IMPACT OF PROCESSING ON NUTRITIONAL, ANTINUTRITIONAL AND PHYTOCHEMICAL COMPOSITION OF FENUGREEK SEEDS *(TRIGONELLA FOENUM-GRAECUM L.)*

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Impact of Processing on Nutritional, Antinutritional and Phytochemical Composition of Fenugreek Seeds *(Trigonella Foenum-Graecum L.)*

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Approval Letter

This ***dissertation*** entitled ***"Impact of Processing on Nutritional, Antinutritional and Phytochemical composition of Fenugreek Seed (Trigonella Foenum-Graecum L.)"*** presented by **Shonu Rai** has been accepted as the partial fulfillment of the requirement for the degree of **Bachelors of Science in Nutrition and Dietetics**.

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Abstract

Phytate and tannin are more pronounced antinutrients limiting the nutritional quality of fenugreek. Fenugreek *(Trigonella foenum-graecum L.)* collected from Biratnagar was studied to explore the effect of different treatments roasting (130±5°C for 7 minutes), soaking (12 hrs. at room temperature) and germination (72 hrs. at 25°C) on its bioactive compounds (polyphenol and anti-oxidant activity), nutritional and anti-nutritional compounds (phytate and tannin). Experimental data was analyzed using software GenStat 12th Edition.

The mean value of moisture, protein, fat, crude fiber, ash, carbohydrate was found to be 9.39%, 22.05% (db), 4.33% (db), 8.51% (db), 3.89% (db), 51.81% (db) respectively while iron and calcium content value were found to be 11.45 and 78.29 mg/100gm (db). Protein increased significantly during roasting, soaking and germination whereas fat decreased significantly during all treatments. Iron and calcium increased during roasting whereas decreased during soaking and germination. The mean value of phytate and tannin content were found to be 85.09 and 674.84 mg/100gm (db). Maximum reduction of anti-nutrients: phytate (54.55%) and tannin (66.73%) were found when fenugreek seeds were germinated (72 hours). The reduction percentage of antinutrients by soaking (12 hour) and roasting (130±5ºC for 7 mins) were found to be less effective method compared to germination. Statistical analysis for antinutrients showed that there was significant difference ($p<0.05$) found in between raw and treated sample. All the treatments had significant impact on its bioactive components. Phenolic content increased significantly ($p<0.05$) from 80.85mg GAE/g to 85.70, 83.38 and 117.70 mg GAE/g during roasting, soaking and germination. Antioxidant activity (IC50=1.28) was found to be high in germinated sample as compare to raw, roasted and soaked sample. IC50 value from ascorbic acid standard curve was 55.18 mg/ml. Hence, Germination of fenugreek seeds for 72 hours is concluded as the most effective and promising method for the reduction of antinutrients and increasing the nutritional components, phytochemicals and antioxidant, compared to the roasting and soaking techniques.

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Symbols and abbreviations	Full form
%	Percentage
μg	Microgram
ºC	Degree Celsius
ANOVA	Analysis of variance
Db	Dry basis
DM	Dry matter
Et. al	Et alibi and others
USDA	United States Department of Agriculture
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DW	Dry weight
Fig.	Figure
g	gram
MC	Moisture content
DFTQC	Department of Food Technology and Quality Control
GAE	Gallic Acid Equivalent
AOAC	Association of Analytical Chemist
D.F	Degree of Freedom
LSD	Least significant Difference
S.D.	Standard Deviation

Part I

Introduction

1.1 Background

Fenugreek (*Trigonella foenum graecum L.*) is an annual plant belongs to the family Leguminosae or Fabaceae and widely grown in many parts of the world including North Africa, Mediterranean Europe, West and South Asia and some parts of Australia (Petropoulos *et al.*, 2002). Fenugreek seed has a central hard and yellow embryo which is surrounded by a corneous and comparatively large layer of white & semi-transparent endosperm (Betty, 2008). It is the famous spices in human food. The seeds and green leaves of fenugreek are used in food as well as in medicinal application that is the old practice of human history. It has been used to increase the flavoring and color, and also modifies the texture of food materials. Seeds of fenugreek spice have medicinal properties such as hypocholesterolemic, lactation aid, antibacterial, gastric stimulant, for anorexia, antidiabetic agent, galactagogue, hepatoprotective effect and anticancer (Srinivasan, 2006).

Fig 1.1 Fenugreek (*Trigonella Foenum Graecum L.*) (Kor *et al.*, 2013a)

Fenugreek seeds can be a good supplement to cereals because of its high protein (25%), lysine, soluble (20%) and insoluble dietary fiber (28%) besides being rich in calcium, iron and beta-carotene (Shalini and Sudesh, 2002). In Ayurvedic and Unani medicine (the traditional medicine systems used in the Middle-East and South-Asian countries), fenugreek is used in the treatment of various ailments including diabetes, epilepsy, paralysis, gout, edema, chronic cough and piles wounds, arthritis, bronchitis, hypocholesterolemic, cancer, ulcer and digestive problems, anorexia (Bin-Hafeez. *et al.*, 2003). In Oman the fenugreek seeds are traditionally used to cure baldness, alleviate certain kidney problems, hepatoprotective effect, to maintain blood sugar level and in cosmetic preparations. Fenugreek seeds are added to the porridge given to new mother to help her sweat out infections, impurities and poisons from the body. Fenugreek seeds are also given to the nursing mothers as they act as galactagogue in augmentation of milk production. It is a useful legume crop that is incorporated into short-term crop rotation systems as it helps in Nitrogen Fixation in the soil through symbiosis with nodule bacteria, and enhances the soil fertility (Acharya. *et al.*, 2008; Sadeghzadeh-Ahari *et al.*, 2009).

However, the seeds are bitter in taste due to presence of bitter saponins, which limit their acceptability in foods (Pandey and Awasthi, 2015; Sharma., 1986b). It has been possible to debitter fenugreek seeds by employing various processing methods such as soaking, germination, roasting, etc. (Kala, 1997; Pandey and Awasthi, 2015; Sharma., 1986a). These days it is used as food stabilizer, prebiotic effect, thickening agent, adhesive and emulsifying agent due to its high fiber, protein and gum content. The seeds are available in any form whether whole or ground form is used to flavor many foods mostly curry powders, seasonings, teas and spice blend. Seeds are used as condiments in most parts of India, as a supplement to wheat and maize flour in Egypt and in Yemen, they are one of the main constituents of the normal daily diet. However, their acceptability in food is limited due to bitter taste (Pandey and Awasthi, 2015). Although fenugreek belongs to the family Fabaceae, it is not classified as a legume from culinary perspectives as the other genera of this family. From culinary perspectives, it has basically 3 main uses: as herb (dried or fresh leaves); as spice (seeds); and as vegetable (fresh leaves and sprouts). Since fenugreek has long been used as a leafy vegetable, spice or condiment and medicinal herb, the Food and Drug Administration (FDA) has approved it as seasonings, spices, flavoring

agent and has classified it in the category of food as "Generally Recognized as Safe (GRAS)" (AmanatAli *et al.*, 2015).

1.2 Problem statement

The medicinal and nutritional property of fenugreek has been getting popularity among the people. Fenugreek has the significant potential for being staple spices in Nepal due to its medicinal and traditional importance. Fenugreek seeds have also been reported to exhibit pharmacological properties such as anti-tumor/anticancer, anti-diabetic, antibacterial, hypocholesterolemic, hypolipidemic, hepatoprotective, lactation aid paralysis, gout, edema, chronic cough, antioxidant activity and cures digestive problems and anorexia. Undesirable taste of fenugreek seeds is due to components like coumarin, diosgenin, saponin and steroids. Antinutrients (Phytate, Tannin, Enzyme and Protease inhibitors, Saponin, alkaloids, Oxalates, Haemaggluttinins (lectin), Cyanogen,) are present in fenugreek seeds which hinders the digestion, absorption and utilization of vitamins, minerals (Iron, Calcium, Zinc) and nutrients (Protein, Carbohydrate) and also inhibit activity of digestive enzymes (α-amylase, Pepsin, Trypsin and Pancreatin).

Phytic acid is a major anti nutritional factor that binds with cationic nutrients like zinc and iron, and makes them unavailable for human intestinal absorption. Phytic acid also inhibit or hinders activity of some digestive enzymes like trypsin, α-amylase, pepsin and pancreatin which are needed for the digestion of protein and starch. Tannins had been reported to affect protein digestibility, adversely influencing the bioavailability of non-haem Iron leading to poor iron and calcium absorption, also carbohydrate is affected leading to reduced energy value of a diet containing tannin. High oxalates absorbed into body and overloaded is linked to several health issues including fibromyalgia, autism, kidney stones, vulvodynia and hypothyroidism. Calcium oxalate, can have deleterious effect on human nutrition and health, particularly by decreasing calcium absorption and aiding the formation of kidney stones. Protease inhibitors have the ability to inhibit the activity of proteolytic enzymes within the gastrointestinal tract of animals. The antinutrient activity of protease inhibitors is associated with growth inhibition and pancreatic hypertrophy. Trypsin inhibitor and chymotrypsin inhibitor are protease inhibitors occurring in raw legume seeds. Trypsin inhibitors that inhibit the activity of the enzyme's trypsin and chymotrypsin in the gut, thus preventing protein digestion, are found in many plant species

mainly in different grain legumes. Consumption of alkaloids will cause rapid heartbeat, paralysis and in fatal case, lead to death. Alkaloids cause gastrointestinal and neurological disorders and also causes infertility. Hypothyroidism is increasing daily worldwide as the thyroid gland is highly sensitive to stress and environmental stimuli. Goitrogens interfere with iodine uptake and thus, affect thyroid function.

There are limited studies for lowering of anti-nutrients and toxicants of fenugreek seeds, the information on comparative effectiveness of these methods is still the subject matter of research. Powdered forms of fenugreek seeds that are available in groceries are made by just sun drying the seeds which doesn't reduce the antinutrients effectively and scientific procedures and validation of traditional processing methods in terms of food quality and safety has not been attempted and are not followed. So, the nutrient along with phytochemicals, antioxidant and functional activities in such available powder is highly degraded. Hence efforts are to be made to enhance the reduction of anti-nutrition by house hold treatments towards improving the nutritional properties of fenugreek which is practiced worldwide and is more than justified.

1.3 Objective of the study

1.3.1 General objective

The general objective of this work was to study the impact of processing on nutritional, antinutritional and phytochemical composition and antioxidant of fenugreek seed.

1.3.2 Specific Objective

1. To determine the nutritional value, antinutrients (tannin, phytate) bioactive components polyphenol and antioxidant content of raw fenugreek.
2. To process fenugreek seed with various treatments (soaking, germination and roasting) on the retention of nutritional values, phytochemicals, antioxidant capacity and reduction of anti-nutritional factors.
3. To determine the best method of processing of the fenugreek seeds to preserve nutritional values and phytochemicals those are medicinally important and also have reduction pattern of anti-nutrients in processed fenugreek.
4. To conclude the type and the level of treatment that could potentially reduce the highest amount of anti-nutrients.

1.4 Significance of the study

In the present world, interest has been increased in search of additional foods to meet the demand of overly increased population (Siddhuraju *et al.*, 2000).This study specifically determines anti-nutritional contents of raw fenugreek and the effect of various processing to reduce those anti-nutrients. The results of this study might help in the establishment of the effective and optimized way for the use of fenugreek in household level and may provide opportunities to promote and support use of fenugreek into various traditional recipes of namely Laddo, Methi, suhali, bread etc. prepared from wheat-fenugreek blends, mainly consumed by the diabetic and hypercholesterolemic people, which can help to improve its production and utilization potentials (Mohammad *et al.*, 2015). Adding fenugreek fiber to refined flours helps to fortify with a balance of soluble and insoluble fiber. Flour fortified with 8– 10% fenugreek fiber has been used to prepare bakery foods such as pizza, bread, muffins, and cakes with acceptable sensory properties (Srinivasan, 2005b). As well as fenugreek has been used as food stabilizer, food adhesive, food emulsifier and gum. Raw and processed fenugreek seed flours were analyzed for nutritional composition, anti- nutritional, and antioxidant activity. Therefore, outcome of this study can be useful in finding the best effective treatment in reduction of antinutrients, which is lacking at present scenario.

1.5 Limitations of the study

Due to time limit this study has the following limitations:

- Medicinal component in fenugreek could not be studied.
- Only one variety of fenugreek was taken for study.

Part II

Literature Review

2.1 Background

Spices are natural food adjuncts that have been in use for thousands of years to enhance the sensory quality of foods. Herbs and spices have been extensively used as food additives for natural antioxidants. In comparison to chemical or synthetic additives herbal additives are preferred as these are safer, flavor enhancer and without any side effects (Das *et al.*, 2012). Spices are considered as rich source of bio-active antimicrobial compounds, preservatives or additives (Akarpat *et al.*, 2008; Lia and Roy, 2004). Medicinal plants have been in trend in developing and developed countries as well as around the globe. Plant derived-medicinal product have attracted the attention of scientist across the world since many years due to their minimum side effects and dominating positive effects on human health. These drugs have been continuously reported advantages remedies pre-clinically and approved by various health authorities for the treatment of different disorders including central nervous system (CNS) disorders (Zameer *et al.*, 2017).

Fenugreek *(Trigonella foenum-graecum L.)* is an annual crop and dicotyledonous plant belonging to the sub family Papilionaceae, family Leguminacae (Fabaceae) with trifoliate leaves, branched stem, white flowers, roots bearing nodules and golden yellow seeds (Acharya. *et al.*, 2008). Although grown as a spice in most parts of the world, the species name "foenum graecum" means "Greek hay" indicating its use as a forage crop in the past (Acharya. *et al.*, 2008; Petropoulos *et al.*, 2002). It is an erect hairy annual of the bean family, reaching 30-60 cm. The plant grows to a height of about three feet, has three-part leaves, the long slender stems bear tripartite, toothed, grey-green obovate leaves, 20-25 mm (3/4-1 in) long. The thin, sword-shaped pods are 10-15 cm (4-6 in), with a curved beak-like tip, each carrying 10-20 seeds. Mild Mediterranean climates are most suitable. Plants mature in about four months. The flowering season for the herb fenugreek is generally midsummer. Fenugreek seeds are small (5 mm. long), 2.5-4 mm width, hard, and brownish yellow the color may varies (Kor *et al.*, 2013b).

Linnaeus (1753) has described the species Trigonella foenum-graecum first. It is one of the oldest medicinal plants originated in central Asia ∼4000 BC (Altuntas *et al.*, 2005). Although fenugreek is a native of the Mediterranean region of Europe, it extends to central

Asia and North Africa, Europe, UK and USA. This wide distribution of its cultivation in the world is characteristic of its adaptation to variable climatic conditions and growing environments. Temperate or low rainfall and cool growing season without extreme temperatures is favorable for the best development of fenugreek. It is being commercially grown in India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Turkey, North Africa, Middle East and Argentina (Flammang. *et al.*, 2004). Over 80% of the total world's production of this seed is contributed by India, one of the major producers and exporters of fenugreek legume in the world (Hooda and Jood, 2003).

Medicinal applications of fenugreek were documented in ancient Egypt, where it was used in incense and for embalming mummies. In modern Egypt, fenugreek is still used as a supplement to wheat and maize flour for bread-making (Zia *et al.*, 2001). This method increases the protein, fat, lysine, minerals, and dietary fiber contents of the bread proportionately to the level of fenugreek substitution (Shalini and Sudesh, 2004). Fenugreek seeds reduce the amount of calcium oxalate in the kidneys which often contributes to kidney stones and has been applied to relieve muscle aches and gout pain (Charles and Soetan, 2014). Other traditional uses have informed extensive study on this medicinal plant and biological activities have been associated with fenugreek in various studies: antidiabetic, antiplasmodic, hypolipidemic, antibacterial, anthelmintic, anti-inflammatory and analgesic activity, antioxidant, anticarcinogenic, antiulcer, antifertility, immunomodulatory effect, enzymatic pathway modifier (Hannana *et al.*, 2003). Galactomannan, a constituent of soluble dietary fibers and sapogenin has antihyperlipidemic effects by lowering total cholesterol, low density lipoprotein cholesterol in serum and liver. It was observed in obese rat model of hyperlipidemia due to the enhanced activity of HMG-CoA reductase with additional excretion of bile acids and neutral sterols in feces (Ramulu *et al.*, 2011).

2.2 Classification and nomenclature

According to Dilkash *et al.* (2016), the Taxonomy hierarchy of fenugreek is given below:

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Fabales

Family: Fabaceae / Leguminosae

Sub-family: Trifoliae

Genus: *Trigonella L.*

Species: *foenum-graecum*

Bionomial name: *Trigonella foenum-graecum L.*

Source: (Dilkash *et al.*, 2016)

2.3 Structure of fenugreek seeds

Although there are as many as seventy-two Trigonella species, most studies on seed structure and physiology have been performed on the *Trigonella foenum-graecum L*. Fenugreek seeds are small, hard, and brownish yellow the color may vary. Seeds are small, angular and flattened, 4-5 mm in length, 2.35-2.60 mm in width and 0.2 cm in thickness with a characteristic oblong rhomboidal outline (Altuntas *et al.*, 2005). A seed holds a central hard yellow embryo bound by large, corneous white and semi-transparent endosperm (Betty, 2008). Seeds are rectangular, square, flattened and have a very characteristic irregular rhomboidal outline with a specific concavity on the outer surface separating the radicle from the cotyledon (Slinkard *et al.*, 2009). Nearly in the center of one of the long, narrow sides is a small depression in which hilum and micropyle are situated, the former being distinctly visible as a whitish point; this depression is continued in the form of a furrow running diagonally across part of each of the adjoining sides, thus dividing the seed into two unequal lobes. If the seed is cut in a direction transverse to the side in which the hilum lies, so as to pass through both lobes of the seed, it will be found that the larger lobe contains two accumbent cotyledons-the smaller, the radical. Both are yellowish in color, and surrounded by a darker horny, translucent endosperm, which

separates the radicle from the cotyledons. When it is soaked in water the endosperm swells up to a thick gelatinous sac and yields mucilage to the surrounding liquid. Entire seeds macerated in warm water burst their seed-coats by the swelling of the mucilage, and disclose the structure of the seed (Snehlata and Payal, 2012).

Fenugreek seeds varies in color from yellowish brown to luteous when mature, but genotypes that miss polyphenolic tannins manifest inconspicuous (yellowish to white) coloration and are surrounded by the seed coat (Basu, 2006; Lee, 2009). There are also some varieties with the ability to produce mature seeds which are either green or yellowish green in color (McCormick *et al.*, 2009). The seed coat (testa) is separated from the embryo by a well-developed semi-transparent endosperm, which is the principal storage organ in the mature seed (Spyropoulos, 2002). In mature seeds the majority of the endosperm cells are nonliving, the cytoplasmic contents of which are occluded by the store reserves: galactomannan (Petropoulos *et al.*, 2002). This tissue is surrounded by a one cell layer of living tissue: the aleurone layer (Spyropoulos, 2002). The aleurone layer cells are small and thick walled and contain aleurone grains, which disappear during the course of seed germination (Zandi *et al.*, 2015). The role of endosperm galactomannan is dual: it serves as a reserve material that will support the seedling growth during the early post-germination phase, but also, due to its high-water retention capacity regulates the water balance of the embryo during germination (Petropoulos *et al.*, 2002). The embryo, as in all dicotyledons is composed of a cotyledon pair and the embryo axis. Apart from the endosperm reserves, there are also reserves in the embryo (protein, lipid, sugar) that will be metabolized upon seed germination and will be used for the growth need of the young seedling (Petropoulos *et al.*, 2002). The fenugreek seed coat apart from its protective character seems also to play a regulatory role in the mobilization of the endospermic food reserves (Petropoulos *et al.*, 2002; Spyropoulos and Reid, 1985; Zambou and Spyropoulos, 1993).

