PROXIMATE COMPOSITION, PHYSICAL PROPERTIES AND BIO-CHEMICAL TRAITS OF FRESH FENUGREEK LEAFY VEGETABLES: A COMPREHENSIVE STUDY

Abstract

The present investigation was undertaken in the Department of Processing and Food Engineering, College of Agricultural Engineering, Raichur to study the different properties of fresh fenugreek leafy vegetable like proximate composition, physical and bio-chemical properties. Fresh fenugreek leaves, also known as methi leaves, are not only a flavourful ingredient but also offer several nutritional benefits. Objective of this study is to determine nutritional compounds of fenugreek leaves and their health benefits. Fresh fenugreek leaves are a rich source of vitamins and minerals. These are essential for maintaining a healthy immune system, promoting good vision, supporting blood clotting, and aiding in the production of new cells. Fenugreek leaves are packed with antioxidants, such as flavonoids (559.81 mg/100g), polyphenols (147.82 mg/100g), and carotenoids (20.25 mg/100g). These compounds help protect the body against damage from harmful free radicals, which can contribute to chronic diseases, including cancer and heart disease. Fenugreek leaves are a good source of dietary fiber. Fiber aids in digestion, helps regulate blood sugar levels, promotes a feeling of fullness, and supports healthy cholesterol levels. These leaves contain high amount of fibers about 2.20% consuming an adequate amount of fiber is important for maintaining a healthy digestive system.

Key words: Fenugreek, Proximate composition, Bio-chemical properties and Fibers

1. Introduction

In order to satisfy human demands for vitamins and minerals, vegetables are crucial. The leafy vegetables offer the highest nutritional value for protection of all the veggies. They are known as "Mines of Minerals" because they are abundant in minerals. Leafy vegetables are a good source of dietary fibre, vitamins, and minerals. (Vorster *et al.,* 2005). These leaves are a cheap supply that a big population can use to fulfil their dietary needs. However, due to their

high surface area to volume ratio and propensity for mechanical harm when handled, leaves are particularly perishable. (Kakade *et al.,* 2015). The most widespread and debilitating nutritional disorders, including birth defects, mental and physical retardation, weakened immune systems, blindness and even death has resulted from the habit of non-consumption of fruits and vegetables (Mwangi and Mumbi, 2006). Leafy vegetables such as fenugreek, parsalene, chard, spinach, lettuce and coriander provide appeal, flavor and desired taste to the consumers. The protein found in leafy vegetables has nutritionally better proportion of amino acid when compared to many cereals and some leguminous crops (Anon., 1999).

A typical Indian's diet is now unbalanced because it consists primarily of cereals. As opposed to the usual dietary advice of 328 g of cereal and 316 g of vegetables, the average Indian consumes 375 g of cereal and 30 g of vegetables every day. The daily recommended allowance for vegetables is 316 grammes, of which 200 grammes should be green vegetables (Anon., 2018). Every year, about 16 million people in underdeveloped nations pass away from preventable causes, with 60% of these deaths resulting from hunger and malnutrition. Most impoverished people struggle with hunger and malnutrition, especially when there are vitamin and mineral shortages, which impede growth, make them weaker, and make them more susceptible to illness (Anon., 2010).

Fenugreek, also known as Trigonella foenum-graecum L., is a member of the Fabaceae family and is cultivated for its seeds, delicate shoots, and fresh leaves. It is an annual plant that is widely grown as a food crop in Yemen, North Africa, the Mediterranean region, and India. A large number of bioactive substances, such as ascorbic acid, carotenoids, chlorophyll, polyphenols, and other phytochemicals, are present in fenugreek (Wani and Kumar, 2018). By quenching singlet oxygen and scavenging free radicals, the flavonoids and carotenoids in fenugreek are beneficial for the retina and lens of the eye, preventing macular degeneration illness (Simopoulos and Gopalan, 2003).

