.63 mg/g, respectively), with hot air oven drying again resulting in the lowest phenolic levels.Freeze drying resulted in the highest fat content in dry seeds (5.46%) and sprouted seeds (3.72%), with solar tunnel drying and hot air oven methods producing comparatively lower fat percentages.

3.2.3 Crude fibre and diosgenin content

Freeze-dried samples had the highest crude fibre content in both seed types, 14.26% in dry seeds and 12.06% in sprouted seeds. Diosgenin content was also influenced by the drying method. The highest value in dry seeds (0.572%) and sprouted seeds (0.483%) was observed under freeze drying. The least content was recorded in hot air oven drying and solar tunnel drying treatments.

Table 4: Effect of different drying methods on catalase activity, protein and phenol content

Treatment	Catalaseactivity (µg of H_2O_2 /g/min)		Protein content (g/100 g)		Phenol content (mg/g)	
	Dry seed	Sprouted seed	Dry seed	Sprouted seed	Dry seed	Sprouted seed
T (Solar tunnel drying)	8.45	7.69	8.43	8.93	4.57	3.28
T_1 (Freeze drying)	10.43	9.51	9.28	10.34	4.73	3.63
T_2 (Spray drying)	10.38	9.38	9.09	10.05	4.61	3.35
T_2 (Hot airovendrying@ 40°C) (control)	8.17	7.53	8.67	9.21	3.19	2.74
Mean	9.36	8.53	8.87	9.63	4.28	3.25
SED	0.173	0.191	0.231	0.224	0.086	0.052
CD (*p*=0.05)	0.399**	0.442**	0.533**	0.516**	0.197**	0.119**

Table 5: Effect of different drying methods on fat content, crude fibre content and diosgenin content

Treatment	Total fat content (%)		Crude fibre content (%)		Diosgenin content (%)	
	Dry seed	Sprouted seed	Dry seed	Sprouted seed	Dry seed	Sprouted seed
T (Solar tunnel drying)	3.17	2.94	12.58	10.47	0.453	0.317
T_1 (Freeze drying)	5.46	3.72	14.26	12.06	0.572	0.483
T_2 (Spray drying)	5.34	3.65	14.18	11.94	0.546	0.471
T_2 (Hot airovendrying@ 40°C) (control)	3.29	2.83	12.34	10.32	0.481	0.308
Mean	4.32	3.29	13.34	11.20	0.513	0.395
SED	0.058	0.082	0.238	0.253	0.012	0.008
CD (*p*=0.05)	0.133**	0.188**	0.549**	0.583**	0.027**	0.019**

4. Discussion

The present study comprehensively evaluates the biochemical changes in fenugreek (*T.foenum-graecum*) across various growth stages and the impact of different drying techniques on its biochemical and enzymatic properties. The findings provide insightful implications for optimizing harvesting time and post-harvest processing to maximize nutritional and functional quality in fenugreek seeds and leaves.

4.1 Effect of different growth stages on biochemical properties

Chlorophyll content is a fundamental indicator of photosynthetic capacity and plant health, directly influencing plant growth and metabolic activities. The observed highest chlorophyll 'a' (2.19 mg/g) and chlorophyll 'b' (1.16 mg/g) contents in young seedlings (T1) align with typical patterns seen in many plant species where early vegetative stages maintain elevated photosynthetic pigment levels to maximize energy capture for rapid growth (Kumar *et al*., 2021). The decline in chlorophyll content through maturity, particularly the lowest values in dried leaves at 60-70 days (T5), likely results from natural senescence and degradation of chlorophyll pigments as leaves age and dry (Gupta *et al*., 2019). Chlorophyll breakdown is a hallmark of leaf maturation and stress, which might be exacerbated by desiccation processes in dried samples, leading to diminished pigment concentration (Singh *et al*., 2020). Maintaining high chlorophyll levels in young and fresh leaves is critical as it ensures optimal photosynthetic efficiency, nutrient synthesis, and energy production. The reduced chlorophyll content in mature, dried leaves could also affect the nutritional and medicinal qualities of fenugreek, particularly if leaves are used as dietary components or in herbal formulations.

Catalase and peroxidase enzymes play crucial roles in the antioxidative defense system by scavenging reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), thus protecting cells from oxidative damage (Kumar and Sharma, 2022). The peak catalase activity in dry seeds (T6) and sprouted seeds (T7) suggests an adaptive mechanism to mitigate oxidative stress, which is particularly pertinent during seed desiccation and germination phases where oxidative metabolism intensifies (Bailly, 2020). The relatively lower catalase activity in dried leaves at 30-40 days (T3) might be associated with reduced metabolic activity or lower ROS production at this stage.Conversely, the highest peroxidase activity in sprouted seeds (T7) emphasizes the importance of this enzyme in early seedling development, potentially linked to cell wall strengthening, lignification, and ROS detoxification during germination (Passardi*et al*., 2017). The very low peroxidase activity in older dried leaves (T5) suggests a decline in antioxidative enzyme defenses as the tissue ages and dries, which could reduce the tissue's resilience against environmental stresses. Soluble protein and carbohydrate contents are key indicators of the nutritional and metabolic status of plant tissues. The elevated protein content in sprouted seeds (T7) and dry seeds (T6) is consistent with the accumulation of storage proteins that support seed germination and seedling growth (Bewley *et al*., 2013; Ahmed *et al*., 2021). The low protein content in dried mature leaves (T5) reflects the typical reduction of soluble proteins during senescence and drying (Lim *et al*., 2019). Similarly, the high carbohydrate content in dry seeds (T6) is characteristic of seed storage reserves, mainly in the form of starch and other polysaccharides, vital for seedling energy supply.In contrast, the lower carbohydrate content in dried leaves at 30-40 days (T3) could be attributed to utilization during metabolic processes or conversion into other compounds during leaf maturation and drying.