2.4 Nutritional and medicinal importance of fenugreek seeds

2.4.1 Antidiabetic Influence:

The presence of a chemical called 4- Hydroxyleucine, precursor of the amino acid leucine in the fenugreek seeds that has been possible to isolate and study (Acharya. *et al.*, 2008). This chemical stimulates the release of insulin in the diabetics in the presence of moderate to high levels of blood glucose (Al-Habori and Raman, 2002). Several researchers reported that treatment with a decoction of Fenugreek seed improved diabetes and suppressed glycosuria in mild diabetes and brought improvement in severe diabetic condition (Srinivasan, 2005a). Diosgenin, a steroid saponin in fenugreek also confers emerging application in medicine, as it has a potential to promote adipocyte differentiation and inhibits inflammation in adipose tissue which constitutively improves glucose metabolism (Uemura *et al.*, 2010).

Fenugreek contains fiber to an extent of 51.7%, containing 19.2% mucilaginous fiber, and 32.5% neutral fiber, respectively. It has been well recognized that dietary fiber offers substantial benefits to person with diabetes mellitus. It also contains an alkaloid named trigonelline, which has an effect on glycosuria. The high soluble fiber content and presence of bioactive compounds gum (i.e. galactomannan) in the fenugreek seeds probably accounts for the delay in gastric emptying, suppress release of the gastric inhibitory peptides, insulinotropic hormones and impaired glucose absorption in the gastro-intestinal tract (Al-Habori and Raman, 2002). National Institute of Nutrition, India have demonstrated the beneficial effect of Fenugreek in both type-1 and type-2 diabetes with the help of different clinical trials on animals. There are so many reports available on the antidiabetic property of Fenugreek seeds. Since fenugreek seeds are a source of protein, they can replace pulses in the diets of diabetics. Suggested oral dose of 25-50 g fenugreek seed powder in the diet of diabetic patients (taken daily) can be an effective supportive therapy in the management of diabetes and studies have been made about the glycemic index of fenugreek which showed that soluble fenugreek fiber has significantly reduced the glycemic index (Senthil *et al.*, 2011).

2.4.2 Anticancer Activity:

Apoptosis is a type of cell death. It was identified in previous studies that Flavonoids produced several biological effects, as apoptosis inducing activity and all (Chen. *et al.*, 2003). Flavonoids and catechins were first shown to be apoptotic in human carcinoma cells (Ahmad *et al.*, 2000). Similar observation has since been extended to lung tumor cell lines (Valette *et al.*, 1984), colon cancer cells, breast cancer cells, prostate cancer cells (Hannan *et al.*, 2003), stomach cancer cells (Zia *et al.*, 2001), brain tumor cells, head and neck squamous carcinoma (Ramesh and Srinivasan, 2004) and cervical cancer cells, and it was also published that other food flavonoids inhibits carcinogenesis in animal models. They all induce apoptosis in tumor cells. Fenugreek has been used to treat peptic ulcers and inflamed conditions of the stomach and bowel, it absorbs toxic material and eliminates it. The healing and soothing action create a protective coating, like lubricant, over inflamed areas. Fenugreek seeds contain the neuroprotective alkaloid trigonelline (Tohda *et al.*, 2005), which can be effectively used in the prevention and treatment of neurodegenerative diseases.

Fenugreek seeds also have anti-inflammatory, antipyretic and analgesic properties (Malviya *et al.*, 2010). Fenugreek is a promising protective medicinal herb for complementary therapy in cancer patients under chemotherapeutic interventions because fenugreek extract shows a protective effect by modifying the cyclophosphamide induced apoptosis and free radical-mediated lipid peroxidation in the urinary bladder of mice (Bhatia *et al.*, 2006). Diosgenin in fenugreek prevented cell growth and induced apoptosis in the H-29 human colon cancer cell line and fenugreek seed was found to have hepatoprotective properties (Thirunavukkarasu *et al.*, 2003). Fenugreek seed extract significantly inhibited 7,12-dimethylbenz(α)anthracene-induced mammary hyperplasia and decreased its incidence in rats and suggested that fenugreek's anti-breast cancer protective effect could be due to increased apoptosis (Amin *et al.*, 2005). The chemical constituents of fenugreek possessing anticancer activity are phytoestrogen and saponin. Saponin selectively inhibit cell division in tumor cells and can activate apoptotic programs which can led to programmed cell death (Raju *et al.*, 2004).

2.4.3 Cholesterol-lowering Effects:

Cholesterol and fecal bile acid excretion are increased by Fenugreek. A reaction between bile acid and Fenugreek derived saponin is responsible for the formation of too large micelles in the digestive tract to absorb. Among the sapogenins some of the important components are diosgenin, tigogenin, gitogenin, etc. (Acharya. *et al.*, 2011). These chemicals were found to lower the plasma total cholesterol, LDL, VLDL and TG levels while slightly increasing the plasma HDL level (Al-Habori and Raman, 2002). This hypolipidemic state prevents the occurrence of an atherosclerosis. This atherosclerosis, among diabetic patients, is an important pre-disposing condition to the microvascular and macrovascular complications (Maitra *et al.*, 2010). Another hypothesis attributes the cholesterol lowering where the fiber-rich gum portion of seed reduces the rate of hepatic synthesis of cholesterol, low density lipoprotein cholesterol in serum and liver. It is likely that both mechanisms contribute the overall effect (Yadav *et al.*, 2011). Diosgenin significantly lowers cholesterol levels, and it can also be used in the production of oral hormones and steroids (Oncina *et al.*, 2000). Galactomannan, a constituent present in soluble dietary fibers of fenugreek, exerts its antihyperlipidemic effect. It was observed in obese rat model of hyperlipidemia due to enhanced activity of HMGG-COA reductase with additional excretion of bile acids and neutral sterols in feces (Ramulu *et al.*, 2011).

2.4.4 Antioxidant Effect:

Scientist evaluated that polyphenol-rich extract of Fenugreek seed have potential effect against hydrogen peroxide (H_2O_2) - induced oxidation in normal and diabetic human erythrocytes (RBC) (Yadav *et al.*, 2011). It is a powerful antioxidant and it acts as a mucus solvent and throat cleanser, which also eases the urge to cough. A number of plant-derived antioxidants are flavonoids and polyphenols, which have been shown to be effective in biological systems in reducing oxidative stress. Treatment with *Trigonella* seed has been shown to restore the altered activity of cellular antioxidant enzymes including superoxide dismutase (SOD), glutathione reductase, catalase and glutathione peroxidase in tissue such as heart muscle, liver, kidney and brain during diabetes (Baquer *et al.*, 2011). Supplementation of fenugreek leaves reduces oxidative stress in streptozotocin-induced diabetic rats which significantly reduced lipid peroxidation and on other hand significantly increased antioxidants (Annida *et al.*, 2005). Antioxidant properties of germinated fenugreek seeds, which are considered to be more beneficial than dried seeds by providing

essential amino acids, especially leucine, lysine and tryptophan also seed germination activates proteolytic and lipid degrading enzymes that help improve protein digestibility as well as fat absorption capacity because germination leads to decreased levels of total unsaturated fatty acids, total lipid, triglycerides, phospholipids and unsaponifiable matter while those of saturated fatty acids increased (Umesh and Baquer, 2014). As oxidative stress is involved in the development and progression of diabetic nephropathy, examined antioxidant activity of *Trigonella* seed aqueous extract in restoring the kidney function of diabetic rats. These findings suggested that supplementation of fenugreek extract significantly increased antioxidant enzymes activities in kidney thereby protecting against functional and morphological injuries in diabetic kidneys (Xue *et al.*, 2011).

2.4.5 Antimicrobial Activity:

The leaf and seed extract with different organic solvents were found effective against various bacteria like *Escherichia coli, Salmonella typhi* and *Staphylococus aureus* and fungus like *F. oxysporum f. sp. lycopersici* (FOL) *and F. oxysporum f. sp. radicis-lycopersici* (FORL) etc. (Omezzine *et al.*, 2014; Yadav *et al.*, 2011). As an antiparasitic agent, fenugreek was pitted in a 2008 Oxford Journals article against the malaria-causing organism Plasmodium. In vitro studies found that fenugreek extracts were effective against resistant species of Plasmodium. The 2004 Asia Pacific Journal of Clinical Nutrition article also noted that germination or sprouting of fenugreek seeds increased their antioxidant profile and antimicrobial activity against H-pylori. Finally, a 2006 African Journal of Biotechnology article compared the effectiveness of fenugreek against two common pathogenic bacteria. Fenugreek was found to strongly inhibit the growth of Staphylococcus aureaus and Pseudomonus aeruginosa in a petri dish (Snehlata and Payal, 2012).

2.4.6 Traditional uses:

The Fenugreek herb has been known to help reduce fever when taken with lemon and honey, since it nourishes the body during an illness. Fenugreek teas, which can be used instead of the green tea which is mucilaginous, nutritious, and soothing to the intestinal canal. The nourishing seeds are given during convalescence and to encourage weight gain, especially in anorexia and is helpful in lowering the fever, gastritis, gastric ulcers and remedy for abdominal cramps associated with both menstrual pain, diarrhea or gastroenteritis (Snehlata and Payal, 2012). The Chinese use the seed for reducing

abdominal pain, labour pains, kidney problems, cholechystosis, fever, hernia, impotence, hypogastrosis, nephrosis and rheumatism. In Malaya, they poultice the seeds onto burns and use them for chronic coughs, edema, hepatomegaly and splenomegaly. The seeds also function as a preservative and are added to pickles, chutneys and other similar products. In modern food practice, the seeds or the extract are used in bakery products, frozen dairy products, meat products, relish, condiments, candy, gravy sauces, gelatin puddings and in alcoholic and non-alcoholic beverages. Fenugreek has a beneficial action on cleansing the blood. As a diaphoretic it is able to bring on a sweat and to help detox the body. For relief from the agonizing symptoms of irritable bowel syndrome, colitis and diverticulitis, the 'soak-and-rinse water' is drunk and the sprouts blended to a liquid. It has been called the herb for 'every ailment under the sun'! When soaked in water, the seeds swell and produce a soothing mucilage said to aid digestion (Kor *et al.*, 2013b).

2.4.7 Remedy to Ease Child Birth for Pregnant Women:

Fenugreek stimulates uterine contractions and can be helpful to induce childbirth. However, pregnant women should only use Fenugreek for inducing labor after consulting with their doctor (Snehlata and Payal, 2012).

2.4.8 Lactation Aid:

Fenugreek seeds contain hormone precursor's galactagogue that increase milk supply. Some scientists believe it is possible because breasts are modified sweat glands, and fenugreek stimulates sweat production. It has been found that fenugreek can increase a nursing mother's milk supply within 24 to 72 hours after first taking the herb (Snehlata and Payal, 2012).

2.4.9 Animal food

In India and Turkey, it is used as green fodder and hay for cattle. Also, Hidvegi *et al.* (1984) report that fenugreek seeds are used for feeding cattle. Ground fine and mixed with cotton seed it is fed to cows to increase the flow of milk. An extract of fenugreek seed is added to animal food to increase its palatability (G. A. Petropoulos, 2002).

2.5 Chemical and nutritional composition of fenugreek seed

Table 2.1 Nutritional value of fenugreek seed

Components	Amounts
Proximate	
Moisture%	13.7
Protein%	26.2
Fat%	5.8
Carbohydrate%	44.1
Ash%	2.93
Crude fiber%	7.2
Minerals%	3
Vitamin C (mg/100gm)	0

Source: DFTQC (2012)

Table 2.2 Vitamin and mineral content in fenugreek seed

Components	Amounts
Iron (mg/100g)	6.5
Calcium (mg/100g)	84.17
Phosphorus (mg/100g)	370
Niacin (mg/100g)	1.1

Source: DFTQC (2012)

2.6 Anti-nutritional factor

Foods are complex substances that contain many chemical compounds, more than 50 of which are required to nourish the body. These nutrients include water, proteins, lipids, carbohydrates, minerals and vitamins. Additionally, most plant foods also consist of thousands of natural compounds and antinutrients, depending on the situation may have beneficial or deleterious effect on consuming them (McEwan, 2008). Anti-nutritional factors may be regarded as the class of these compounds that are generally not lethal. They diminish animal productivity but may also cause toxicity during the periods of scarcity or

confinement when the food rich in these substances is consumed by animals in large quantities (Thakur *et al.*, 2019).

Anti-nutrients are compounds which reduce the nutrient utilization and food intake of plants or plant products used as human foods. Plants evolved these substances to protect, prevent and defense themselves from being eaten by herbivorous and pathogens. They are generated in natural feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exerts effect contrary to optimum nutrition (Soetan, 2008). If the diet is not varied, some of these toxins build up in the body to harmful levels. Some vitamins in food may be destroyed by anti-nutritional substances. These anti-nutritional factors must be inactivated or removed, if values of food substances are to be fully maintained (Thakur *et al.*, 2019). They are potentially harmful and give rise to a genuine concern for human health in that they prevent digestion and absorption of vitamins, minerals and other nutrients. They can reduce the nutritional value of a plant by causing a deficiency in an essential nutrient or preventing through digestion when consumed by humans or animals (McEwan, 2008; Prathibha *et al.*, 1995). Several anti-nutritional factors are present in root and tuber crops and are partially neutralized during ordinary cooking (Bhandari and Kawabata, 2004). The remaining anti-nutrients can, however, be responsible for the development of serious gastric distress and may interfere with digestion of nutrients, which inevitably results in chronic deficits in absorption of nutrients (Brune *et al.*, 1989; Jood *et al.*, 1986; Kelsay, 1985; McEwan, 2008).

Anti-nutrients can be divided into two groups: heat-stable group Phytic acid, Tannins, Alkaloids, Saponins, non-protein amino acids etc. and heat labile group include Lectins (hemagglutinins), Cyanogenic glycosides, Isoflavones, Enzyme inhibitors (protease, trypsin and amylase inhibitors), Oxalate, Pyrimidine glycoside and Toxic amino acids. Anti-nutritional factors are present in different food substances in varying amounts, depending on the kind of food, mode of its propagation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances (Thakur *et al.*, 2019).

2.6.1 Tannin

Tannins are water soluble phenolic compounds having molecular weights between 500 to 3000 giving the usual phenolic reactions and having special properties such as the ability to precipitate alkaloids, gelatin and proteins. The dark color and astringent taste of food is often ascribed to tannins. They can have a large influence on the nutritive value of many foods eaten by humans such as vegetables, fruits, chocolate, tea, alcoholic and nonalcoholic beverages, etc. Plant tannin extracts on the other hand, are used worldwide for the industrial production of leather (mimosa or quebracho) (Romer *et al.*, 2011) and to the lesser extend in wine making (Garcia-Ruiz *et al.*, 2012). Foods rich in tannins are considered to be of low nutritional value because they precipitate proteins, inhibiting digestive enzymes and Fe absorption and affect the utilization of vitamins and minerals from meals (Tinko and Uyano, 2001).

Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. They form indigestible complexes with proteins, macromolecules, carbohydrates, gelatin and alkaloids under specific environmental conditions (Saxena. *et al.*, 2014). Tannins are phenolic plant secondary compounds and are widely distributed in the plant kingdom and many species of plants, especially in pulses where they play a role in the defensive system or protection from predation, and perhaps also as pesticides, and might help in regulating plant growth. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripen fruit or red wine or tea. Likewise, the destruction or modification of tannins with time plays an important role when determining harvesting times (McGee, 2004).

Tannins had been reported to affect protein digestibility, adversely influencing the bioavailability of non-haem Iron leading to poor iron and calcium absorption, also carbohydrate is affected leading to reduced energy value of a diet containing tannin (Adeparusi, 2001). This results in growth depression, in all probability owing to enzyme resistant substrates formed by interaction between tannins and protein/starch. Digestibility of the substrates is compromised by interaction between tannin and the enzymes (Thompson, 2006). They can be classified into two groups, the proanthocyanins (or condensed tannins) and the polyesters of gallic acid and (or) hexahydroxy diphenic acid (hydrolysable tannins, respectively, Gallo- and ellagitannins) with an immense structural variability, reaching high degrees of polymerization (Mahmut and Ayhan, 2002; Mueller-

Harvey, 2006). Condensed tannins are derivatives of flavanols and hydrolysable tannins are esters of a sugar, usually glucose (Bartosz *et al.*, 2017). Tannins are found in the leaves, fruits, barks, roots and wood of trees (Mahmut and Ayhan, 2002). The tannin content in the fenugreek is about 718.92 mg/100gm (Atlaw and Kumar, 2018). In the present study, (Shimelis. and Rakshit., 2008) reported that the germination reduced tannin content by more than 77.85%. The structure of tannin is shown below in Fig. 2.1.

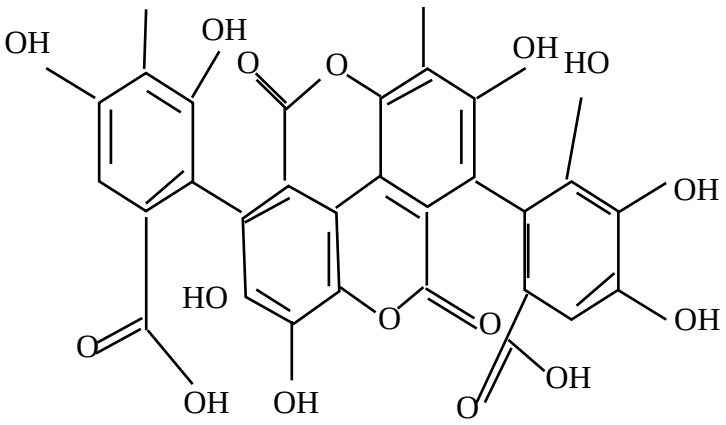


Fig. 2.1 Structure of tannin

2.6.2 Phytate

Phytic acid is a hexaphosphoric ester of the hexahydric cyclic alcohol meso-inositol, phytic acid (known as inositol hexakiphosphate (IP6), or phytate when in salt form) is the principal storage form of phosphorus in many plant tissues especially in all seeds and grains. Inositol penta- (IP5), tetra- (IP4) and triphosphate (IP3) are also called phytates. The chemical description for phytic acid is myoinositol (1,2,3,4,5,6) hexakiphosphoric acid (Kumar *et al.*, 2010). Phytate is formed during maturation of the plant seed and in dormant seeds it represents 60-90% of the total phosphate (Loewus, 2002).

Phytic acid, mostly as phytate, is found within the hulls of seeds, including nuts, grains and pulses. Phytic acid is not digested by humans, and is therefore not a dietary source of inositol or phosphate. In fact, because phytic acid is a good metal chelator, it is believed to have a negative nutritional impact on strongly chelating metals necessary for good health (e.g., Zinc, iron, calcium, magnesium) and could prevent their absorption by the intestine. Phytates are important because they are the major phosphorus form in mature plant seeds, not absorbed from the GI tract, and hydrolyzed only slightly in human and animal

intestines. For this reason and because phytic acid is thought to have a positive dietary impact as an antioxidant to prevent carcinogenesis, determining the phytic acid content of foods is of interest (Phesatcha *et al.*, 2012). In-home food preparation techniques can break down the phytic acid in all of these foods. Simply cooking the food will reduce the phytic acid to some degree. More effective methods are soaking in an acid medium, sprouting and lactic acid fermentation such as in sourdough and pickling (Fardet, 2010).