Considering the above facts, it could be said that these fenugreek leafy vegetables have a high nutritional and dietary importance. Hence, the objective of the research article is to study the detailed quality characteristics of fenugreek leafy vegetables

2. Material and Methodology

Fresh fenugreek *(Trigonella foenum-graecu* L.) bunches were procured after harvesting in the morning hours from Merched village located near the Raichur city, Karnataka. Sorting and grading were done manually to remove diseased and non uniform fenugreek leafy vegetables. Uniformly sized matured and cleaned fenugreek (Var. local Joara) was used for the investigation

2.1 Proximate composition of fresh fenugreek leafy vegetable

The proximate compositions *viz.*, moisture content, carbohydrates, crude protein, crude fat, crude fiber and total ash content of fresh fenugreek leafy vegetables was estimated by following the standard procedures

2.1.1 Moisture content

The moisture content of fresh fenugreek leaves and was determined by using standard procedure (AOAC, 2005, Method No. 945.43) and equation given below

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_2} \times 100$$

Where, W_1 and W_2 are the initial and final weight of sample (g) respectively

2.1.2 Carbohydrate

The estimation of carbohydrate for fresh fenugreek along with stems was carried out as per AOAC (2005, Method No. 975.14).

2.1.3 Crude protein

The nitrogen content in fresh fenugreek leaves along with stem was estimated by using Kjeltech instrument (D-40599; Behr Labor Technik GmbH, Bavaria, Germany) by Kjeldahl's (AOAC, 2005, Method No 991.20) and equating in

$$\text{Nitrogen (\%)} = \frac{\text{Volume of 0.1N HCL(mL)} \times 0.1 \times 14.007}{\text{weight of sample (g)} \times 1000} \times 100$$

Percent protein on total nitrogen basis = N (%) × Conversion factor

Conversion factor for leafy vegetables= 6.00

2.1.4 Crude fat

The crude fat content of the fresh fenugreek along with stems were estimated by soxhlet apparatus method (AOAC, 2005; Method No. 922.06) using SOCS – PLUS apparatus (Pelican Equipments; SCS-08, Tamil Nadu, India).

2.1.5 Total ash

The total ash content of fresh fenugreek leaves along with stem was determined as per the standard procedure (AOAC, 2005; Method No. 925.23) by using muffle furnace (MAC; MSW-251, Tamil Nadu, India).

2.2 Physical properties of fresh fenugreek leafy vegetable

The physical properties of fresh fenugreek leaves was assessed by determining different quality parameters *viz.*, weight, leaf area, leaf thickness, bulk density and colour.

2.2.1 Weight

50 g of fresh, uniformly sized and matured fenugreek leafy vegetables were taken for the study. The digital weighing balance (Sartorius, L- 610, Wisconsin, UK) was used for determination of weight and expressed in g. The experiment was repeated thrice.

2.2.2 Leaf Area

The leaf area of the fenugreek and coriander was determined by taking average area of ten leaves. The digital planimeter (PLACOM, KP-90N, New Delhi, India) was used for determination of leaf area. The leaf was taken and spread over the sheet, the leaf margin was outlined with the help of pencil. Marking an initial point anywhere on the leaf outline the digital planimeter was moved on the same outline till marked point reached. The experiment was repeated by taking ten leaves and the readings were tabulated to calculate the mean value (Schurer, 1971).

2.2.3 Leaf thickness

A digital screw gauge having a least count of 0.01 mm was used to determine the thickness of fresh fenugreek leaves. Ten samples of fenugreek leaves were randomly selected for the measurement. The thickness was measured at the centre of the leaf and readings were tabulated to calculate mean value and expressed in cm (Mohsenin, 1986).

2.2.4 Bulk density

A rectangular plastic box (15×10×10 cm) was taken and its volume was determined and then the box was completely filled with fenugreek leaves. The weight of the leaves taken in fill the box was recorded and the bulk density was determined using the following formula (Mohsein, 1986).

$$\text{Bulk density } (g/cm^3) = \frac{\text{Weight of leaves } (g)}{\text{Volume of wooden box } (cm^3)}$$

2.3 Biochemical traits of fenugreek leafy vegetables

The bio- chemical properties of fresh fenugreek leaves were assessed by determining different quality parameters *viz* total flavonoid, total phenols, chlorophyll content, ascorbic acid, *ß*-carotene and GC-MS analysis of different compounds.

2.3.1 Total flavonoid content of fenugreek leafy vegetables

Total flavonoid content of fresh fenugreek along with the stem was determined by aluminum chloride colourimetric method by using quercetin as a standard. This method was based on formation of flavonoid aluminum complex. 1 g of fenugreek leaves were extracted with 10 mL of pure methanol. 0.5 mL of extract solution was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The solution was then incubated for 30 min. The optical density of the reaction mixture was measured against the blank at 415 nm with the help of UV-Vis spectrophotometer. The standard calibration curve was prepared by using quercetin solutions at concentrations of 12.5 to 100 µg/mL in methanol (Chang *et al.*, 2002).