Phenolic compounds are essential secondary metabolites with significant antioxidant properties, contributing to the medicinal value of fenugreek (Pandey and Rizvi, 2019). The maximal total phenol content in dry seeds (T6) indicates a concentration of these bioactive compounds in mature seeds, which might serve protective roles against oxidative damage during dormancy (Surveswaran *et al*., 2019). The low phenol content in dried leaves at 30-40 days (T3) might be due to dilution effects or degradation during drying.Fat content trends show the seeds as primary lipid storage sites, with dry seeds (T6) having the highest fat content, reflecting their role in energy provisioning during germination. Crude fibre content, higher in dry seeds (T6), is consistent with the structural and protective functions of seed coats and cell walls, while lower fibre in young seedlings (T1) corresponds with less developed structural tissues. Polyphenol oxidase (PPO) is an enzyme involved in the oxidation of phenolic compounds, often linked to browning reactions and defense responses in plants (Mayer, 2019). Its highest activity in dry seeds (T6) may reflect a protective mechanism against pathogens and oxidative stress during seed storage. The low PPO activity in young seedlings (T1) is expected given their high metabolic activity and reduced need for such defense. The relatively high PPO activity in fresh leaves at 60-70 days (T4) might be part of natural senescence or stress responses.

4.2 Influence of drying methods on biochemical parameters

Post-harvest drying techniques critically influence the retention of nutrients and bioactive compounds. The comparative analysis across freeze drying, hot air oven drying, solar tunnel drying, and spray drying reveals the superiority of freeze drying in preserving fenugreek's biochemical integrity. Freeze drying resulted in the highest catalase activity and soluble protein content in both dry and sprouted seeds. This preservation effect can be attributed to the low-temperature dehydration process, which minimizes enzyme denaturation and protein degradation (Ratti, 2020). In contrast, hot air oven drying, involving higher temperatures, induced the greatest reduction in catalase activity and protein content, likely due to thermal denaturation and oxidative degradation (Sharma *et al*., 2021). Solar tunnel drying, which uses moderate temperatures and solar radiation, produced intermediate results but still fell short of freeze drying in enzyme and protein preservation. The data strongly suggest that freeze drying is the optimal method for retaining enzymatic function and protein levels in fenugreek seeds. Carbohydrates and total phenol content were also best preserved by freeze drying, followed by spray drying, whereas hot air oven drying consistently caused the greatest losses. The retention of phenolics under freeze drying is particularly important given their antioxidant and health-promoting properties (Manach *et al*., 2020). Heat treatments can accelerate phenol oxidation and degradation, reducing antioxidant capacity (Cacace and Mazza, 2021). Similarly, fat content was highest in freeze-dried samples, indicating that lipids are vulnerable to oxidation and breakdown during conventional drying, especially at elevated temperatures (Sánchez *et al*., 2021). The maintenance of lipid integrity in freeze-dried fenugreek is beneficial for seed quality and shelf-life.

Freeze drying maintained the highest crude fibre content, possibly because low temperature drying causes minimal structural damage to cell walls. Polyphenol oxidase activity was also highest in freeze-dried samples, reflecting preserved enzyme functionality. Reduced PPO activity in hot air oven drying may result from thermal inactivation, while enzymatic activity retention in freeze drying suggests better maintenance of native protein structures. Diosgenin, a steroidal saponin with significant pharmacological relevance (anti-inflammatory, hypocholesterolemic effects), showed highest retention in freeze-dried samples. Thermal drying methods, especially hot air oven and solar tunnel drying, led to significant diosgenin loss, likely due to heat-induced degradation or oxidation (Wang *et al*., 2022). Preserving diosgenin is critical for maximizing fenugreek's medicinal value.

5. Conclusion

The findings have important implications for both fenugreek producers and processors. Harvesting at optimal growth stages, particularly targeting dry seeds for maximum phenolics, fat, and diosgenin, or fresh leaves at 60-70 days for vitamin C and chlorophyllcan enhance the nutritional and functional profile of fenugreek-based products. Additionally, adopting freeze drying as the preferred post-harvest processing method ensures maximal retention of bioactive compounds, enzymes, and nutrients, though it requires higher energy and cost investments. For commercial applications where cost constraints exist, spray drying may offer a viable alternative while still preserving significant nutrient content compared to traditional oven drying. Future research could explore the cost-benefit analysis of these drying methods at industrial scale, investigate the bioavailability of retained compounds post-processing, and expand biochemical profiling to other fenugreek varieties.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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