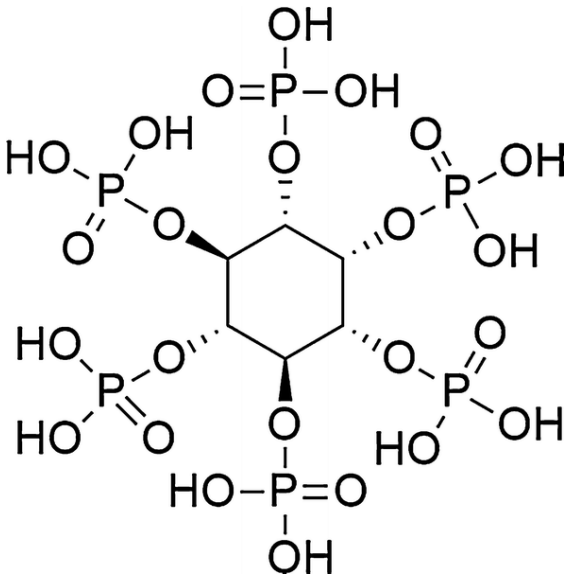


Fig 2.2 Structure of Phytic Acid (Pyarasani *et al.*, 2018)

Phytic acid is a major anti nutritional factor that binds with cationic nutrients like zinc and iron, and makes them unavailable for human intestinal absorption. (Pedersen *et al.*, 2007; Sandberg and Svanberg, 1991). When iron and zinc bind to phytic acid they form insoluble precipitates and reduce bioavailability of these ions through lower absorption rate in the intestines. This process can therefore contribute to iron and zinc deficiencies in people whose diets rely on these foods for their mineral intake, such as those in developing countries and vegetarians (Baskota, 2019). They form digestion-resistant complexes with carbohydrate, protein and minerals in the feed under both acidic and alkaline pH conditions and make them unavailable. These interactions were found to affect the protein's structure, thus reducing the enzymatic activity, protein solubility and proteolytic digestibility (Kies *et al.*, 2006; Yao *et al.*, 2011).

Phytic acid also inhibit or hinders activity of some digestive enzymes like α-amylase, pepsin and pancreatin (Mabrouki *et al.*, 2015). Phytic acid not only grabs on to or chelates

important minerals, but also inhibits enzymes that we need to digest our food, including pepsin, needed for the breakdown of proteins in the stomach, and amylase, needed for the breakdown of starch into sugar. Trypsin, needed for protein digestion in the small intestine and stomach, is also inhibited by phytate. Although indigestible for many animals, phytic acid and its metabolites as they occur in seeds and grains have several important roles for the seedling plant. Most notably, phytic acid functions as a phosphorus store, as an energy store, as a source of cations and as a source of myoinositol (a cell wall precursor). Phytic acid is the principal storage form of phosphorus in plant seeds (Fardet, 2010). As humans and animals are dependent on plant-based foods for their nutrient requirement except Vit B12, the deficiency of any nutrient can lead to malnutrition or under nutrition (White and Brown, 2010). Phytate is a common constituent of plant derived foods like cereals or legumes, which are the main staple food of people in developing countries. The daily intake of phytate for humans or vegetarian diets, on an average, is 2000-2600 mg whilst, for inhabitants of rural areas in developing countries, on mixed diets, it is 150-1400 mg (Reddy, 2002).

2.6.3 Oxalate

The plant oxalis, commonly known as wood sorrel, gave rise to oxalic acid (chemical formula HOOC-COOH), a strong, organic acid which has been found to be widely distributed in plants (Liebman, 2002). Oxalates are found most commonly in dark-coloured fruits and vegetables like berries, spinach and also cereals and legumes like wheat, rye, soybean, tofu, lentils, and kidney beans. Consumption of high oxalate foods exerts a negative effect on calcium and iron absorption in the body (Chai and Liebman, 2005). Consumption of high oxalates binds to calcium in body and forms crystal resulting in kidney stones. Legumes, nuts, and different types of grain-based flours are commonly consumed throughout the world. Soybeans and other legumes such as lentils, red kidney beans, and white beans have been previously analyzed for oxalate (Fox *et al.*, 2015; Massey *et al.*, 2001).

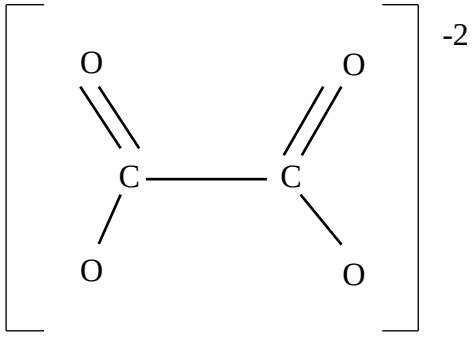


Fig 2.3 Structure of oxalate

Oxalate is of primary concern among the anti-nutritional factors due to its strong oxidizing and corrosive nature with good chelating activity, synthesized by a broad range of animals, plants and microorganisms (Stewart *et al.*, 2004). Oxalate-producing plants, which include many crop plants, accumulate oxalate in the range of 3%-80% (w/w) of their dry weight (Nakata and McConn, 2000). The diversity of calcium oxalate crystal shapes and sizes, as well as their prevalence and spatial distribution, have led to a number of hypotheses regarding crystal function in plants. The proposed functions include roles in ion balance, in plant defense, in tissue support, in detoxification, and in light gathering and reflection (Franceshi and Horner, 1980; Nakata and McConn, 2000). Recently, Nakata and McConn (2000) hypothesized the roles of calcium oxalate formed in plants in supporting tissue structure and in regulating excess tissue calcium. Oxalic acid occurs as the free acid, as soluble salts of potassium and sodium and as insoluble salts of calcium, magnesium and iron (Noonan and Savage, 1999). Oxalic acid content in foodstuffs has long been a concern in human diets, due to the negative health effects connected to a high intake of oxalic acid. Incidences of kidney stones, hypocalcemia and hyposideremi (low plasma levels of calcium and iron) correspond strongly with the intake of oxalic acid that perform as an absorption inhibitor are common (Palaniswamy *et al.*, 2002). High oxalate content in urine and blood causes several diseases such as hyperoxaluria and vitamin deficiencies. Soluble oxalates can have toxic poisoning effects on grazing animals and are associated with calcium oxalate accumulation in rumen walls, arteries and kidneys (Franceschi and Nakata, 2005). Oxalates crystallizes with calcium in the renal vasculature and infiltrates vessel walls causing renal tubular obstruction, vascular necrosis and hemorrhage, which leads to anuria, uremia, electrolyte disturbances or even rupture. Oxalic acid may cause greater decreases in mineral availability if consumed with a high fiber diet, although the decrease may only be temporary (Savage *et al.*, 2000).Prevention of calcium oxalate stone formation

can be achieved by avoiding large amounts of oxalate containing foods and consuming calcium rich foods such as dairy products together with oxalate containing foods (Martensson and Savage, 2008).

2.7 Polyphenols

Phenols are the aromatic compounds with hydroxyl groups attached directly to an aromatic ring, are widespread in plant kingdom. It is well known that the phenolic acids are the derivatives of benzoic and cinnamic acid; and are generally classified into two types, hydroxybenzoic and hydroxycinnamic acid. Phenols are said to offer resistance to diseases and pest in plants. Grains containing high number of polyphenols are resistance to bird attack occurring in all vegetative organs, as well in flowers and fruits, vegetables, cereals, grains, seeds, drinks. Phenols include an array of compounds like tannins, flavanols, etc. (Sadasivam and Manickam, 2016). Phenolic compounds are characteristics of plants and as a group they are usually found as esters or glycosides rather than as free compounds. Perhaps the oldest medical application of phenolic compounds is the use of phenol as an antiseptic. Because of its negative side effects on living tissues, including blister formation, especially at the higher concentrations, it is no longer used in this capacity. Another very common use of phenolic compounds is in sunscreen. The presence of the aromatic ring in the effective absorbance of the UV-B radiations from the sun and thus prevents sunburn. Aside from the medical applications, polyphenols, including the flavonoids and tannins, are an integral part of human and animal diets, because they represent one of the most numerous and ubiquitous groups of plants metabolites. Although traditionally regarded as anti-nutrients, because of their bad taste, unappealing color, or cause of browning of tissues, polyphenols and other food phenolics are the subject of increasing interest because of their possible beneficial effects on health (Vermerris and Nicholsomn, 2008).

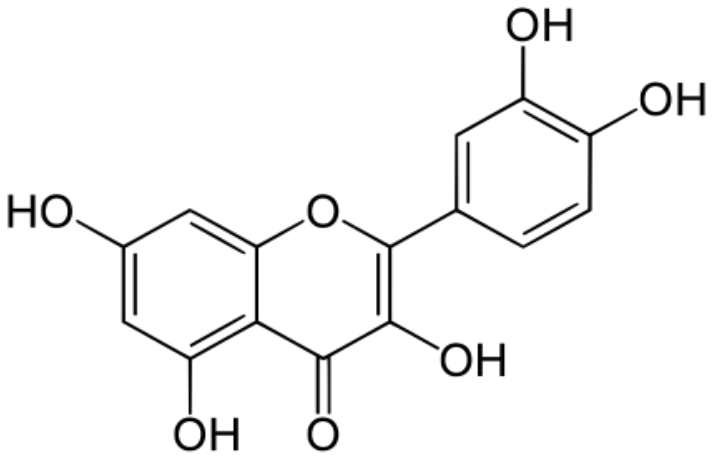


Fig 2.4 Structure of polyphenol

The possible health benefits of dietary phenolics depend on their absorption and metabolism, which in turn are determined by their structure including their conjugation with other phenolics, molecular size and solubility. It occurs at various point during the passage through the wall of the small intestine into the circulatory system and transport to the liver. The metabolites of polyphenols are rapidly eliminated from plasma, thus, daily consumption of plant products is essential to supply high metabolite concentrations in the blood (Crozier *et al*., 2009). Up to now epidemiological knowledge emphasize that polyphenols display important functions like inhibition of pathogens and decay microorganisms, anti-deposition of triglycerides, reduces the incidence of non-communicable diseases, diabetes, cancer and stroke, anti-inflammation, anti-mutagenic and anti-allergic effect. Initially the protective effect of dietary phenolics was thought due to their antioxidant properties which are the lowering of the levels of free radicals present in the body. The majority of data on health benefits of polyphenols-rich diet is still observational and some are contradictory. Some epidemiologic studies have indicated a negative correlation between consumption of polyphenols with diet and the risk of infectious, degenerative and chronic diseases. Some phenolic compounds, when ingested at high concentrations may exhibits roles in genotoxicity, thyroid toxicity, interactions with pharmaceuticals, and estrogenic activity. Nevertheless, most researchers stated that the health effects of polyphenols depend on the amount consumption and on their bioavailability leading more controlled and clinical studies (Ozcan *et al*., 2014).

2.8 Antioxidant

Any substance which is capable of delaying, retarding or preventing the development of the rancidity or other flavors deterioration due to oxidation is called antioxidant (Bhattarai, 2010). Oxidation reactions are chemical reactions that involve the transfer of electrons from one substance to an oxidizing agent. Antioxidants can slow these reactions either by reacting with intermediates and halting the oxidation reaction directly, or by reacting with the oxidizing agent and preventing the oxidation reaction from occurring (Pokorny, 2007). Much is known about antioxidants because of their functional importance, interest in antioxidants is high to protect edible oils, their derived products and also when used in food products to provide baking and culinary characteristics and nutritional benefits. Antioxidants are substances that generally prevent, delay or retard the onset of rancidity in food products due to oxidation of the unsaturated fatty acids incorporated in food products. The use of antioxidants helps to extend the shelf life of a food, minimizes waste and nutritional losses, and extends the scope of use of various fats/oils (Bhattacharya., 2003).

An antioxidant is a substance that when present at low concentrations compared to that of an oxidizable substrate significantly delays or prevents oxidation of that substrate. Antioxidants can act by scavenging biologically important reactive oxygen species (O_2, H_2O_2, OH, HOCl, ferryl, peroxyl, and alkoxyl), by preventing their formation, or by repairing the damage that they do (Halliwell, 1991; Sato *et al.*, 2013). The antioxidant defense system in most cells is composed of two components, the antioxidant enzymes component which includes enzymes such as superoxide dismutase, catalase and glutathione peroxidase, and the low molecular weight antioxidant component that includes vitamins A and E, ascorbate, glutathione and thioredoxin.

Oxygen, an element indispensable for life, can under certain circumstances, adversely affect the human body. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called ‘free radical’. Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. ROS are free radicals produced as by-products of oxidation–reduction (redox) reactions (Dowling and Simmons, 2009). Processes, like protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity, and differentiation, are all dependent on a proper ROS production

and presence inside cells that need to be kept at a low level (Rajendran *et al.*, 2014) . When ROS production increases, they start showing harmful effects on important cellular structures like proteins, lipids, and nucleic acids (Wu *et al.*, 2013). ROS are implicated in many clinical disorders and may cause human diseases such as cancer, diabetes, atherosclerosis, heart diseases, metabolic disorder and cerebrovascular diseases through multiple mechanisms (Yoshikawa *et al.*, 2000). Therefore, antioxidants can help in therapies for these diseases. Fenugreek is used as an antidiabetic agent and has been shown to have antihyperglycemic(Mowla *et al.*, 2009),hypolipidemic and hypocholesterolemic activities (Xue *et al.*, 2007). As ROS are implicated in the pathophysiology of diabetes and its secondary complications, along with its other properties, the antioxidant property of fenugreek would be an added advantage for its use as an antidiabetic drug.

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. ROS can play several physiological roles (i.e., cell signaling), and they are normally generated as by-products of oxygen metabolism; despite this, environmental stressors (i.e., UV, ionizing radiations, pollutants, and heavy metals) and xenobiotics (i.e., antiblastic drugs) contribute to greatly increase ROS production, therefore causing the imbalance that leads to cell and tissue damage (oxidative stress). Oxidative stress occurs when the production of ROS exceeds the body's natural antioxidant defense mechanisms, causing damage to biomolecules such as lipids, proteins and DNA (Alessandra *et al.*, 2017). The shift in balance between oxidant/antioxidant in favor of oxidants is termed "oxidative stress". Oxidative stress contributes to many pathological conditions including cancer, neurological disorders, atherosclerosis, hypertension, ischemia, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease and asthma (Andreadis *et al.*, 2003). Medicinal plants and herbs are known to be an important source of various natural antioxidants. Many other non-nutrient food substances, generally phenolic or polyphenol compounds, display antioxidant properties and thus may be important for health. One of the ways to deal with such oxidative damage and disease is the adequate oral intake of antioxidants from external sources. The best-known external antioxidants are vitamin E, vitamin C, flavonoids, polyphenols and the carotenoids (Chen *et al.*, 2016; Lobo *et al.*, 2010).

2.8.1 Mechanism action of antioxidant

The antioxidants are active in lengthening the induction period in the process of oxidation of fats, probably due to the absorption of the activating energy of the chain reaction that result in the oxidation of antioxidants (Bhattarai, 2010). An antioxidant act by reacting with free radical fatty acid (free radial or peroxide free radical) as they are formed, converting them back to the original substrate and then by terminating the chain propagation (or initiation). Free radicals of the antioxidant molecule are formed in the process, but the structure of an antioxidant so such that these are relatively stable and do not have enough energy to react with the fat to form further free radicals (Bhattarai, 2010; Coppen, 1983). An antioxidant (AH) apparently reacts with free radicals in following manner (Dugan, 1976; Lundberg, 1961).

$$R\bullet + AH \longrightarrow RH + A\bullet$$
$$RO\bullet + AH \longrightarrow ROH + A\bullet$$
$$ROO\bullet + A\bullet \longrightarrow ROOH + A\bullet$$
$$R\bullet + A\bullet \longrightarrow RA$$
$$RO\bullet + A\bullet \longrightarrow ROA$$
$$A\bullet + ROO\bullet \longrightarrow ROOA \text{ (Non radical product)}$$
$$A\bullet + A\bullet \longrightarrow AA \text{ (Non radical product)}$$

Fig 2.5 Mechanism of action of antioxidant

At high concentrations the antioxidant may have a peroxidant effect (Pokorny, 2007) and one of the reactions may be as follows:

$$A\bullet + RH \longrightarrow AH + R\bullet$$

The mode of action of all antioxidants (artificial or natural) is similar to the above mechanism. The antioxidant should be added to the fat as early as, if possible, in its life to produce the maximum effect. It follows that an antioxidant will only really be effective if it is added during the initiation period (Bhattarai, 2010).

2.8.2 DPPH radical scavenging assay

DPPH is a stable free radical that has ability to accept an electron or hydrogen from antioxidant compounds and is then converted to a DPPH stable molecule. The potential health benefits of plant phenolics are mainly due to their free radical-scavenging activities through donating a hydrogen atom and or an electron from an aromatic hydroxyl group to free radicals (Win *et al.*, 2011). DPPH assay measures the ability of a substance to scavenge the DPPH radical, reducing it to hydrazine. When a substance that acts as a donor of hydrogen atoms is added to a solution of DPPH, hydrazine is obtained, with a change in color from violet to pale yellow (Formagio *et al.*, 2014). A number of methods are used to determine the radical scavenging effects of antioxidants. The DPPH method is a preferred method because it is fast, easy and reliable and does not require a special reaction and device (Fukumoto and Mazza, 2000). The DPPH antioxidant assay is based on the ability of 2,2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. DPPH free radical, which is at its maximum wavelength at 517 nm, can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule (Marxen *et al.*, 2007). The DPPH radical is one of the few stable organic nitrogen radicals, which produces solutions colored deep purple. A solution of DPPH radicals prepared in methanol is converted into DPPH-H (diphenyl hydrazine) molecules in the presence of an antioxidant agent. When the antioxidants in plant extract react with DPPH, it reduced to DPPH-H and results in decolorization to yellow color with respect to the number of electrons captured. The color absorbance corresponds inversely to the radical scavenging activity of the sample extract. Discoloration occurs due to the decreasing quantity of DPPH radicals in the environment. The discoloration of the DPPH therefore reflects the radical scavenging activity of the analyzed extract (Molyneux, 2004).The scavenging of DPPH by radical scavengers can be summarized as:

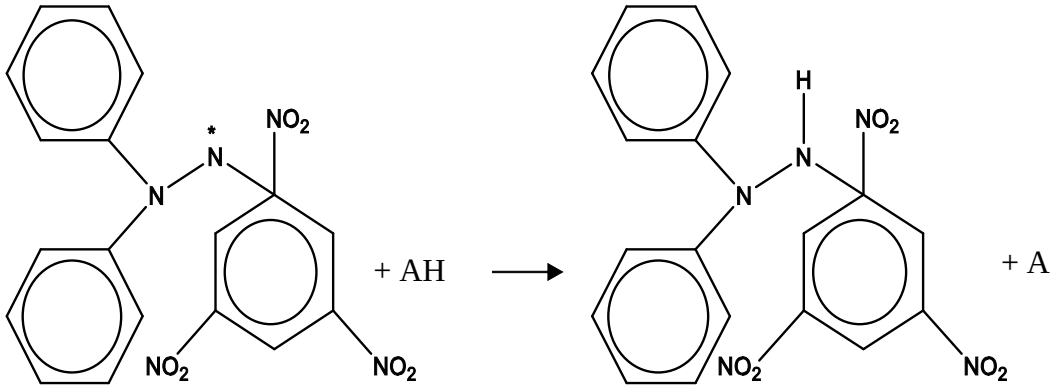
$$DPPH\bullet + FE \longrightarrow DPPH - H + A\bullet \quad (1)$$

$$DPPH + A\bullet \longrightarrow DPPH - A \quad (2)$$

$$A\bullet + A\bullet \longrightarrow A - A \quad (3)$$

Where, **FE** is a scavenger of the extract and **A•** is a radical.

The newly formed radical (A•) can mainly follow radical-radical interaction to render stable molecules, via radical disproportionate, collision of radicals with abstraction of an atom by one radical from another equations (Chandra and Goyal, 2014).