2.3.2 Total phenolic content of fenugreek leafy vegetables

The concentration of total phenols in fresh fenugreek along with stem was estimated with Folin Ciocalteau reagent. 1 g of fenugreek was extracted with 10 mL of methanol: water (50:50, v/v). 0.5 mL of the diluted (1:10) extract or the standard phenolic compound (Gallic acid) was mixed with 5 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water) and 4 mL of aqueous Na_2CO_3 (1 M). The mixture was allowed to stand for 15 min and the optical density of the mixture was determined against the blank at 765 nm with the help of UV-Vis spectrophotometer. Standard curve was prepared using 0, 50, 100, 150, 200, 250 µg solutions of gallic acid per mL of methanol:water (50:50, v/v). Total phenol values were expressed in terms

of the standard reference compound as gallic acid equivalent (g/100 g fresh weight of leaves) and the procedure was repeated for the determination of total phenols for fresh coriander (McDonald *et al.*, 2001).

2.3.3 Chlorophyll

The pigments (total chlorophyll) of fenugreek leaves along with stem were determined and quantified using the procedure proposed by Nagata and Yamashita (1992). 1g of shredded sample was homogenized with 10 mL of acetone and n-hexane (4:6), using a tissue homogenizer (Labco, India) for 30 s. The homogenized solution was allowed to settle down. Then, 1 mL of supernatant was taken and was diluted with 9 mL of acetone and n-hexane (4:6). The resulting solution was analyzed spectrophotometrically with the help of an UV-Vis spectrophotometer. The optical density was measured at 453, 505, 663 and 645 nm using acetone and n-hexane (4:6) as a blank.

Chlorophyll concentration (mg/100 g) was quantified using the following equations

$$\text{Chlorophyll A} = 0.999A_{663} - 0.0989A_{645}$$

$$\text{Chlorophyll B} = -0.328A_{663} + 1.77A_{645}$$

$$\text{Total chlorophylla} = \text{Chlorophyll A} + \text{Chlorophyll B}$$

Where, A_{663} and A_{645} are the absorbance's at 663 and 645 nm respectively

2.3.4 *ß*-carotene content

The *ß*-carotene content of fenugreek leaves along with stem were determined and quantified using the procedure proposed by Nagata and Yamashita (1992). 1g of shredded sample was homogenised with 10 mL of acetone and n-hexane (4:6), using a tissue homogenizer (Labco, India) for 30 s. The homogenized solution was allowed to settle down. Then, 1 mL of supernatant was taken and was diluted with 9 mL of acetone and n-hexane (4:6). The resulting solution was analyzed spectrophotometrically with the help of an UV-Vis spectrophotometer. The optical density was measured at 453, 505, 663 and 645 nm using acetone and n-hexane (4:6) as a blank.

ß-carotene concentration (mg/100 g) was quantified using the following equation

$$ß - carotene = 0.216A_{663} - 1.220A_{645} + 0.452A_{453} - 0.304\,A_{505}$$

Where, A_{453}, A_{505}, A_{663}, A_{645} are the absorbance's at 453, 505,663 and 645 nm respectively

2.3.4 Ascorbic acid

The fenugreek samples were analyzed for the ascorbic acid content, using 2, 6-dichlorophenol indophenol dye titrimetrically as per the method suggested by Sadasivam and Manickam (1992). Two grams of the fenugreek along with stems were first crushed with 10 mL of 4 % oxalic acid and filtered through muslin cloth and then with Whatman No. 4 filter paper. An aliquot of extract (2 mL) of the samples were titrated against 2, 6-dichlorophenol indophenol dye till the pink end point persist for at least 15. Similar procedure was followed for acid mixture to get blank titre value and against standard solution made in 4 % oxalic acid to get standard titre value. The results were expressed in terms of mg/100 g.

Gas chromatography analysis of various compounds found in fenugreek and coriander

GC analysis of the methanol extract of fresh fenugreek and coriander leafy vegetables samples were carried out using Shimadzu Make QP-2020 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 300 °C and held for three min and the final temperature of the oven was 250 °C with rate at 150 °C/ min. One micro litre samples were injected with split mode of 1:50. Mass spectra was recorded over 35-1050 amu range with electron impact ionization energy 70 eV. The total running time for a sample was 45 min. The bioactive volatile compounds from the methanolic extract of fenugreek leaves were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC (Pasricha and Gupta 2014).