2, 2-diphenyl-1-picrylhydrazyl 2, 2-diphenyl-1-picrylhydrazine

Fig 2.6 Reaction of DPPH-free radical with an antioxidant

The test is simple and rapid and needs only a UV-vis spectrophotometer to perform, which probably explains its widespread use in antioxidant screening (Prior *et al*., 2005; Sochor et al., 2010).

Antioxidant efficiency is measured at ambient temperature and thus eliminates the risk of thermal degradation of the molecules tested. An antioxidant can be defined as “any compared to that of an oxidizable oxidation of that substrate” substance that, when present in low concentrations substrate, significantly delays or inhibits the oxidation of that substrate". Although the DPPH method is widely used, it does have some limitations. The radical portion of the molecule is a nitrogen atom located at the center of the structure. While this centralized location is freely accessible to small molecules, larger molecules may have limited access to the radical portion due to steric hindrances (Molyneux, 2004).

2.9 Effect of processing on antinutritional properties in food

Different processing techniques are often utilized in order to reduce anti-nutritional factors. Some techniques are performed on household level or domestically and others are performed on a larger scale in industry (Raes *et al.*, 2014). According to many studies in literature, soaking, cooking and boiling have generally achieved significant reduction of anti-nutrients. Therefore, foods high in anti-nutrients should be processed adequately in order to make them wholesome for consumption (ILeke, 2014).

The term "Food Processing" covers an enormous field, from simple boiling to the use of irradiation. The types of cooking methods differ in countries around the world and also vary with the ethnic background of the family. Processing (cooking) can be both beneficial and detrimental to nutrient composition of foods. It is known that processing techniques may decrease the food value of some nutrients (Tyagi *et al.*, 2015): for example, there is some inevitable leaching of nutrients into the cooking water during processing. The cooking water may or may not be discarded, depending upon cultural and personal preference.

Cooking may enhance the nutritional quality of food by reducing or destroying the anti-nutrients present in it, as well as increasing the digestibility of proteins and starches. Elimination of inactivation of anti-nutritional compounds is absolutely necessary to improve the nutritional quality and effectively utilize human foods to their full potential. A typical example is the protein in legumes, which is made more digestible by heating because of inactivation of anti-nutrients such as trypsin inhibitors (Siddhuraju and Becker, 2001). The use of some processing methods, such as boiling, baking, microwave and pressure cooking are known to achieve reduction or elimination of anti-nutritional factors (Bhandari and Kawabata, 2006; Habiba, 2002; Khokhar and Chauhan, 1986; Udensi *et al.*, 2007).

Extrusion cooking was found to be a versatile, quick and efficient method to reduce anti-nutrients when compared with other traditional processing methods (Alonso *et al.*, 2000). Soaking, sprouting, fermentation and cooking methods have also been investigated. Combination of cooking and fermentation improved nutrients quality and drastically reduced the antinutrient factors to safe levels much greater than any of the other processing methods tested (Obizoba and Atii, 1991). Excessive heat processing, however, should be

avoid, since it adversely affects the protein quality of foods. It is therefore important that processing is done within the recommended guidelines e.g. for heat, pH, as over processing will further destroy not only nutrient content but also taste and appearance (Morris *et al.*, 2004).

2.9.1 Soaking

Beans and other legumes are often soaked in water overnight to improve their nutritional value (Fernandes *et al.*, 2010). Most of the antinutrients in these foods are found in the skin. Since many antinutrients are water-soluble, they simply dissolve when foods are soaked. In legumes, soaking has been found to decrease phytate, protease inhibitors, lectins, tannins and calcium oxalate. It is done by submerging biological material in a water at a specific temperature of 4-80ºC. Upon soaking, water is absorbed by cells and the pH changes, which results in the activation of endogenous enzymes (Raes *et al.*, 2014). For example, a 12 hours soak reduced the phytate content of peas by up to 9% (Bishnoi *et al.*, 1994). Another study found that soaking pigeon peas for 6-18 hours decreased lectins by 38%-50%, tannins by 13%-25% and protease inhibitors by 28-30% (Onwuka, 2006). However, the reduction of antinutrients may depend on the type of legume. In kidney beans, soybeans and faba beans, soaking reduces protease inhibitors only very slightly. Not only is soaking useful for legumes, leafy vegetables can also be soaked to reduce some of their calcium oxalate. Soaking is typically used in combination with other methods, such as sprouting, fermenting and cooking (Arnarson, 2017).

2.9.2 Germination

Sprouting is a period in the life cycle of plants when they start emerging from the seed. This natural process is also known as germination. This process increases the availability of nutrients in seeds, grains and legumes. Sprouting takes a few days. During sprouting, changes take place within the seed that lead to the degradation of antinutrients such as phytate and protease inhibitors (Samtiya *et al.*, 2020). Sprouting has been shown to reduce phytate by 37-81% in various types of grains and legumes. There also seems to be a slight decrease in lectins and protease inhibitors during sprouting. Germinated cereals showed enhanced activity of phytase-degrading enzyme while in non-germinated cereals the endogenous activity of phytase enzyme was observed in diminished amounts (Vashishth *et al.*, 2017). Germination is one of the methods used in elimination of various anti-nutritional

factors present in foods. It is a natural process in which dormant but viable seeds are induced to start growing into seedlings. This is the process by which amylase degrade starches into dextrin and maltose. The enzymes convert the stored foods such as insoluble carbohydrates and proteins into soluble components. Germination resulted in greater retention of all minerals and B-complex vitamins compared to cooking treatment in chickpeas (El-Adawy, 2002).

Germination of the grain has important effects on the chemical composition, nutritive value, and acceptability characteristics of products for human consumption. It is the most effective process for the reduction of phytic acid in legumes. Reddy *et al.* (2006) noticed that phytic acid was hydrolyzed during germination resulting in an increase in available inorganic phosphorus. Sharma and Sehgal (1992) reported that 48 hours germination of two faba bean varieties (VH-131 and WF) reduced tannin content by 90 and 91%, respectively. The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase. Similar losses of phytic acid during soaking and germination have been reported by Grewal and Jood (2006). Khattab *et al.* (2009) reported that soaking caused a 42.82 –48.91% reduction in phytic acid content. Reduction of anti-nutrients like tannin and phytic acid in germinated cereals increase the bioavailability of several minerals, which led to increased nutritional value of the food products (Ogbonna *et al.*, 2012; Oghbaei and Prakash, 2016).

Significant quantities of tannic acids have been reported in the raw fenugreek seeds (718.92 mg/100g). In the present study, germination reduced tannin content by more than 77.85%. The observed reduction in tannin content after germination was a result of formation of hydrophobic association of tannins with seed proteins and enzymes. In addition, loss of tannins during germination also may be due to the leaching of tannins into the water (Shimelis. and Rakshit., 2008). The raw fenugreek seeds contained 64.22 mg/100g phytate. Comparing the germinated and non-germinated fenugreek seed, the phytate content decreased significantly ($p<0.05$) from 64.22 to 18.99 with 53.96% reduction. Because phytate is water soluble, a significant phytate reduction can be realized by discarding the soak water. In addition, decrease in phytic acid content during germination could be due to increase in phytase activity as reported for other germinated cereals including sorghum (Tizazu *et al.*, 2010).

A significant decrease in oxalate content was observed in the initial hours of germination i.e., 24 hrs. followed by a non-significant change in the later stages and the oxalate content of raw horse gram was 466 mg/100 g which decreased to 308 mg/100 g during 18 h germination and 341 mg/100 g during 12 hrs. of germination. Decrease in oxalate during germination is because of the activation of oxalate oxidase which breakdown oxalic acid into carbon dioxide and hydrogen peroxide consequently releasing calcium and same has been previously investigated by Murugkar *et al.* (2013).

2.9.3 Boiling

Food legumes are generally cooked by boiling or by using a pressure cooker prior to consumption. Previous studies also reported that boiling or cooking highly improved the nutritional value of foods by reducing their anti-nutritional (e.g., tannins and trypsin inhibitors) contents (Patterson *et al.*, 2017). High heat, especially when boiling, can degrade antinutrients like lectins, tannins and protease inhibitors. One study showed that boiling pigeon peas for 80 minutes reduced protease inhibitors by 70%, lectin by 79% and tannin by 69%. Additionally, calcium oxalate is reduced by 19-87% in boiled green leafy vegetables. Steaming and baking are not as effective. In contrast, phytate is heat-resistant and not as easily degraded with boiling. The cooking time required depends on the type of antinutrient, food plant and the cooking method. Generally, a longer cooking time results in greater reductions of antinutrients (Arnarson, 2017).

2.9.4 Roasting

Roasting can improve protein digestibility. Heat can kill or inactivate potentially harmful organisms including bacteria and viruses. Thermal treatments significantly improve the nutritional quality and digestibility of pulses by inactivating or eliminating heat-labile antinutrients (Patterson *et al.*, 2017). The goal of roasting is to improve sensory qualities and achieve inactivation of destructive enzymes which improves the storage and nutritional quality of the product (Dronachari and Yadav, 2015). Friedman reported reduced trypsin inhibitor activity when seed temperatures reached 90-100°C and also lipoxygenase activity was lost at temperatures of 75-80°C (Coulibaly *et al.*, 2011). Sade reported that during roasting total phenols and tannins decrease (Sade, 2009). Malik observed the reduction in mineral contents during roasting; he said that might be due to the loss of nutrients while heating at high temperature. It should be noted that, the drying effect of roasting reduces

the moisture content of the flour. Reduced moisture allows a larger concentration of solids by weight, resulting in an increased viscosity (Malik *et al.*, 2002).

2.9.5 Combination of several processing methods

Legumes contains different anti-nutritional factors. Eliminating the anti-nutritional factors by single processing method only gave partial detoxification and that the use of one method of processing may not affect the desired removal of the antinutritional factors. Therefore, combination of two or more methods is required. According to Effiong and Umoren (2011), soaking the seeds in water prior to cooking was more effective in improving the nutritional value of the legumes than cooking alone of legumes. They reasoned that soaking prior to cooking may have open up more surface area for heat penetration.

2.10 Malting of fenugreek seeds

Malting is the controlled germination process followed by drying and terminating the growth of the embryo, which activates the enzymes of the resting grains resulting the conversion of cereal starch into fermentable sugar and other particles, partial hydrolysis of protein and macromolecules into micro molecules (Rosentrater and Evers, 2018). Germination is done for the purpose of developing an active enzyme content which will later convert starches into sugars in the malted barley or other cereal grains, which can be easily fermented during fermentation step (Neylon *et al.*, 2020).

It was found that malting had significant effect on dry matter content, fat content, starch content, total free amino acids content, ascorbic acid content and amylase activity except protein in which it did not had significant effect. An increase in reducing sugars during malting could be due to starch hydrolysis by hydrolytic enzymes such as α-amylase (Traoré *et al.*, 2004). This increased solubility could be as a result of the increase in amount of soluble sugars which directly affect the water absorption capacity of malted flour (Gernah *et al.*, 2011). Both the smaller granular size and its higher amylase content formed during the malting process is responsible for the slightly increase in gelatinization temperature (Brenda *et al.*, 2019; Greenwood, 1959).

2.10.1 Outline of malting

Malt is produced by germinating cereal grains, usually barley for a limited period of time. It is then dried to arrest the physical germination process and accompanying biochemical processes of enzyme modification (Visser, 2011). The aim of malting is to activate and produce enzymes able to degrade endosperm cell wall components and storage proteins. This action allows starch granules to be released from the endosperm protein matrix. Malting is also important to develop the desirable color and flavor of malt (Briggs, 1998; Home *et al.*, 2001; Visser, 2011). The production of malt entails three processes: steeping, germination and kilning. The condition of each process is dependent on the malting barley cultivar, age of the grain, specification of the grain (Visser, 2011).

2.10.2 Malting Operation

The sequence of operation in malting is as follows:

1) The collection of stocks of suitable grains or legumes.
2) The storage of the cereals until it is required.
3) Steeping the grains in water.
4) Germination of the grains.
5) Drying and curing on a kiln.

2.10.2.1 Storing

If grains or cereals has been harvested by a combine harvester at moisture contents above 15%, it is usually dried either at the farm or at the malting. Too high temperature is avoided and speeding up of the drying must be based on increasing airflow as well as additional heating. A typical treatment is to dry to 12% moisture and then to store at 25°C. It is then usual to permit the temperature to fall to 15°C while carrying out cleaning and grading of corn size (Visser, 2011).

2.10.2.2 Steeping

Typically, a clean fenugreek will be steeped in a tank filled with water at 15°C. Steeping allows water to enter the kernel through or near the micropyle, after which it penetrates through the husk. The embryo hydrates quickly; water then distributes through the aleurone layer and finally reaches the endosperm through which it slowly penetrates (Visser, 2011).

This encourages the emergence of the root tip (MacGregor and Bhatty, 1993; Visser, 2011). It is the purpose of steeping to achieve a sufficient moisture content able to activate metabolism in the embryonic and aleurone tissues, thus stimulating the production of hydrolytic enzymes (Bamforth and Barclay, 1993). Grains cultivars differ in the amount of moisture required to germinate kernels. Most require a steeping regime that allows a moisture content of at least 42 - 46% to be reached which is sufficient to support growth and biochemical alteration in the grains during the malting period, without, however, allowing excessive growth (Bamforth and Barclay, 1993; Visser, 2011). CO_2 and ethanol are produced by the respiratory metabolism of the embryo & aleurone tissues, which can inhibit germination. The steeping process is interrupted periodically after 6 hrs. by draining off steep water to allow air rests, which permits the removal of these substances (Bamforth and Barclay, 1993). Additional aeration is achieved by bubbling oxygen through the steep water (French and McRuer, 1990; Visser, 2011). Any cracks in the husk, fruit and seed coat permit more rapid moistening of the endosperm material. This loss of material is one aspect of 'malting loss'; another component is the loss due to respiration by the embryo, consuming food reserves to yield energy, CO_2 and water.

2.10.2.3 Germination

Germination is traditionally carried out in darkness at relatively low temperature 12-15°C for choice but this could not be easily controlled. During germination, the kernel develops rootlets and an acrospire in the presence of sufficient water. The aim of germination is to generate a maximum amount of extractable material by encouraging endosperm modification through the development, activation and action of starch-degrading, proteolytic, cytolytic and hydrolytic enzymes (Bamforth and Barclay, 1993; Visser, 2011). These enzymes are essential for the breakdown of large molecules such as starch, proteins, and β-glucans occurring during mashing (Phiarais and Arendt, 2008). The starchy endosperm of a barley kernel consists of separate cells, bound by walls constituting mainly β-glucans and pentosans. The material inside these cells consists of starch granules embedded within a protein matrix. Embryo exposure to moisture stimulates secretion of plant hormones (i.e., gibberellins), which diffuse to the aleurone layer. Gibberellin production reaches a maximum after the first two days of germination with gibberellic acid playing a major role in controlling endosperm modification which stimulate enzyme synthesis in the aleurone layer (Bamforth and Barclay, 1993; Briggs *et al.*, 2004; Hough,

1985). Enzymes develop in the sequence of (1) cell wall degrading enzymes, (2) proteases and (3) amylases (MacLeod *et al.*, 1964). These are secreted into the starch endosperm to attack the cell walls, protein matrix and starch granules inside the cells (Wainwright, 1997). At the beginning of germination, the starch granules are covered by the protein matrix. However, within a day of the start of germination, degraded proteins leave these granules exposed (Hough, 1985). This process facilitates the degradation of starch granules at discrete points until larges holes, penetrating to the inside, are formed. Gummy polysaccharides are also degraded so that the work derived from malt has a low viscosity compared with extracts of raw barley. Simple water-soluble products of hydrolysis accumulate in the grain during malting. Gradual solubilization of the inside eventually causes the outer shell to collapse, rendering the grain friable and readily milled (Bamforth, 1999). Enzymatic breakdown of the endosperm therefore proceeds from the embryo (proximal) end of the kernel to the distal end, and from the outer to the inner regions (Bamforth, 1999; Hough, 1985). This physical weakening of the endosperm structure and the accompanied biochemical degradation are referred to as modification, a term commonly used to describe the extent of enzymatic degradation (Hough, 1985; Visser, 2011). As a result of the intensive respiration, the steeped grain must be provided with sufficient aeration to allow for cooling and the removal of CO_2 (Phiarais and Arendt, 2008).

2.10.2.4 Kilning

Kilning is the controlled drying process of green malt to reach a final moisture content of 2-5%, rendering the malt stable for storage. During kilning, the temperature and humidity of the air is strictly controlled to ensure the survival of heat liable enzymes. A gradual increase in temperature combined with a gradual decrease in malt moisture content is required to allow most enzymes (present in malt) to survive (Bamforth and Barclay, 1993; Visser, 2011). Exposure to excessive heat, when the grain is still wet, results in heat inactivation of enzymes (Bamforth and Barclay, 1993; Hough, 1985). Higher temperatures help to facilitate the development of more prominent flavor and color characteristics, mainly through non-enzymatic reactions (i.e. Maillard reaction), accompanied by other chemical reactions involving reductones (Bamforth and Barclay, 1993; Visser, 2011) and in addition to drying, kilning removes a raw flavor from the green malt. Different kilning cycles are used for different types of malt. Pale malt contains a large content of active enzymes due to lower kilning temperatures, while darker, more flavorful malt, contains

little to no active enzymes due to higher kilning temperatures (Bamforth and Barclay, 1993; Briggs *et al.*, 2004; Hough, 1985). Malt may be stored for several months or even years after processing, depending on storage conditions (ideal conditions: moisture content under 5% and temperatures between 10-15°C) (Bamforth and Barclay, 1993; Visser, 2011). Thus, formed malt is dried in a cabinet drier at 50±5ºC for 16-18 hours.

Cabinet drier consist of an insulated cabinet fitted with shallow mesh or perforated trays, each of which carries a thin layer of food. Hot air is circulated through the cabinet tray. A system of duct and baffles is used to direct air, over and/ or through each tray, to promote uniform air distribution either horizontally between the trays of food materials or vertically through the trays and food (Fellows, 2000). Air Heaters may be direct gas burners, steam coil exchangers or electrical resistance heaters LFO heat sources, or use indirect heat sources such as steam or thermal coil. The fresh air is blown past the heaters by a fan, filtered by the screen to remove dust and then air passes across and between the materials on trays or may be directed up through the perforated trays to an exhaust system and thus heated air is used for dying. The air is heated to the required temperature in this region, then distributed from side-to-side (cross-flow) or bottom-to-top (through-the-bed flow) in a circular motion, according to specific drying requirements. The motive force for the air movement typically is a plug-type fan that acts as both an induced draft and forced draft machine. The fan sucks the air through the heating chamber and blows it across the trays, which act as channels or ducts to direct the air. The fresh air inlet is located on the suction side and the exhaust is located on the pressure side. The inlet and typically exhaust will have dampers on them, enabling the percentage of recycled air to be controlled. This recycle improves the efficiency of the unit operation, conserving energy in the gas stream. It is relatively cheap to build and maintain, flexible in design, and produces variable product quality due to relatively poor control. It is used singly or in groups, mainly for small- scale production (1-20 ton/day) and is comparatively inexpensive and is commonly used to dry fruits and vegetables (Smith *et al.*, 2007).

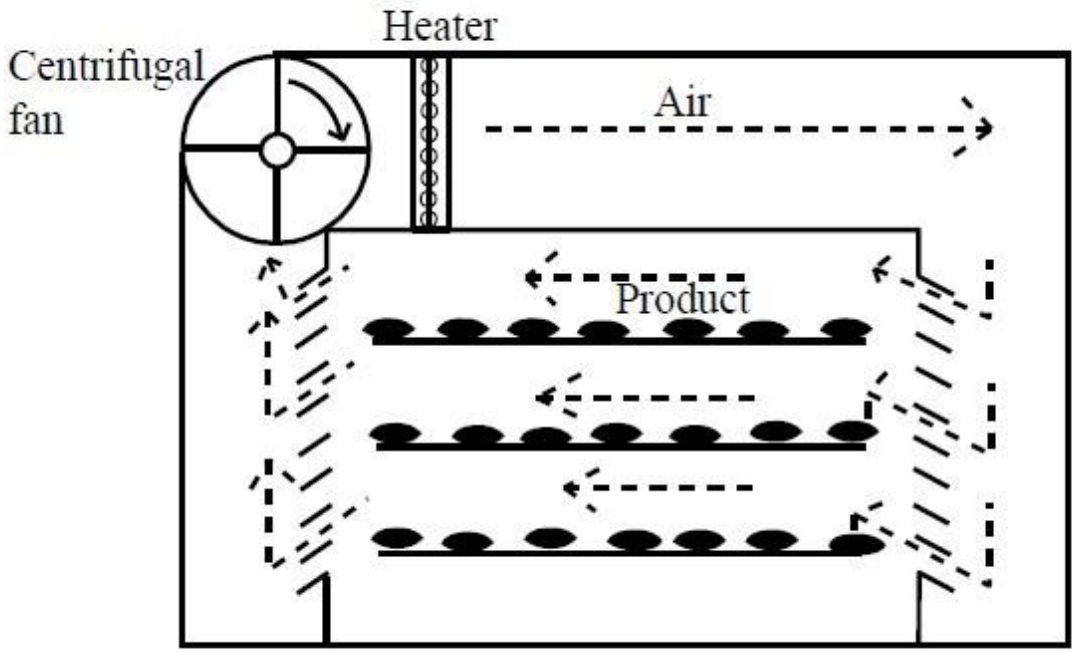


Fig 2.7 Cabinet dryer (Ghimire, 2017)

2.11 Effect of treatment on proximate composition of the fenugreek seeds

Raw fenugreek seeds contain 6.92% moisture, 4.2% during roasting, 5.2% during soaking, 5.5% during germination (Pandey and Awasthi, 2015). Mounir. *et al.* (1978) reported 9.3% moisture content in raw fenugreek seeds. Amankwah *et al.* (2009) reported that the removal of moisture generally increased concentrations of nutrients and can make some nutrients more available.

Raw fenugreek seeds contain 45-60% carbohydrates, mainly mucilaginous fiber (galactomannans), on roasting total carbohydrate decreased to 43.9%, on soaking total carbohydrate also decreased marginally 44.8%, on germination 35.7% (Pandey and Awasthi, 2015). The effects of germination on carbohydrate constituents in seeds are influenced by many factors such as the amount of oxygen and other constituents in the steep medium, the temperature, and the procedure of hydration from dry seed. The decreased carbohydrate content in the sprouted fenugreek flour might be attributed to use of the nutrient as a readily available energy source during sprouting (Atlaw and Kumar, 2018).

Protein content in seeds varied from 18.1 to 24.63% with an average mean of 20.78 per cent high in lysine and tryptophan. Similarly, 36.8% during roasting, 35.1% during soaking and significant increase in the protein 41.2% during germination, this might be due to reduction of seed nitrates into plant protein or ammonium compounds during germination (Pandey and Awasthi, 2015). The crude protein content of fenugreek seeds increased with germination time. The highest crude protein recorded with 72 h germination time. This is because germination is a biotechnological process, in which metabolic enzymes, such as proteinases, are activated. As a result of this process, some amino acids and peptides can be released, and the synthesis or utilization of others, to form new proteins, can occur. As a consequence, the nutritional quality of proteins can be enhanced, that is why the germination process is suggested as a technological procedure for improving the nutritional quality of legumes and other seeds (Atlaw and Kumar, 2018).

Similarly, raw seed contain 4.96% fat, 4.725% during roasting, 4.75% during soaking and 3.82% during germination. The decrease in fat content on roasting may be attributed to loss of volatile oils on dry heating of fenugreek seeds (Mathur and Chaudhary, 2008). Loss of fat during germination may be due to its consumption as an energy source in the process of germination (Mansour and EL-Adway, 1994). El-Aal (1986) and (Pandey and Awasthi, 2015) reported decrease in total fat content along with decrease in free fatty acids, monoglycerides and polar lipids upon germination.

Ash content of unprocessed seed is 3.95%, 3.8% during roasting, 4.31% during soaking, and 4.6% during germination. The observed decrease in ash content of fenugreek flour samples during germination might be due to leaching of minerals during steeping and washing (Ahmadzadeh and Prakash, 2006).

Fiber content of raw seed is 6.07%, 6.2% during roasting, 6.10% during soaking and 8.8% during germination. As the time of germination increased the crude fiber content slightly decreased. This decrease is accompanied by a drop in galactan content. An enzyme α-galactosidase from germinated fenugreek seeds partially attacks galactomannan to yield galactose (Shalini and Sudesh, 2004). Increase in crude fiber content upon germination, a major constituent of cell walls, might be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicelluloses during germination (Anonymous, 2012).

Iron content in raw fenugreek seed is 11.6 mg/100gm, similarly in roasted seeds 13.1 mg/100gm, 10.7mg/100gm in soaking and 11.5 mg/100gm in germination (Pandey and Awasthi, 2015). Decrease in Fe content in germinated fenugreek seed flour might be due to leaching of Fe in to soaking medium (Duhan *et al.*, 2002; El-Shimi *et al.*, 1984). Reduction of mineral content because of germination, indicated the transfer of nutrients from the seed material to the growing embryo (Atlaw and Kumar, 2018). Calcium content in raw seed is 70.5 mg/100gm, 71.2 mg/100gm in roasting, 68.2 mg/100gm in soaking and 70.7 mg/100gm in germination (Pandey and Awasthi, 2015). The increase of mineral may be related to break down of antinutritional compounds such as phytates and oxalates which bind these mineral and reduce their availability (Reddy *et al.*, 2006). Comparative lower contents of mineral when soaked in water might be due to leaching of some amount in to soaking water (Pandey and Awasthi, 2015).

2.12 Antinutrients of fenugreek seeds during treatment

Significant quantities of tannic acids have been reported in the raw fenugreek seeds (718.92 mg/100g). In the present study, 72 hours at 25°C germination reduced tannin content by more than 77.85% (Atlaw and Kumar, 2018). The observed reduction in tannin content after germination was a result of formation of hydrophobic association of tannins with seed proteins and enzymes. In addition, loss of tannins during germination also may be due to the leaching of tannins into the water, as well as washing during germination and binding of polyphenols with other organic substances such as carbohydrate or protein (Shimelis. and Rakshit., 2008). Loss of tannins during germination is attributed to the presence of polyphenol oxidase and enzymatic hydrolysis (Rao and Deosthale, 2006). During roasting, the tannin content decreased to 34% (Saini *et al.*, 2016) and during soaking there was reduction of 39.64% (Ojha *et al.*, 2018).

The raw fenugreek seeds contained 64.22 mg/100g phytate. Comparing the germinated and non-germinated fenugreek seed, the phytate content decreased significantly ($p<0.05$) from 64.22 to 18.99 with 70.43% reduction (Atlaw and Kumar, 2018; Tizazu *et al.*, 2010). Because phytate is water soluble, a significant phytate reduction can be realized by discarding the soak water. In addition, decrease in phytic acid content during germination could be due to increase in phytase activity as reported for other germinated cereals including sorghum (Tizazu *et al.*, 2010). In the case of fenugreek seed, phytic acid significantly decreased on soaking and germination, which ultimately caused significantly

increase in protein and starch digestibility (Ojha *et al.,* 2018). Germination may mobilize starch, seed proteins are metabolized and antimetabolites are catabolized, thereby resulting in improved digestibility of starch and protein. Sprouting causes mobilization of protein with the help of protease leading to the formation of peptides, oligopeptides and free amino acid (JOOD *et al.,* 1988; Kataria, 1986). Roasting of fenugreek seeds decreased phytic acid content 40.77%. Decrease in phytic acid after roasting might be due to thermolability of phytic acid. During roasting there is also breakdown of the bound between phytates and phosphorus (Pandey and Awasthi, 2015). Phytic acid content decreased significantly after 12 hour soaking up to 9.03% but not more than that of germination (Hooda and Jood, 2003).

2.13 Antioxidant and Phenol during treatments of fenugreek seeds

Antioxidant activity 18.1 % has been observed in raw fenugreek seed flour as compared to processed. The antioxidant activity of the extracts of soaked, germinated and roasted fenugreek seed flours was 60.7 %, 73.9 % and 32.0 %. Germinated fenugreek seeds had significantly ($P \leq 0.05$) higher DPPH radical-scavenging activity compared to raw seeds. Polyphenols have high free radical scavenging activity. This increase might be due to the synthesis of compounds and tocopherols which are responsible for antioxidant activity (Sharma. and Gujral., 2010). Randhir *et al.* (2004a) reported higher antioxidant activity during early germination, which correlates to high phenolic content suggesting that initially phenolics are antioxidant in nature. Cevallos. *et al.* (2010) reported that phenolic compounds are directly correlated and responsible for the antioxidant properties of the sample. Jeong *et al.* (2004) reported that antioxidant activities of sesame seed increased as the roasting temperature increased at 200°C for 60 minutes which significantly increased the total phenolic content, radical scavenging activity. Saxena *et al.* (2016) reported that the phenol content of raw fenugreek was 72.99 mg GAE/g. Similarly, Naidu *et al.* (2011) also reported 85.88 mg GAE/g phenol in raw fenugreek seeds. Manju *et al.* (2016) increased phenol from 67.32 mg GAE/g to 73.12 mg GAE/gm during roasting at 130±5°C for 7 minutes. Ojha *et al.* (2018) reported increase in phenolic content during 12 hours soaking of fenugreek seeds. These differences in the total contents of phenolic of the extracts obtained from fenugreek seeds are dependent on the polarity of the solvents used, extraction method and extraction time and difference in cultivar, geographical region, season and weather may contribute to the variation of phenolic compounds profile (Lim and Murtijaya, 2007).

Part III

Materials and Methods

3.1 Materials

All chemicals used were reagent grade unless specified otherwise and distilled water was used throughout the work.

3.1.1 Collection of raw materials

Fenugreek seeds (*Trigonella foenum graecum L.*) was collected from the local market of Biratnagar located at geographic coordinate latitude 26º27′18.2″ North and longitude 87º16′12.3″ East in Morang district.

3.1.2 Equipment and chemicals

The following equipment and chemicals used were available in Central Campus of Technology (CCT). The list of chemicals and equipment used for the analysis is shown in Appendix A respectively.

3.2 Methodology

Fenugreek seeds (*Trigonella foenum graecum L.)* was processed as per the research and then converted to powder form. There were Roasting, Soaking and Germination method applied in this work to analyze the nutritional composition such as moisture, ash, fat, protein, fiber and antinutrients like tannin, phytate and phytochemical components like antioxidant, phenol in raw and treated sample. The general outline for processing of fenugreek seeds (soaking, roasting, and germination) is presented in Fig. 3.1

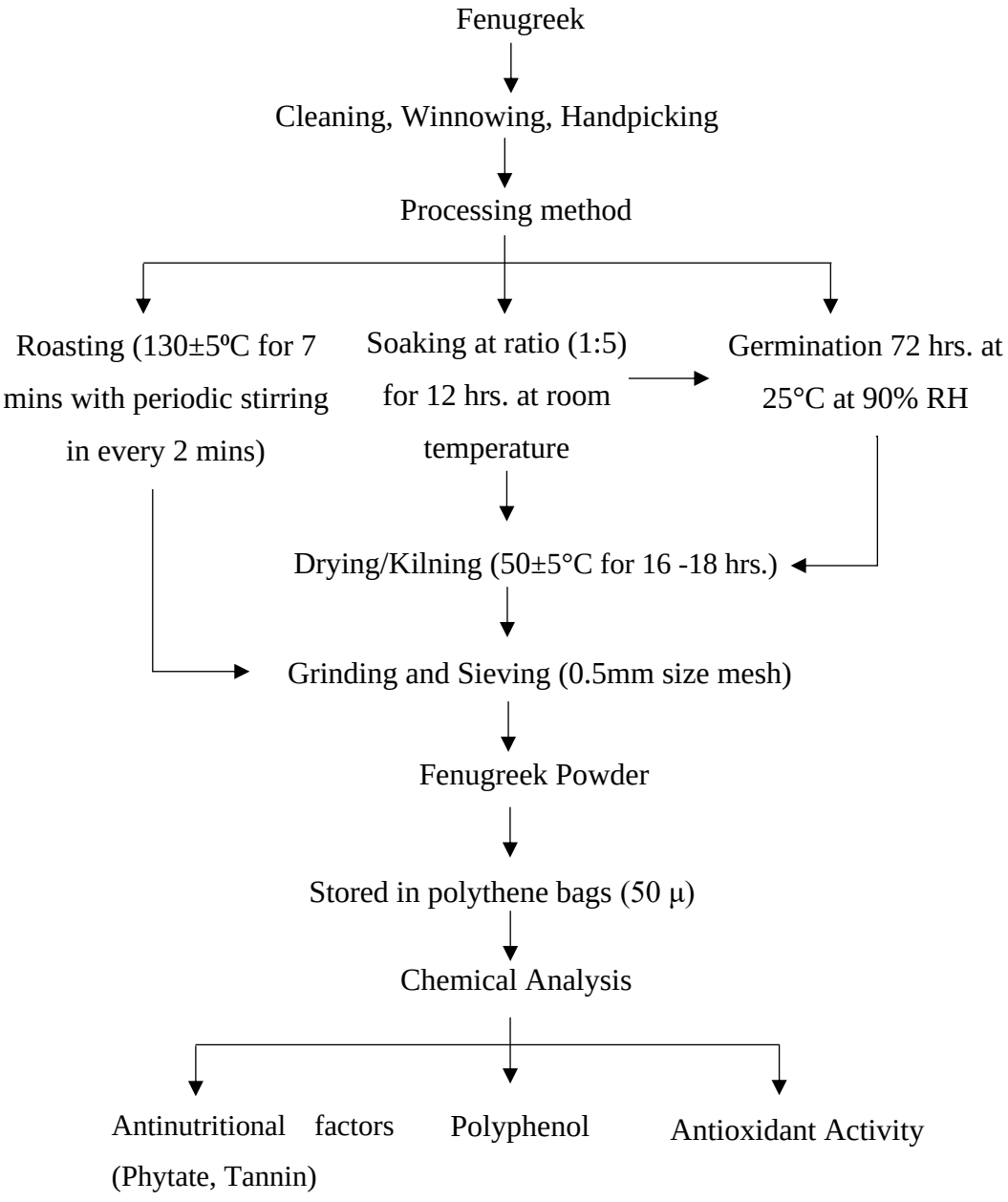


Fig 3.1 General flow sheet for processing of fenugreek seeds powder

3.2.1 Processing method

3.2.1.1 Cleaning

The fenugreek sample was first cleaned screening to remove impurities such as stones, strings, weed seeds, or similar size in seeds like flax, canary seed, or small wheat etc. and then by winnowing with nanglo (flat round woven bamboo tray) to remove dusts, husk, immature grains and other light particles. Then shifting, hand picking and finally washing with water was done to remove adhering dust and finer impurities.

3.2.1.2 Treatment

3.2.1.2.1 Soaking

Fenugreek seeds were soaked in distilled water for 12 h at room temperature. A seed to water ratio of 1:5 (W: V) was used (Ojha *et al.*, 2018). Light materials present in the sample were skimmed off. Agitation was done to clean the seed. The soaked seeds were rinsed twice in distill water. Then it was dipped in KMS solution for 10 minutes to prevent the mold growth (Jood *et al.*, 1987).

3.2.1.2.2 Germination

The soaked seeds were germinated in sterile petri-plates lined with wet whatman no. 1 filter paper for 72 hours at 25°C at 90% RH. The drying out of seeds was prevented by moistening muslin cloth and spraying the potable water and the seeds were rinsed in distilled water (Ojha *et al.*, 2018).

3.2.1.2.3 Drying for soaked and germinated sample

Drying for soaked sample was carried out in a cabinet drier at 50±5°C until the constant weight was obtained. Samples were grinded in grinder and sieved using 0.5 mm size mesh and stored in plastic containers at room temperature until required for further analysis (Jood *et al.*, 1987; Ojha *et al.*, 2018). Similarly, drying for germinated seeds was carried out by drying in a cabinet drier at 55±5°C till moisture content goes below 14%. Samples were grinded in grinder and sieved using 0.5 mm size mesh and stored in plastic containers at room temperature until required for further analysis (Ojha *et al.*, 2018).

3.2.1.2.4 Roasting

Fenugreek seeds (50 g) were roasted in an open pan at 130±5°C for 7 minutes. It was continuously stirred with ladle for proper and uniform roasting until it became slight brown and left a peculiar aroma. Raw and processed (soaked, germinated and roasted) fenugreek seeds were ground in grinder and passed through standard test sieve to get uniform sized flour. Flours were collected and stored in air tight food grade containers separately for further use at ambient temperature (Pandey and Awasthi, 2015).

3.2.1.3 Preparation of methanolic extract of the samples

One gram of each sample of fenugreek seed was ground with 30 ml of methanol (80%) in mortar and pestle for homogenization. After recovery of the homogenate, 15 ml methanol (80%) was used to wash the mortar and pestle and then pooled with the first homogenate. The mixture was refrigerated for half an hour and allowed to centrifuge at 4,500 rpm for 15 min at room temperature (27°C), the clear supernatant solution was filtrated by Whatman filter paper No. 42 (125mm). Collected the extract in 50ml volumetric flask. And, last volume was made up 50 ml with 80% methanol. Finally, extracts were transferred to brown colored glass bottles, sealed by using caps and was stored in refrigerator maintained at temperature <7˚C (Karakaya, 2004).

3.3 Chemical analysis

3.3.1 Proximate analysis

3.3.1.1 Determination of moisture content

The moisture content was determined by using hot air oven method. 5g of sample was weighted and heated in an insulated oven at 110°C to constant weight. The difference in weight was the water that has evaporated as Rangana (2001). The results were expressed in terms of percentage.

3.3.1.2 Determination of crude protein

Crude protein was determined by the Kjeldahl method, total protein was calculated by multiplying the nitrogen content by a factor of 6.25 (Rangana, 2001). The calculated data were presented per 100 g on dry basis.

$$\text{Nitrogen \%} = \frac{(\text{Sample titre} - \text{Blank Titre})\text{xNormality of HCl x}14100}{\text{Wt of sample} \times 100}$$

3.3.1.3 Determination of ash content

The ash content was determined by incinerating the fenugreek seeds (5 g) in a muffle furnace at 525ºC for 4-6 hours (Rangana, 2001). The calculated data were presented as g/100 g on dry basis.

3.3.1.4 Determination of crude fat

The fat content of the samples was determined as described in Rangana (2001). The calculated data were presented as gram per 100 g on dry basis.

$$\text{\% Crude fat} = \frac{\text{Wt of ether soluble material} \times 100}{\text{Wt of sample}}$$

3.3.1.5 Determination of crude fiber

Crude fiber was determined by using chemical process, the sample was treated with boiling dilute Sulphuric acid, boiling sodium hydroxide and then with alcohol as standard method of Rangana (2001). The calculated data were presented as g/100 g on dry basis.

$$\text{\% Crude fiber} = \frac{(\text{ Loss in weight noted})}{\text{Wt of sample}} \times 100$$

3.3.1.6 Determination of carbohydrate

Total carbohydrate content of the samples was determined by difference method.