3. Results and Discussion

3.1 Proximate composition of fresh fenugreek leafy vegetables

The mean values of proximate composition for fenugreek leafy vegetable *viz.*, moisture content, carbohydrate, crude protein, crude fat, crude fiber and ash content fenugreek were

determined using different standard analytical methods and the data obtained are presented in Table 1. The moisture content, carbohydrate, crude protein, crude fat, crude fiber and ash content of fresh fenugreek leafy vegetable were found to be 86.12, 4.9, 4.5, 0.8, 2.2, 1.48 %, respectively. The proximate composition of the fenugreek leafy vegetable is depicted in Table 1. The high crude fiber content of fenugreek suggests that it could serve as an important source of dietary fiber. The high ash content observed in fresh fenugreek might serve as essential mineral elements. Similar results were obtained by Srinivasan, (2006) who conducted experiment for fenugreek leaves.

3.2 Physical properties of fresh fenugreek leafy vegetables

Physical properties of fenugreek leafy vegetables *viz.*, leaf area, leaf thickness and bulk density determined are presented in Table 2. The fenugreek leafy vegetable had an average leaf area, leaf thickness and bulk density values of 2.88 cm^2, 0.280 cm, 0.08 g/cm^3, respectively. The colour values of fresh fenugreek leafy vegetable *viz.*, *L**, *a** and *b** vales were found to be 49.31, -21.23, 14.21

3.3 Biochemical traits of fresh fenugreek leafy vegetables

The mean values of biochemical composition for fenugreek leafy vegetable *viz.*, total flavonoids, total phenolic content, chlorophyll content, ascorbic acid and *ß*-carotene data obtained are presented in Table 3.The total flavonoids, total phenols, chlorophyll content, ascorbic acid and *ß*-carotene content of fresh fenugreek leafy vegetable were found to be 559.81, 147.82, 287.69, 87.12, 20.25 mg/100 g, respectively. The bio-chemical properties of fresh fenugreek leafy vegetable are presented in Table 3. High content of flavonoids in fenugreek observed might help to reduce the risk of cancer, heart diseases and other age related degenerative diseases. These compounds are also called phytonutrients and are importance in the human diet (Sellappan and Akoh, 2002).

3.4 Gas chromatography analysis of various compounds found in fenugreek leafy vegetable

The various functional compounds in fenugreek leafy vegetable were analyzed using GC technique are presented in Table 4. The results revealed that, fenugreek leafy vegetable are rich source of bioactive compounds, which are necessary to enhance the nutritional and health status

of human beings. The compounds also have the potential antioxidant and good therapeutic property (Pasricha and Gupta, 2014). The chromatograph for fenugreek leafy vegetable is shown in Fig.1

Table 1. Proximate composition of fresh fenugreek vegetables

Sl. No.	Proximate composition	Fenugreek
1	Moisture content (%)	86.12
2	Carbohydrate (%)	4.90
3	Crude protein (%)	4.50
4	Crude fat (%)	0.80
5	Crude fiber (%)	2.20
6	Ash(%)	1.48

Table 2. Physical properties of fresh fenugreek leafy vegetables

Sl. No.	Physical properties	Fenugreek
1	Leaf area, cm^2	2.88
2	Leaf thickness, cm	0.28
3	Bulk density, g/ cm^3	0.08
4	Colour value	
	*L**	49.31
	*a**	-21.23
	*b**	14.21

Table 3. Biochemical traits of fresh fenugreek leafy vegetables

Sl. No.	Biochemical traits properties	Fenugreek
1	Total flavonoids content (mg/100 g)	559.81
2	Total phenolic content (mg/100 g)	147.82
3	Chlorophyll (mg/100 g)	287.69
4	Ascorbic acid (mg/100 g)	87.12
5	ß-carotene (mg/100 g)	20.25