Carbohydrate (%) = 100 – [sum of moisture, protein, total ash, fiber and fat].

3.3.1.7 Determination of energy value

One of the methods specified by FDA was employed. This uses the general factors of 4, 4 and 9 calories per gram of protein, total carbohydrate, and total fat, respectively, to calculate the calorie content of food (Bassey *et al.*, 2013).

Energy value per 100g = [carbohydrate $*$ 4 + protein $*$ 4 + Fat $*$ 9] Kcal

3.3.2 Ultimate analysis

3.3.2.1 Determination of iron

Iron in the sample was determined by converting all the iron into ferric form using oxidizing agents like potassium per sulphate or hydrogen per oxide and treating thereafter with potassium thiocyanate to form a red ferric thiocyanate which was measured calorimetrically at 480 nm (Rangana, 2001).

$$\text{Iron } \left(\frac{\text{mg}}{100}\right) = \frac{\text{Abs. of sample} \times 0.1 \times \text{Total vol. of ash solution} \times 100}{\text{Abs. of standard} \times 5 \times \text{Wt of sample taken for ashing}}$$

3.3.2.2 Determination of calcium

Calcium was precipitated as calcium oxalate. The precipitate was dissolved in hot dilute Sulphuric acid and titrated with standard potassium permanganate (Rangana, 2001).

$$\text{Calcium } \left(\frac{\text{mg}}{100}\right) = \frac{\text{Titre} \times 0.2 \times \text{Total volume of ash solution } \times 100}{\text{Vol. taken for estimation xWt of sample taken for ashing}}$$

3.3.3 Quantitative determination of Phytochemical

3.3.3.1 Determination of total Phenol

Determination of total phenol in fenugreek was carried out with Folin-Ciocalteau reagent as mentioned by Sadasivam and Manickam (1996) with slight modification. Phenols react with Phosphomoybdic acid in Folin-Ciocalteau reagent in alkaline medium producing a blue colored complex (molybdenum blue). 0.5 ml of the extract and 1ml of Folin- Ciocalteu reagent was mixed and incubated at room temperature for 15 minutes. Then 2.5 ml of saturated sodium carbonate was added and further incubated for 30 minutes at room temperature and absorbance measured at 760 nm. Also, the standard curve was prepared

using 0-1000 µg/ml solutions of Gallic acid in methanol by half dilution. Total phenol values were calculated using the standard curve equation and expressed in terms of Gallic Acid equivalent (mg/ml) of dry mass (Jaradat *et al.,* 2015).

3.3.4 Quantitative analysis of Anti nutritional factors

3.3.4.1 Determination of Phytate

Young and Greaves (1940) method was used for the determination of Phytic acid content. 0.2 g of the sample was weighed into 250 ml conical flask. It was soaked in 100 ml of 20% concentrated HCL for 3 hours, the sample was then filtered 50 ml of the filtrate was placed in a 250 ml beaker and 100 ml beaker and 100 ml distilled water added to the sample. Then 10 ml of 0.3% ammonium thiocyanate solution was added to the sample. Then 10 ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution that contained 0.00195 g iron per ml.

Percentage of phytic acid is calculated by formula:

$$\% \text{ Phytic acid } = \frac{\text{Titer value x } 0.00195 \text{ x } 100}{2}$$

3.3.4.2 Determination of tannin

The tannins were determined by Folin-Dennis method. 0.5 g powdered sample was transferred to 250ml conical flask and 75ml water was added and boiled for 30 min. It was centrifuged and 1ml of the sample extract was transferred to 75 ml water and 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate was added. The mixture was shaken well and kept at room temperature for 30min. a set of reference standard solution of tannic acid was prepared in same manner. Colorimetric estimation of tannins is based on the measurement of the blue color formed by the reduction of phosphor-tungsto-molybdic acid by tannin-like compounds in alkaline condition. The intensity was measured in a spectrophotometer at 700nm (Sadasivam and Manickam, 2016).

3.3.5 Determination of DPPH radical scavenging activity

3.3.5.1 Total Antioxidant Activity Using 1,1-Diphenyl-2Picryl Hydrazyl (DPPH) Method

Antioxidant activity was measured utilizing 2, 2-diphenyl-1-1 picrylhydrazyl (DPPH) radical scavenging capacity. Free Radical Scavenging Activity Using 1, 1-Diphenyl-2Picryl Hydrazyl (DPPH). DPPH, a commercial oxidizing radical is reduced by antioxidants. The disappearance of the DPPH radical absorption at a characteristic wavelength is monitored by decrease in optical density (Singh *et al.*, 2002). Different concentrations of the methanolic extract were taken in different test tubes and the volume made to 1 ml with methanol. 4 ml of 0.1 mM methanolic solution of DPPH was added. The tubes were shaken vigorously and allowed to stand for 20 min at room temperature. A control was prepared as above without any sample and methanol was used for base line correction. Changes in absorbance of samples were measured at 517 nm. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

$$\%\ \text{Free radical Scavenging Capacity} = \frac{\text{Control}_{\text{OD}} - \text{Sample}_{\text{OD}} * 100}{\text{Control}_{\text{OD}}}$$

3.4 Statistical analysis

For all chemical analysis, triplicates of the sample were used for determination of each constituent. Mean values with standard deviation were computed and were graphically represented using Microsoft excel-2016. Data on processing different techniques were subjected to analysis of variance (ANOVA) and considered at 95% confidence level using statistical software GenStat (Twelfth Edition developed by VSN International Limited). Means of the data were compared by using Fisher's Unprotected LSD method at 5% level of significance.

Part IV

Result and discussion

A common variety of fenugreek (*Trigonella foenum-graecum L.)* was collected from the local market of Biratnagar to study the impact of processing on nutritional, antinutritional and phytochemical composition. The different processing techniques were carried out soaking for 12 h at room temperature, germination for 72 hours at 25°C and roasting at 130±5°C for 7 minutes. Then, obtained processed samples were analyzed to study the effect of different processing techniques on its nutritional composition, antinutrients (phytate, oxalate, tannin) and phytochemicals (phenol, antioxidant). The findings are described in the sections to follow.

4.1 Analysis of raw fenugreek seeds

4.1.1 Measurement of Physical parameters of raw fenugreek seeds

Fenugreek was analyzed for the physical properties which are presented in table 4.1. Physically, the length, width, and height are 0.41 cm, 0.24 cm and 1.7 mm respectively measured by screw gauge according to Digvir. and Stefan. (2006). The procedure as given by the Balasubramanium (1985) followed by Narvani. and Panwar. (1993), was used to measure the bulk density of the fenugreek seed. For the fenugreek seed, a measuring cylinder of known volume (recorded in cubic meter) was filled with the fenugreek seeds, then the weight of same material was measured and the bulk density was calculated. The bulk density of fenugreek seeds ranged from 682.56 to 691.62 kg/m^3. The average bulk density of fenugreek seeds was found to be 687.62 kg/m^3. Similar results have been reported by Altuntas *et al.* (2005). Mabrouki *et al.* (2015) also reported 690 kg/m^3 bulk density of raw fenugreek seeds.

Table 4.1: Physical properties of fenugreek seeds

Physical property	Mean
Length	4.1±0.1mm
Width	2.4±0.1 mm
Height	1.7±0.1 mm
Bulk density	687.62±4.62 kg/m^3

4.1.2 Proximate composition of raw fenugreek seeds

Moisture content of the raw fenugreek seed sample was found to be 9.39%. This result was closer to the journal by Mounir. *et al.* (1978) (9.3%) on dry basis. Similarly, Sulieman *et al.* (2008) also reported (9%) moisture content in raw fenugreek seeds. Other similar result in fenugreek reported by Atlaw and Kumar (2018) was (7.59%). Also, the same type of result reported by Dwivedi *et al.* (2019) was (7.96%) in raw fenugreek seeds. Rasheed *et al.* (2015) conducted study on nutritional properties of fenugreek seeds and found that seeds contained (11.21%) moisture on dry basis whereas, Buba *et al.* (2015) reported average moisture value of (10.91%). Similarly Hooda and Jood (2003) reported that raw fenugreek seeds contain (13.70%) moisture. Moisture of fresh processed foods gives an indication of its freshness and shelf life and thus high moisture content increase microbial spoilage, deterioration and short shelf life (Tressier *et al.,* 1980). Amankwah *et al.* (2009) reported that the removal of moisture generally increased concentrations of nutrients and can make some nutrients more available. Low moisture content of flours prevents microbial activity and extends the shelf life of the flour.

Protein content in the raw fenugreek sample was found to be 22.05% (db). This value is very closer to the value reported by (Rasheed *et al.,* 2015) (23.30%) on dry basis in raw fenugreek seed. The above result also agrees with (Dwivedi *et al.,* 2019) (23.13%) as dry basis. Hooda and Jood (2003) reported that the protein content of raw fenugreek seed was (25.90% db). Kavitha *et al.* (2015) also reported that the protein content in raw fenugreek seeds was 25.5% (db).

In the raw fenugreek sample, the fat content was found to be 4.33% as dry basis. This was very closer to the result reported by Rahmani *et al.* (2014) (4.32%) as dry basis. Other similar result was reported by (Mabrouki *et al.*, 2015) (5.36%) db. Similarly, Kavitha *et al.* (2015) reported that the fat content of raw fenugreek was (6.7%) db. Other similar results was reported by Kochhar *et al.* (2006) (6.53%) db. Hooda and Jood (2003) reported that the fat content of raw fenugreek seed was (6.90%) db.

In raw fenugreek sample, the crude fiber content was found to be 8.51% as dry basis. This value is closer to the value reported by Sulieman *et al.* (2008) (8%). In contrast, the value found in the present study was nearly similar to that reported by Kavitha *et al.* (2015) who reported a value of 7.2% crude fiber as dry basis. Rahmani *et al.* (2014) also reported that the crude fiber content of raw fenugreek sample was (9.27%) as dry basis.

In raw fenugreek sample, the ash content was found to be 3.89% as dry basis. This value was very close to the value reported by Mabrouki *et al.* (2015) (3.96%) db. Similarly, Kochhar *et al.* (2006) reported that the ash content of raw fenugreek seed was found to be (3.26%) db. Mahmooda and Yahya (2017) also reported that the ash content of raw fenugreek seeds was found to be (3.566%) db. Similarly, Kavitha *et al.* (2015) reported that that the ash content of raw fenugreek seeds was (3.6%) as dry basis. Rasheed *et al.* (2015) also reported that the ash content of raw fenugreek seeds was 3% as dry basis.

In raw fenugreek sample or control, the total carbohydrate and the energy value was found to be 51.81% as dry basis. This value was very close to the value reported by Mahmoud et al. (2012) 50.06% as dry basis. The similar value was reported by Kavitha et al. (2015) was 52.5% db carbohydrate. Rasheed et al. (2015) also reported that the carbohydrate content of raw fenugreek was 55.49% db. Kochhar et al. (2006) also reported the carbohydrate content to be 58.13% as dry basis. Result for proximate composition of the raw, roasted soaked and germinated seeds are presented below in Table 4.2.

4.2 Impact of different processing method on proximate composition of fenugreek seeds

The proximate analysis gives information about the nutritional and biochemical composition of foods. The proximate composition such as moisture, crude fat, crude protein, crude fiber and total ash in different treatment fenugreek samples were determined as per the standard procedure and results expressed in dry basis percentage. Result for proximate composition of the raw, roasted soaked and germinated seeds are presented in Table below.

Table 4.2: Proximate composition of Raw / Roasted / Soaked / Germinated Fenugreek seeds.

Sample	Moisture (%)	Protein (% db)	Fat (% db)	Crude fiber (%db)	Ash (%db)	Carb (%db)
Raw	9.39^{a} ± 0.01	22.05^{a} ±0.82	4.33^{a} ± 0.26	8.51^{ab} ± 0.46	3.89^{a}± 0.01	51.81^{a} ± 0.39
Roasted	5.56^{b} ± 0.67	23.07^{a} ±0.66	4.15^{a} ± 0.15	8.31^{a} ± 0.32	3.94^{a} ± 0.19	54.95^{b} ± 0.37
Soaked	9.64^{a} ± 0.15	24.95^{b} ±0.31	4.19^{a} ± 0.10	8.50^{ab} ± 0.27	4.12^{a} ± 0.13	48.58^{c} ± 0.52
Germinated	8.15^{c} ± 0.18	31.47^{c} ±0.72	2.90^{b} ± 0.10	9.34^{b} ± 0.76	4.58^{a} ± 0.85	43.54^{d} ± 0.89

*Values presented are means of triplicate determination ± SD in dry basis

*Means having similar super script in a column are not significantly different by LSD at 5% significance

Table 4.3: Percentage loss and gain of nutrients

Sample	Moisture (%)	Protein (%)	Fat (%)	Crude fiber (%)	Ash (%)	Carb (%)
Roasted	40.74	4.62*	4.17	2.31	1.19*	6.06*
Soaked	2.72*	13.15*	3.31	0.12	5.67*	6.22
Germinated	13.17	42.71*	33.04	9.79*	17.52*	15.96

Note: *Indicate that gain of moisture, protein, crude fiber, ash and carbohydrate

4.2.1 Moisture

The moisture content of the different treatment on the fenugreek sample are shown in table 4.2. Statistical analysis shows that the moisture content in fenugreek treatment sample like roasted, soaked, germinated samples were found to be significantly different ($p<0.05$). However, LSD indicates the moisture content in the fresh raw sample and soaked sample was not significantly different ($p>0.05$). The moisture content of both raw and soaked fenugreek was higher than the roasted and germinated sample.

The raw fenugreek seeds were roasted in an open pan at 130 ± 5º C for 7 min, the moisture content was found to be 5.56%. The similar type of result purposed by Dwivedi *et al.* (2019) was 5.96% in fenugreek seeds by roasting at 130 ±5º C for 7 min. Roasting of fenugreek seed causes decrease in moisture content 4.25% reported by (Saini *et al.*, 2016). Pandey and Awasthi (2015) reported the moisture content of roasted fenugreek seeds was 4.2%. Among the treatments minimum moisture content was found in seeds which was roasted. The process of roasting also resulted in decrease in moisture content that gave rise to increase in ash content reported by (Folasade. *et al.*, 2014). Statistical analysis showed that the moisture content in roasted sample was significantly decreased ($p<0.05$) from the raw sample. The percentage loss of moisture in roasted as compared to raw was found to be 40.74% respectively.

In soaked fenugreek sample for 12 hours, the moisture content was found to be 9.64%. The result was quite closer to the raw fenugreek sample. An increase in moisture content (8.81%) from (6.92%) was observed in fenugreek seeds due to hydration of seeds during soaking by Saini *et al.* (2016). But this value was lower than the value reported by Hooda and Jood (2003) 13.96% as in soaked fenugreek sample. Statistical analysis showed that

there was no significant difference ($p>0.05$) of moisture in between the raw and soaked sample. The percentage gain of moisture was 2.72% in soaked sample as compared to raw sample respectively.

The moisture content of germinated fenugreek seeds for 72 hours was found to be 8.15%. In similar study carried by Kavitha *et al.* (2015) the moisture content of 72 hours germinated fenugreek sample was found to be 7.5%. Atlaw and Kumar (2018) reported the moisture content of germinated fenugreek seeds was 7.71%. Statistical analysis showed that the moisture content of germinated fenugreek sample was significantly decreased ($p<0.05$) from the raw sample. The percentage loss of moisture in germinated sample as compared to raw was 31.17% respectively.

4.2.2 Protein

The protein content of the different treatment on fenugreek sample is shown in table 4.2. Statistical analysis shows that the protein content in the fenugreek treated sample like roasted, soaked and germinated sample were found to be significantly different ($p<0.05$). However, LSD indicates the protein content in raw and roasted sample was not significantly different ($p>0.05$). The protein content of both soaked and germinated fenugreek was higher than the raw and roasted sample.

The protein content of roasted fenugreek was found to be 23.07% on dry basis. Dwivedi et al. (2019) reported that the protein content of roasted fenugreek seed (27.48% db). This value was very closer to the result reported by Khan and Saini (2016) (22.83) in flaxseeds at 180º C for 10 mins roasting. Similarly, Tenyang *et al.* (2016) reported that the protein content of roasted white sesame seeds at 180º C for 10 minutes was 22.97%. LSD indicates that there was no significant difference ($p>0.05$) found in both raw and roasted sample. The percentage gain of protein in roasted sample as compared to raw was found to be 4.62% respectively.

In soaked fenugreek sample, the protein content was found to be 24.95% on dry basis. Hooda and Jood (2003), reported the amount of protein 26% as the dry basis is similar to the above result. While soaking, biological breakdown of various complex compounds into simpler compounds takes place as suggested by Narsih *et al.* (2012) and thus a significant increase in total protein content was observed with enhancement of the soaking from

22.05% to 24.95%. Statistical analysis shows that there was significant difference ($p<0.05$) of protein in between raw and soaked fenugreek sample. The percentage gain of protein in soaked sample as compared to raw was 13.15% respectively.

In germinated sample, the protein content was found to be 31.47% on dry basis. Mahmoud *et al.* (2012) reported that germinated fenugreek seed contain (32.04%) protein as dry basis which agrees with above result. This value was closer to the result reported by (Atlaw and Kumar, 2018) (29.89%) db in germinated fenugreek sample for 72 hours. But this value is lower than the value reported by (Kavitha *et al.,* 2015) (37.4%) db where crude protein is increased by 40% according to this journal. Statistical analysis shows that there was significant difference ($p<0.05$) between the raw and germinated fenugreek sample. The percentage gain of protein in germinated sample as compared to raw was 42.71% respectively. Among the treatment, there was significant increase in the protein during germination, this might be due to reduction of seed nitrates into plant protein or ammonium compounds during germination (Pandey and Awasthi, 2015). The highest crude protein recorded with 72 h germination time. This is because germination is a biotechnological process, in which metabolic enzymes, such as proteinases, are activated. Improvement in protein digestibility during germination may be attributed to modification and degradation of storage proteins of the grain. Sprouting causes mobilization of proteins with the help of activated proteases, leading to the formation of polypeptides, oligopeptides and amino acids. Furthermore, hydrolytic reduction of phytates during germination may also partly account for the improved protein digestibility of millet sprouts because phytates are known to inhibit proteases reported by (Kumar. and Chauhan., 1993). As a result of this process, some amino acids and peptides can be released, and the synthesis or utilization of others, to form new proteins, can occur. As a consequence, the nutritional quality of proteins can be enhanced, that is why the germination process is suggested as a technological procedure for improving the nutritional quality of legumes and other seeds (Atlaw and Kumar, 2018).

4.2.3 Fat

The fat content of different treatment in fenugreek sample are shown in table 4.2. Statistical analysis shows that there was no significance difference ($p>0.05$) found in raw, roasted, soaked fenugreek sample.

In roasted fenugreek sample, the fat content was found to be 4.15% as dry basis. But this value is lower than the value reported by (Dwivedi *et al.*, 2019) (9.01%) db where crude fat is increased by 2.38% according to this journal. Statistical analysis showed that there was no significant difference ($p>0.05$) of fat content in between the raw and roasted sample. The percentage loss of fat in roasted sample as compared to raw was 4.17% respectively. Roasting caused decrease in fat content which may be due to reduction in actual weight with the shrinkage. This may be due to loss of volatile oil on open dry heat treatment (Mathur. and Chaudhary., 2009).

In soaked fenugreek sample, the fat content was found to be 4.19% as dry basis. This value was closer to the result reported by Pandey and Awasthi (2015) (4.6%) as dry basis. (Hooda and Jood, 2003) reported that the fat content in soaked fenugreek seeds was found to be (6.45%) as dry basis. Statistical analysis showed that there was no significant difference ($p>0.05$) of fat content in between the raw and soaked sample. The percentage loss of fat in soaked sample as compared to raw was 3.31% respectively.

In germinated fenugreek sample, the fat content was found to be 2.90%. This value was very close to the result reported by Kavitha *et al.* (2015) (2.3%) as dry basis. Statistical analysis showed that there was significant difference ($p<0.05$) of fat content in between the raw and germinated sample. The percentage loss of fat in germinated sample as compared to raw was 33.04% respectively. Loss of fat during germination may be due to its consumption as an energy source in the process of germination by Mansour and EL-Adway (1994). The observed decrease in the fat content of the germinated seeds might be due to the increased activities of the lipolytic enzymes during germination Mahmoud *et al.* (2012).

4.2.4 Crude fiber

The crude fiber content of different treatment in fenugreek sample are shown in table 4.2. Statistical analysis shows that the significant difference ($p < 0.05$) was found in roasted and germinated sample. And there was not significant change ($p>0.05$) found in raw, roasted and soaking of fenugreek sample treatment.

The total crude fiber content of roasted fenugreek sample was found to be 8.31% as dry basis. But this value is lower than the value reported by Dwivedi *et al.* (2019) (13.71%) in roasted fenugreek seeds. In contrast, the value found in the present study was nearly similar to that reported by Tenyang *et al.* (2016) that the crude fiber content of roasted brown sesame seeds at 180º C for 10 mins was (9.04%). Similarly, Khan and Saini (2016) reported that the crude fiber content of roasted flaxseed was (9.63%). Statistical analysis showed that the crude fiber content of roasted sample was not significantly different ($p>0.05$) found with raw and soaked sample treatment. The percentage loss of crude fiber in roasted sample as compared to raw was found to be 2.31% respectively.

In soaked fenugreek sample, the crude fiber was found to be 8.50% as dry basis. This value was very closer to the value reported by Saini *et al.* (2016) (7.22%). Similarly, Hooda and Jood (2003) reported that the crude fiber in soaked fenugreek seeds was 6.95% as dry basis. Statistical analysis shows that there was no significant difference ($p > 0.05$) of crude fiber in between raw and soaked fenugreek sample. The percentage loss of crude fiber in soaked sample as compared to raw was 0.12% respectively. Significant decrease in total dietary fiber was observed during soaking. This decrease might be attributed to enzymatic degradation of seeds during soaking (Mathur. and Chaudhary., 2009).

In germinated fenugreek sample, the crude fiber was found to be 9.34%. This value was closer to the value reported by Kavitha *et al.* (2015) (10.2%) as dry basis in germinated fenugreek seeds. Similarly, Atlaw and Kumar (2018) reported that the crude fiber content of the germinated fenugreek seeds was (11.34%) db. LSD indicates that there was significant difference ($p<0.05$) found in between germinated and roasted sample. The percentage gain of crude fiber in germinated sample as compared to raw was 9.79% respectively. Increase in crude fiber content upon germination, a major constituent of cell walls, might be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicelluloses during germination (Anonymous, 2012).

4.2.5 Ash

The ash content of different treatment in fenugreek sample are shown in table 4.2. Statistical analysis shows that the ash content in the fenugreek treated sample like roasted, soaked and germinated sample were not found to be significantly different ($p>0.05$) at 5% level of significance.

In roasted sample, the ash content of fenugreek sample was found to be 3.94% as dry basis. This value was close to the value reported by Pandey and Awasthi (2015) (3.8%). Dwivedi *et al.* (2019) reported that the ash content of roasted fenugreek seeds was found to be 5.57%. similarly, Tenyang *et al.* (2016) reported that the ash content of roasted white sesame seeds at 180º C for 10 mins was 3.55%. LSD indicates that there was no significant difference ($p>0.05$) found in both raw and roasted sample. The percentage gain of ash in roasted sample as compared to raw was found to be 1.19% respectively.

In soaked sample, the ash content of fenugreek sample was found to be 4.12% as dry basis. Saini *et al.* (2016) reported that the ash content in soaked fenugreek seed was 4.17% which was similar to the above result. Pandey and Awasthi (2015) also reported the ash content 4% in soaked fenugreek seed. Hooda and Jood (2003) reported the ash content in soaked fenugreek seeds was 2.92% db which was lower than the present value. Statistical analysis showed that there was no significant difference ($p>0.05$) of ash content in between the raw and soaked sample. The percentage gain of ash in soaked sample as compared to raw was 5.67% respectively.

Similarly, in germinated fenugreek sample, the ash content was found to be 4.58% as dry basis. This value was closer to the value reported by Mahmoud *et al.* (2012) (4.48%) as dry basis in germinated fenugreek seed. Kavitha *et al.* (2015) also reported (6.5%) ash content as dry basis. Similarly, Atlaw and Kumar (2018) reported that ash content of 72 hours germinated seeds was 2.94% as dry basis. Statistical analysis shows that there was no significant difference ($p > 0.05$) between the raw and germinated fenugreek sample. The percentage gain of ash in germinated sample as compared to raw was 17.52% respectively.

4.2.6 Carbohydrate

The total carbohydrate was calculated by subtracting the nutrient (protein, fat, crude fiber and ash) out of total. The total carbohydrate value was shown in table 4.2. Statistical analysis shows that the Carbohydrate content in the fenugreek treatment sample like raw, roasted, soaked and germinated sample were found to be significantly different ($p<0.05$).

In roasted fenugreek sample, the total carbohydrate was found to be 54.95% as dry basis. Statistical analysis shows that there was significance change ($p<0.05$) between raw and roasted treatments. The percentage retain of carbohydrate as compare to fresh was found to be 6.06% respectively.

Carbohydrate content of the soaked fenugreek sample was found to be 48.58% as dry basis. During soaking, leaching of other nutrients takes place leading to modification in structural components of the legume and thereby increasing the availability of starch, as reported by Marconi *et al.* (2000). Another reason for the reduction in carbohydrate content can be due to the use of carbohydrate as a source of energy for embryonic growth (Vidal-Valverde *et al.*, 2002). Statistical analysis shows that there was significant difference ($p<0.05$) between the raw and soaked treatments. The percentage loss of carbohydrate as compared to raw was found to be 6.22%.

Carbohydrate content of the germinated fenugreek sample was found to be 43.54% as dry basis. Similar value was reported by Mahmoud *et al.* (2012) (41.31%) as dry basis in germinated fenugreek seeds. Statistical analysis shows that there was significant difference ($p<0.05$) between the raw and germinated sample. The percentage loss of carbohydrate as compared to raw was found to be 15.96% respectively. The effects of germination on carbohydrate constituents in seeds are influenced by many factors such as the amount of oxygen and other constituents in the steep medium, the temperature, and the procedure of hydration from dry seed. These factors profoundly influence respiration, and breakdown and synthesis of seed carbohydrates. The decreased carbohydrate levels of the germinated seeds might be due to increase in amylase activity, which breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination (Inyang and Zakari, 2008).

4.3 Effect of processing methods on Mineral Composition

The iron and calcium content of different treatment in fenugreek sample are shown in table 4.4. Statistical analysis shows that there was no significant difference ($p>0.05$) of iron content in between the raw, soaked and germinated fenugreek sample. Whereas statistical analysis shows that there was no significant difference ($p>0.05$) of calcium content in between the raw and roasted fenugreek sample.

Table 4.4: Mineral composition of fenugreek seed

Sample	Iron mg/100gm	Calcium mg/100gm
Raw	$11.45^{a} \pm 0.70$	$78.29^{a} \pm 1.01$
Roasted	$12.79^{b} \pm 0.60$	$79.07^{a} \pm 0.97$
Soaked	$10.85^{a} \pm 0.37$	$74.97^{b} \pm 1.02$
Germinated	$11.18^{a} \pm 0.98$	$72.01^{c} \pm 1.00$

Values are the mean ± SD of three determinations.
All values are expressed on dry basis.

Table 4.5: Percentage loss and gain of minerals

Sample	Iron%	Calcium%
Roasted	11.75*	0.99*
Soaked	5.20	4.25
Germinated	2.32	8.03

Note: *Indicate the gain of iron and calcium

4.3.1 Iron

The iron content of raw fenugreek seeds was found to be 11.45 mg/100gm. This value was very close to the value reported by Abbas Ali *et al.* (2012) (11.6 mg/100gm) as dry basis. Similar value was reported by El-Shimi *et al.* (1984) (10mg/100gm) as dry matter basis. Hooda and Jood (2003) reported that the iron content in raw fenugreek seed was (12.60 mg/100gm) as dry basis. El-Mahdy and Sebaiy (1982) also reported that raw fenugreek has (10.9 mg/100gm) iron as dry basis. Iron is an essential component of the respiratory pigments, haemoglobin, myoglobin and various enzymes system. Its deficiency

has been indicated to cause anaemia which is one of the most common nutritional problems worldwide (Loumouamou *et al.*, 2010).

Similarly, iron content in roasted fenugreek was found to be 12.79 mg/100gm. Pandey and Awasthi (2015) reported that the iron content of roasted fenugreek seed was 13.1 mg/100gm which was similar to the above result. Tenyang *et al.* (2016) also reported that iron content of roasted sesame seeds at 180ºC for 10 mins was 14.08 mg/100gm. LSD indicates that there was significant difference ($p<0.05$) found in both raw and roasted sample. The percentage gain of iron in roasted sample as compared to raw was found to be 11.75% respectively. The increase of mineral may be related to break down of antinutritional compounds such as phytates and oxalates which bind these mineral and reduce their availability (Pandey and Awasthi, 2015; Reddy *et al.*, 2006).

The iron content in soaked sample was found to be 10.85 mg/100gm. This was closer to the value reported by Hooda and Jood (2003) (11 mg/100gm) as dry basis. Saini *et al.* (2016) also reported that iron content of soaked fenugreek was (9.75 mg/100gm) as dry basis. Statistical analysis showed that there was no significant difference ($p > 0.05$) of iron content in between the raw and soaked sample. The percentage loss of iron in soaked sample as compared to raw was found to be 5.20%. Soaking and germination did not show any significant change in total minerals. Comparatively lower contents of minerals when soaked in water might be due to leaching out of some minerals into the soaking water (Nolan and Duffin, 1987; Pandey and Awasthi, 2015).

The iron content in germinated sample was found to be 11.18 mg/100gm. This was very close to the value reported by Atlaw and Kumar (2018) (11.51 mg/100gm) and Pandey and Awasthi (2015) (11.5mg/100gm). Hooda and Jood (2003) reported that the iron content of fenugreek seeds germinated for 48 hours was found to be (11.20 mg/100gm). Statistical analysis showed that there was no significant difference ($p>0.05$) of iron content in between the raw and germinated sample. The percentage loss of iron in soaked sample as compared to raw was found to be 2.32%. Decrease in Fe content in germinated fenugreek seed flour might be due to leaching of Fe in to soaking medium reported by Duhan *et al.* (2002). Reduction of mineral content because of germination, indicated the transfer of nutrients from the seed material to the growing embryo reported by (Atlaw and Kumar, 2018).

4.3.2 Calcium

The calcium content of different treatment in fenugreek sample are shown in table 4.4. Statistical analysis shows that there was no significant difference ($p > 0.05$) of calcium content in between the raw and roasted fenugreek sample.

The calcium content of raw fenugreek sample was found to be 78.29 mg/100gm. Hooda and Jood (2003) reported the calcium content of raw fenugreek sample to be (72.50 mg/100gm) as dry basis which was closer to the above value. Jasass and Jasser (2012) also reported calcium content (75 mg/100gm). Pandey and Awasthi (2015) also reported calcium content (70.5 mg/100gm) of raw fenugreek seed.

Similarly, calcium content in roasted fenugreek was found to be 79.07 mg/100gm. This value was similar to the value reported by Saini *et al.* (2016) (87.21 mg/100gm) and Pandey and Awasthi (2015) (71.2 mg/100gm) in roasted fenugreek seeds. LSD indicates that there was no significant difference ($p>0.05$) found in both raw and roasted sample. The percentage gain of calcium in roasted sample as compared to raw was found to be 0.99%. During roasting (high temperature), anti-nutrients like phytate, oxalates are partially or completely destroyed. Their destruction is responsible to the increase of Ca concentration in the medium (Makinde and Akinoso, 2013).

The calcium content in soaked fenugreek was found to be 74.97 mg/100gm. Hooda and Jood (2003) reported the calcium content of soaked fenugreek seeds was (70.60 mg/100gm) as dry basis which was similar with above result. Saini *et al.* (2016) also reported the loss of calcium from (84.17 to 76.10 mg/100gm). Pandey and Awasthi (2015) has reported that calcium content of soaked fenugreek was (68.2 mg/100gm). LSD indicates that there was significant difference ($p<0.05$) found in both raw and soaked sample. The percentage loss of soaked in soaked sample as compared to raw was found to be 4.25% respectively.

The calcium content of germinated fenugreek sample was found to be 72.01 mg/100gm. This value was similar to the value reported by Hooda and Jood (2003) (71.22 mg/100gm) in germinated fenugreek seeds. Mahmoud *et al.* (2012) reported the calcium content in germinated fenugreek seeds was (84.83 mg/100gm) as dry basis. Pandey and Awasthi (2015) also reported the calcium content of germinated fenugreek was (70.7 mg/100gm). Statistical analysis showed that there was significant difference ($p<0.05$) of calcium content

in between the raw and germinated sample. The percentage loss of calcium in germinated sample as compared to raw was 8.03%.

4.4 Effect of processing methods on phytochemical composition and percentage gain of polyphenol

The effects of roasting, soaking and germination on the phenolic content in fenugreek was studied and are shown in table 4.6. The content of total phenolics in the methanol plant extract was determined using the Folin-Ciocalteau reagent. The result of total phenolic content was calculated from the regression equation of the standard plot (y =0.0095x - 0.0539, R^2 = 0.9957) (Table 4.6). Generally, the total phenolic content increased significantly ($p<0.05$) for all treatments. Statistical analysis shows that there was significant different ($p<0.05$) found in raw, soaked, roasted and germinated sample.

Table 4.6: Quantitative determination of phytochemical on raw, soaked, roasted and germinated fenugreek seeds.

Sample	Polyphenol (mg GAE/g db)	Percentage gain of polyphenol
Raw	$80.85^{a} \pm 0.85$	
Roasted	$85.70^{b} \pm 0.54$	5.99*
Soaked	$83.38^{c} \pm 0.31$	3.12*
Germinated	$117.70^{d} \pm 0.39$	45.56*

Note: *Indicate the gain of phenol

Total phenol content of the raw fenugreek seeds was found to be 80.85 mg GAE/g as dry basis. Manju *et al.* (2016) reported the total phenol content of raw fenugreek sample to be 67.32 mg GAE/g as dry basis which was slightly lower than our result. During research conducted by Saxena *et al.* (2016) he reported the phenol content of raw fenugreek was 72.99 mg GAE/g which was closer with our findings. Our findings was closer to the value reported by Naidu *et al.* (2011) (85.88 mg GAE/g) in raw fenugreek seeds. Agrawal *et al.* (2016) reported that maximum phenolic content was observed in methanol seed extract of all cumin genotypes with an average value of 85.64 mg GAE/g. Dubey. *et al.* (2016) reported phenolic content in various genotypes of cumin from India that ranged

between 70.4-92.4 mg GAE/g seeds. Dubey *et al.* (2017) reported the phenol content of cumin seed was 58.61 mg GAE/g. The difference in the extraction yield could be the result of using different extraction solvents in other works and methanol in ours. The difference in cultivar, geographical region, season & weather may contribute to the variation of phenolic compounds profile (Lim and Murtijaya, 2007; Zou *et al.*, 2012). These differences in the total contents of phenolic of the extracts obtained from fenugreek seeds are dependent on the polarity of the solvents used, extraction method and extraction time. Furthermore, it may be due to the chemical diversity of phenolic and flavonoid compounds and the complexity of composition in plant sources (Wijekoon *et al.*, 2011)

The total phenol content of roasted fenugreek sample was found to be 85.70 mg GAE/g as dry basis and percentage gain of phenol in roasted sample as compared to raw was found to be 5.99% respectively. According to the data of Manju *et al.* (2016), there was increase in total phenol content of fenugreek seeds during roasting at 130±5°C for 7 mins i.e. 73.12 mg GAE/gm which was similar to the above result. Similarly, Pandey and Awasthi (2015) reported the phenolic content of roasted fenugreek in an open pan at 130±5°C for 7 min was 48.5 mg GAE/100gm which significantly increased 6.82% from raw data. Our result obtained shows slightly higher as compared to this research. Statistical analysis shows that there was significant change ($p<0.05$) found in between raw and roasted sample. Boateng *et al.* (2008) explained that disruption of the cell wall through heating broke the covalently bound phenolic compound or by the breakdown of insoluble phenolic compounds as function of thermal treatments to release the phenolic compound, which was readily soluble in methanol. In addition, the increase in total phenolics of fenugreek during roasting in this study may also be linked to the development of Maillard reaction products which leads to the release of bound polyphenols that have been reported to possess scavenging activity on reactive oxygen species (Segev *et al.*, 2012). Maillard reaction involves condensation reactions between sugars and amino acids and has been found to be linked to polyphenols Jokic *et al.* (2004) via inhibition of polyphenol oxidase. The thermal treatment applied to foods of plant origin by heating or roasting causes evaporation of intracellular water, triggering chemical reactions that can change the lignocellulosic structure and promotes protein denaturation, which may result in a greater availability of plant phenolic compounds (Rizki *et al.*, 2015). A hypothesis that may explain the increase of the level of some phenolics compounds in plant food after heat treatment is that the heat alters the structure of some molecules, including proteins that are associated with the phenolic

compounds resulting in increased levels of free phenolic compounds. This hypothesis could explain the different effects of roasting process on the content of individual phenolic compounds and the antioxidant activity found in the present study (Hii *et al.*, 2009; Wollgast and Anklam, 2000).

Phenolic content of the soaked fenugreek treated sample was found to be 83.38 mg GAE/gm as dry basis which increased slightly in compared to the raw sample by 3.12%. These results agrees with those reported by Pandey and Awasthi (2015) 54.4 mg GAE/gm db who also found an increase of 19.82% of total phenols in fenugreek seed after soaking for 12 hours. Ojha *et al.* (2018) reported increase in phenolic content during 12 hours soaking of fenugreek seeds (9.43 mg GAE/g) db. Our result obtained shows slightly higher as compared to this research. Statistical analysis shows that there was significant change ($p<0.05$) found in between raw and soaked sample. The TPC observed here were may be due to different extraction conditions, growth conditions, and genotypes.

Phenolic content of the germinated fenugreek sample was found to be 117.70 mg GAE/gm as dry basis which increased significantly by 45.56%. Total phenolic contents were significantly ($p<0.05$) increased by germination process. Cevallos. *et al.* (2010) reported that germinated edible seed spices are an excellent source of dietary phenolic content. Phenolic content increased from 67.32 mg GAE/gm DB in raw fenugreek seeds to 93.27 mg GAE/gm during 48 hour germination reported by Manju *et al.* (2016). Dixit *et al.* (2005) also reported the total phenolic content of 24 hour germinated fenugreek was 64.61 mg GAE/gm db. Pandey and Awasthi (2015) also reported increase in phenolic content 77.97% i.e. 80.8 mg GAE/gm during 24 hour germination which agrees to our findings. Similarly, Sharara (2017) reported that the phenolic content increased from 1186 mg GAE/100gm in raw fenugreek seeds to 1815 mg GAE /100gm during 4 days germination. Our result obtained shows slightly higher as compared to this research. Ojha *et al.* (2018) reported 14.50 mg GAE/gm phenol during 72-hour germination. According to Fouad and Rehab (2015) Phenolic content increased from 1341.13 mg/100 g DW in raw lentil seeds to 1411.50 in 3 days germinated seeds. These increases could be due to the biosynthesis and bioaccumulation of phenolic compounds as a defensive mechanism to survive under environmental stresses, like cold exposure reported by Randhir *et al.* (2004b), and to degradation of polymerized polyphenols, specifically hydrolysable tannins, and the hydrolysis of other glycosylated flavonoids (Monagas *et al.*, 2005). The high

phenolic content observed during early germination for all treatments corresponds to high antioxidant activity during the same period. This suggests that the phenolics are antioxidant in nature during early germination when the oxidative stress in the germinating sprouts is naturally high due to the multitude of biological processes that are initiated with seed imbibition and growth (Randhir *et al.*, 2004a). Data obtained from present study confirmed the effect of germination process on polyphenols increment in fenugreek which indicates that a new polyphenols synthesis may be occurred within germination of fenugreek seeds (Sharara, 2017). Lopez-Amoros *et al.* (2006) indicated that germination modifies the quantitative and qualitative phenolic compounds of legumes, and the changes depend on the type of legume and the germination conditions. These changes influence the functional properties of the legumes as the consequence of variation in antioxidant activity.