Table 4. GC chromatogram extract of fresh fenugreek leafy vegetable

Sl. No.	Retention time (min)	Peak area	Name of the compound	Fresh Fenugreek leafy Vegetables
1	1.314	1.18	Ethanol	+
2	1.450	0.04	Silanol, trimethyl-	+
3	1.484	0.76	2-Butanone	+
4	15.537	12.00	4-O-Methylmannose	+
5	19.545	0.29	Phytol	+
6	18.680	0.13	1- Nonadecene	+
7	10.817	1.29	Tetradecane	+
8	14.357	0.55	1- Hexadecanol	+
9	18.390	0.25	n-Hexadecanoic acid	+
10	17.645	0.21	9- Heptadecanone	+
11	19.669	0.10	Methyl stearate	+
12	11.120	0.07	Cyclohexane, octyl-	+
13	11.783	0.28	D-Allose	+
14	4.541	0.10	Hexanoic acid	+
15	10.038	0.56	1- Tetradecenol	+

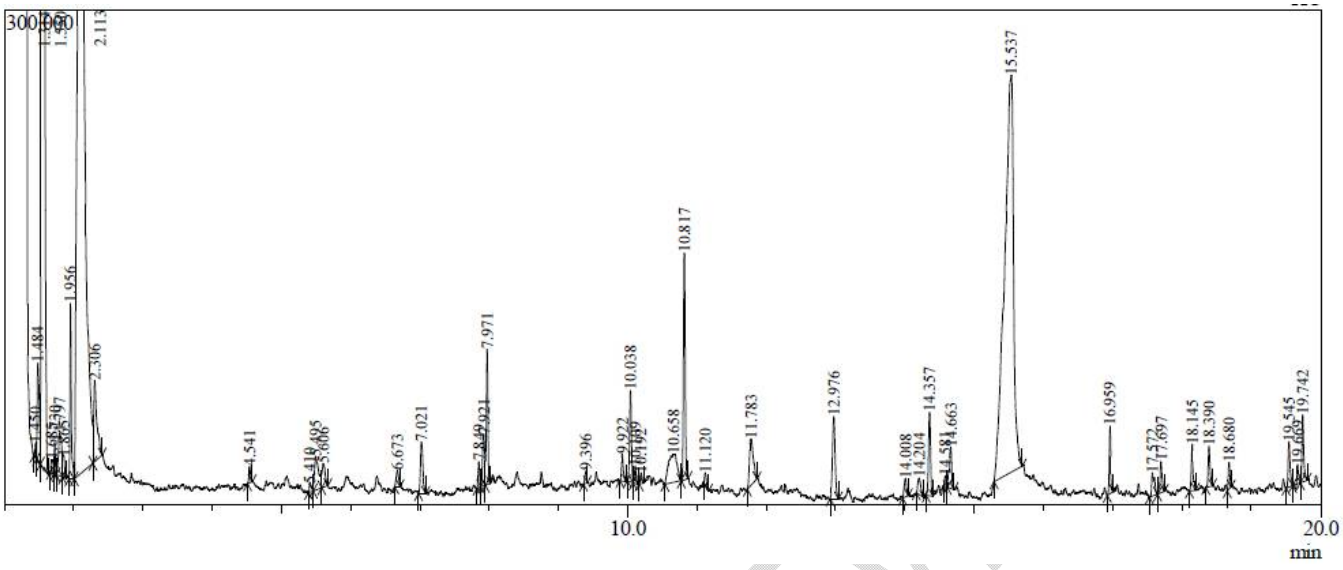

Fig. 1. GC chromatogram extract of fresh fenugreek

Conclusion

In conclusion, the fresh fenugreek leafy vegetables exhibit a favorable proximate composition, physical characteristics, and bio-chemical properties, making them an excellent choice for enhancing the nutritional value of a balanced diet. These vegetables have a moisture content of 86.12%, along with 4.9% carbohydrates, 4.5% crude protein, 0.8% crude fat, 2.2% crude fiber, and 1.48% ash content. Furthermore, the bio-chemical analysis reveals the presence of beneficial compounds in fresh fenugreek leafy vegetables. They contain a considerable amount of total flavonoids (559.81 mg/100 g) and total phenols (147.82 mg/100 g), which contribute to their antioxidant properties. The fresh leaf contained high level of chlorophyll (287.69 mg/100 g), while the presence of ascorbic acid (87.12 mg/100 g) and ß-carotene (20.25 mg/100 g) adds to their nutritional value. The combination of these favorable proximate composition and bio-chemical properties makes fresh fenugreek leafy vegetables a valuable addition to a balanced diet. Their richness in essential minerals, antioxidants, and dietary fiber promotes overall health and well-being. Including these vegetables in one's regular meals can help ensure a nutrient-dense diet and potentially contribute to the prevention of various chronic diseases.

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