4.5 Effect of different processing method on the antinutritional factors of fenugreek seeds

The effects of roasting, soaking and germination on the phytate and tannin content in fenugreek was studied and are shown in table 4.7. All the treatments significantly reduced ($p<0.05$) the phytate and tannin content of the fenugreek seeds, but to the varying extent. Germination had most pronounced effect than other treatments in reduction of antinutrients contents. Statistical analysis shows that there was significantly difference ($p<0.05$) found in raw, soaked, roasted and germinated sample.

Table 4.7: Effect of different processing methods on the antinutrients of fenugreek seeds.

Sample	Phytate (mg/100gm)	Tannin (mg/100gm)
Raw	$85.09^{a} \pm 6.69$	$674.84^{a} \pm 5.76$
Roasted	$54.15^{b} \pm 6.69$	$481.13^{b} \pm 3.77$
Soaked	$73.48^{a} \pm 6.69$	$435.84^{c} \pm 3.77$
Germinated	$38.67^{c} \pm 6.69$	$224.52^{d} \pm 3.77$

(Values presented above are the average of triplicates ± standard deviation)

Table 4.8: Percentage loss of antinutrients.

Sample	Phytate %	Tannin %
Roasted	36.37	28.70
Soaked	13.64	35.41
Germinated	54.55	66.73

4.5.1 Phytate

Phytate content of the raw fenugreek seed was found to be 85.09 mg/100gm. During research conducted by (Atlaw and Kumar, 2018) , he reported that the phytate content of raw fenugreek seeds was 64.22 mg/100gm which was closer to our findings. Similarly, Kavitha *et al.* (2015) also reported the phytate content of fenugreek seeds was 23 mg/100gm which was lower than our result. Burham (2017) reported the phytate content of fenugreek seeds 152.91 mg/100gm which was slightly higher than the current study. Similarly, El-Shimi *et al.* (1984) also reported the phytate content in raw fenugreek seeds 164 mg/100gm. Suri *et al.* (2019) reported that the cumin seeds contains 64.08 mg/100gm phytate and Folasade. *et al.* (2014) reported the phytate content of sesame seeds was 62.67 mg/100gm. The variation in phytic acid contents of different varieties could be due to phosphorus utilization efficiency of the varieties, which is the result of the genetics behind this trait. Tesfaye *et al.* (2017) and Oomah *et al.* (1996) reported that phytic acid content is influenced by difference in location and year of production, variety (cultivar), post-harvest treatment and their interaction.

Phytate content of the roasted fenugreek was 54.15 mg/100gm. Phytate was reduced by 36.37% respectively. Similar result was obtained by Folasade. *et al.* (2014), he found that roasting of sesame seeds at 160 ºC for 25 mins, significantly reduced phytic acid content by 38.9%. Also, the result obtained by El-Mahdy and Sebaiy (1982) shows the reduction of the phytate of fenugreek seeds roasted at 185 ºC for 15 mins by 39.85% which agreed to our finding. Pandey and Awasthi (2015) reported similar reduction of phytate in fenugreek seeds when roasted at 130±5 °C for 7 min that is 40.77%. The only factor that could account for the lower concentrations of phytate in roasted fenugreek was the heat applied as these antinutrients are thermo labile in nature reported by Folasade. *et al.* (2014). Statistical analysis shows that there was significant difference ($p<0.05$) found in raw and roasted sample.

Effect of soaking on phytate content of fenugreek was studied and the value obtained showed that there is significant reduction ($p<0.05$) in phytate content. Our result shows great reduction range from 85.09 mg/100 g to 73.48 mg /100 g after soaking for 12 h (13.64% reduction). During research conducted by Hooda and Jood (2003), he reported that soaking of fenugreek seeds during 12 hours reduced the phytate content by 9.03%. A loss of 8.7% phytate content of fenugreek has been reported by Pandey and Awasthi (2015) during 12 hours soaking. Statistical analysis shows that there was no significant difference ($p>0.05$) found in raw and soaked sample. Soaking can be seen to remove soluble antinutritional factors by deactivating the enzyme inhibitors. Phytate is water soluble so the seeds that soaked in water for overnight shown considerable removal of phytates in water in addition to that it also enhances the naturally occurring phytase (Kumar *et al.*, 2010). Some authors like Luo *et al.* (2013) suggested that diffusion is a cofactor which affected the hydrolysis of phytic acid in the soaking process. The loss in phytate may be due to the leached down of phytate ion in soaking liquid under the influence of difference in chemical potential which manage the diffusion rate (Deshpande and Cheryan, 1984; Kumar *et al.*, 2010). The phytate content of fenugreek may vary depending on the variety, growth, season, soil conditions, time of harvest and many factors. Differences observed in the reported values and the values determined in this study could be attributed to these factors.

Phytate content of germinated fenugreek seeds were 38.67 mg/100gm which was reduced by 54.55% during 72 hours germination. Our results showed substantial decrease in the concentration of phytic acid in the seeds processed by germination and are in agreement with the earlier studies. Our results are in well agreement with those of (Kavitha *et al.*, 2015), where the phytate was reduced by 50% during 3 days germination of fenugreek. A loss of 44% phytate content was reported by (Pandey and Awasthi, 2015) during 24 hours germination. Hooda and Jood (2003) reported the phytate reduction by 42.14% during 48 hours germination. El-Mahdy and Sebaiy (1982) also reported 68.11% reduction of phytate in fenugreek during 96 hours germination. Loss of 70.42% phytate was reported by Atlaw and Kumar (2018) during 72 hours germination. Phytic acid is water soluble and the reduction of the phytic acid during germination is attributed due to hydrolysis of the legumes by enzymes broadly designated as phytases synthesized in germinating seedlings as reported for several plants. Plant seed utilize phytate as a source of inorganic phosphate during germination and thus tend to increase palatability and

nutritional value (Wang *et al.*, 1997). Generally, germination is preceded or combined with soaking in water which decreases phytic acid level due its leaching out into soaking water under the concentration gradient and to its hydrolysis following the activation of the enzyme phytase and phosphatase activity during the germinating process (Mabrouki *et al.*, 2015). It was reported that phytase activity originates after germination and the phosphatase activity was increased in the germinated seeds which results in the reduction of phytic acid content in fenugreek seeds after germination and roasting (El-Mahdy and Sebaiy, 1982; Pandey and Awasthi, 2015). During germination enzymatic hydrolysis of phytate phosphorus takes place which decreases phytic acid content (Raju *et al.*, 2001). Pawase *et al.* (2021) and Archana and Kawatra (1998) reported that malting with 72 hours of germination was most effective in reducing the antinutrient levels of pearl millet grains. Because germination is mainly a catabolic process that supplies important nutrients to the growing plant through hydrolysis of reserve nutrients, reduction in phytic acid was expected. Since phytic acid may be one of the factors responsible for reducing mineral bioavailability its reduction during germination may enhance the nutritional quality with respect to mineral bioavailability of nutrients. The analysis of variance (Appendix D) showed that there was a significant difference ($p<0.05$) between the raw and germinated sample.

4.5.2 Tannin

The effects of roasting, soaking and germination on the tannin content in fenugreek seeds was studied and are shown in table 4.7. All the treatments significantly reduced ($p<0.05$) the tannin of the fenugreek seeds, but to the varying extent. The result of total tannin content was calculated from the regression equation of the standard plot ($y=0.0053x+0.0085$, $R^2=0.9725$). Germination had most pronounced effect than other treatments in reduction of tannin contents. Statistical analysis shows that there was significant different ($p<0.05$) found in raw, soaked, roasted and germinated sample.

Tannin content of raw fenugreek sample was found to be 674.84 mg/100gm. Atlaw and Kumar (2018) reported the tannin content of fenugreek seed was 718.92 mg/100gm which agrees to our result. In the study conducted by Tesfaye *et al.* (2017), the tannin content of sesame seed was found to be 660 mg/100 gm and that of flaxseed was 695 mg/100gm which is similar to our result. Mabrouki *et al.* (2015) reported the tannin content of

fenugreek seeds 353 mg/100gm which was slightly differ from the present study. Similarly, Saini *et al.* (2016) also reported the tannin content of fenugreek seeds 394 mg/100gm.

Tannin content of roasted fenugreek seeds was found to be 481.13 mg/100gm which was reduced by 28.7% respectively. Effect of roasting on fenugreek seeds at 130 ºC for 7 mins studied by Manju *et al.* (2016) reported the similar reduction of tannin by 26.36% respectively. Similar result was obtained by Saini *et al.* (2016), he found that roasting of fenugreek seeds at 130±5 ºC for 7 min reduced the tannin content by 34%. During roasting breakdown of the bond between phytate and might takes place which results in destruction of phytates, tannins (Reddy *et al.*, 2006). The decrease may be attributed due to the heat labile and water-soluble nature of tannins (Mittal *et al.*, 2012). Siddhuraju and Becker (2007) also reported the presence of significantly higher level of tannins in raw legumes such as cowpea seeds than in dry heated samples, which are comparable to the results observed for field bean of the present study. The higher levels of tannins observed in raw grains than processed grains could be attributed to increased activity of tannin degradation enzymes such as polyphenol oxidase and other catabolic enzymes during the thermal treatments. The analysis of variance (Appendix D) showed that there was significant difference ($p<0.05$) between the raw and roasted sample.

Tannin content of soaked fenugreek seeds was found to be 435.84 mg/100gm which was reduced by 35.41%. Ojha *et al.* (2018) reported the tannin content of soaked fenugreek seeds for 12 hours was 39.64% which agrees to our result. Similarly, Shweta *et al.* (2010) reported the reduction of tannin content in green gram, red gram, bengal gram and lentil during soaking at 12 hours was 38.9%, 22.5%, 22.1% and 24.1% respectively. The reduction of tannins after soaking is mainly due to the fact that those compounds, in addition to their predominance in seed coats, are water soluble and consequently leach into the liquid medium reported by (N. R. Kumar *et al.*, 1979; Vanshika *et al.*, 2017). The loss of tannin content after soaking may be attributed to leaching out into soaking water under the concentration gradient (Kataria *et al.*, 1988; Saharan *et al.*, 2002). Statistical analysis shows that there was significant change ($p<0.05$) found in between raw and soaked sample.

Tannin content of germinated fenugreek sample was found to be 224.52 mg/100gm which was reduced by 66.73 % respectively. Effect of germination on fenugreek seeds for 72 hours studied by Ojha *et al.* (2018) reported similar reduction of tannin by 60.52%. (Mabrouki *et al.*, 2015) also reported reduction of tannin in germinated fenugreek seeds for

48 hours by 58.6% which was closer with the present study. Atlaw and Kumar (2018) reported 72 hours germinated fenugreek seeds contains 197.62 mg/100gm tannin which was reduced by 72.51% from the raw value. Kavitha *et al.* (2015) also reported reduction of tannin during 72 hours germination of fenugreek seeds by 63.41%. A loss of 57.27% tannin in 72 hours germinated sesame seeds was reported by Maria and Victoria (2018). As the germination period increased tannin content gradually decreased. The reduction might be due to leaching of tannins into water (Shimelis. and Rakshit., 2008) and bonding of polyphenols with carbohydrate or protein (Saharan *et al.*, 2002). The observed reduction of tannin content after germination was a result of formation of hydrophobic association of tannin with seed protein and enzymes (Rusydi and Azlan, 2012). Loss of tannins during germination is attributed to the presence of polyphenol oxidase and enzymatic hydrolysis (Rao and Deosthale, 2006). Statistical analysis shows that there was significant change ($p<0.05$) found in between raw and germinated sample.

4.6 Effect of Processing Methods on Antioxidant Activity and DPPH

Free radical scavenging activity for DPPH radical was expressed as IC_{50} in samples. IC_{50} concentration of methanolic extracts of fenugreek shown in Table 4.9 indicates that the amount of extract needed for 50% inhibition (IC_{50}) of DPPH radicals. The antioxidant activity is inversely proportional to IC_{50} (Qusti and Abo-khatwa, 2010) which were calculated from the linear regression of the % antioxidant activity versus extracts concentration. IC_{50} of extract shows positive antioxidant activity of the plant. These studies can also help the manufacturers for identification and selection of the raw material for drug production.

Table 4.9: IC_{50} concentration of methanolic extracts of plant samples for DPPH inhibition.

Parameter	Total Antioxidant Activity		
	DPPH Radical Scavenging Activity (% inhibition)	**IC_{50} Value (mg dm/ml)**	**mg Ascorbic eqvt/mg dm**
Raw	16.95±1.98[a]	5.95	9.27
Roasted	26.98±1.39[b]	3.38	16.29
Soaked	62.28±0.78[c]	1.54	35.69
Germinated	75.66±1.45[d]	1.28	42.91

Note: IC_{50} value from Ascorbic Acid standard curve = 55.18816 mg/ml

The lowest IC_{50} concentration was found to be 1.28 mg dm/ml in germinated fenugreek seeds. It means that 1.28 mg fenugreek extract is needed to 50% inhibit the 1 ml DPPH. As comparing with the L- ascorbic acid, 42.91 mg L- ascorbic acid is needed. As comparing the sample raw, roasted, soaked and germinated, the highest amount of antioxidant activity was found in germinated sample. Similarly, IC_{50} concentrations were found as 5.95 mg dm/ml for raw, 3.38 mg dm/ml for roasting, 1.54 mg dm/ml for soaking respectively. In above data, antioxidant activity was found to be 16.95% in raw, 26.98% in roasted, 62.28% in soaked and 75.66% in germinated sample.

Our findings were closer to the value reported by Pandey and Awasthi (2015), where he observed 18.1% antioxidant activity in raw fenugreek seeds, 32% in roasted, 60.7% in soaked and 73.9% in germinated fenugreek. In response to phenolic content, antioxidant activity increased significantly after soaking from 18.1–60.7%. Similarly, Ojha *et al.* (2018) reported the dry, soaked and germinated fenugreek seeds powder exhibited free radical scavenging activities of 10.4%, 13.6% and 55.45% respectively. Bukhari *et al.* (2008) revealed antioxidant activity for methanolic extract of raw seed was less than 20%. Randhir *et al.* (2004a) reported higher antioxidant activity during early germination, which correlates to high phenolic content suggesting that initially phenolics are antioxidant in nature. Jeong *et al.* (2004) reported that antioxidant activities of defatted sesame meal extract increased as the roasting temperature of sesame seed increased, but the maximum antioxidant activity was achieved when the seeds were roasted at 200 °C for 60 min. In a

study it was reported that roasting nuts may destroy bioactive compound but it can also form antioxidant compound through Maillard reaction (Kamalaja *et al.*, 2018). Another reason for increased antioxidant content at the time of heating was that heating inactivates the endogenous oxidative enzyme, preventing further oxidation of antioxidant compounds in the raw plant material (Neri *et al.*, 2020; Nicoli *et al.*, 1999). Untreated, pan and microwave roasted kalonji sample showed the antioxidant activity of 81.76%, 86.14% respectively. The phenolic hydroxyl groups present in kalonji seed antioxidants have redox properties (Pietta, 2000), allowing them to act as reducing agents and hydrogen donors in the DPPH assay. The higher antioxidant activity could be attributed to the formation of Maillard products like melanoidins at high temperatures (Wani *et al.*, 2017). Also higher antioxidant activity may be due to higher release of bound phenolics from roasted sample (Rashid *et al.*, 2015).

The increase in antioxidant activity with sprouting is one of the many metabolic changes that take places upon sprouting of seeds, mainly due to an increase in the activity of the endogenous hydrolytic enzymes. This increase may also be due to the synthesis of compounds like vitamin C and tocopherols which are responsible for antioxidant activity (Sharma. and Gujral., 2010). Doblado *et al.* (2007) suggested that during germination, the hydrolytic enzymes modify the endosperm and may liberate some of the bound components that play a role in antioxidant activity. The reducing power is also an indicator of Antioxidant activity (Y. R. Lee *et al.*, 2007). The electron donor compounds are considered as a reducing agent and can reduce the oxidized intermediates of the lipid peroxidation reactions; therefore, they may be primary or secondary antioxidants (Zhao et al., 2008). Legumes contain other bioactive compounds besides phenolic such as vitamins and carotenoids at different concentrations that might also behave as an antioxidant (Prodanov *et al.*, 1997; Tarzi *et al.*, 2012). These compounds might also exert synergetic activities among themselves and with phenolic compounds, which could be the main reason for the observed differences in the antioxidant activities.

Production of phytochemicals in plant are affected by many pre- and post-harvest factors including farming practices, environmental factors, storage and processing conditions, but temperature is the primary factor among all. The metabolism of phytochemical begins right after the harvest and can involve complex biochemical reactions. This reaction can lead to significant changes in plant attributes and health

promoting phytochemicals, such as those with strong antioxidant activities (Hongyan *et al.*, 2012). Some of the vegetables which had high concentrations of phenolic compounds also had higher capacities to scavenge for DPPH radicals. The key role of phenolic compounds as scavengers of free radicals is emphasized in some reports (Komali *et al.*, 1999; Moller *et al.*, 1999). Polyphenolic compounds have an important role in preventing lipid oxidation and are associated with antioxidant activity (Gülçin *et al.*, 2003; Yen *et al.*, 1993). Polyphenolic compounds may contribute directly to antioxidative action. Tanaka *et al.* (1988) and Zineb *et al.* (2012) suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g of polyphenols are daily ingested from a diet rich in fruits and vegetables. The natural antioxidants are the subject of much research and a new breadth to the exploitation of secondary metabolites generally and particularly polyphenols. Hence, the extract of Trigonella foenum graecum exhibited potent DPPH activity (Priya *et al.*, 2011). The high free radical scavenging activity of the fenugreek seeds explained by the presence of polyphenols in this plant methanolic extract, also a polyphenol-rich extract from the seeds of fenugreek reduces the oxidative haemolysis and lipid peroxidation in normal and diabetic human erythrocytes (Dixit *et al.*, 2005).

Part V

Conclusions and recommendations

5.1 Conclusions

Present work was carried out to study the effect of roasting (130ºC for 7 mins), soaking (12 hours) and germination (72 hours) as comparing with control on the nutritional, antinutritional, phytochemical and antioxidant content of *Trigonella foenum graecum* seeds. Based on this research following conclusion can be drawn.

1. Proximate composition of fresh seeds was determined that is Moisture, Protein, Fat, Crude fiber and Ash content was found to be 9.39%±0.01, 22.05%±0.82, 4.33%±0.26, 8.51%±0.46 and 3.89%±0.01.
2. Protein significantly increased during roasting, soaking and germination whereas, fat significantly decreased during all the treatments and there was no significant difference in ash content between the control and treatments.
3. Iron and calcium increased during roasting whereas slightly decreased during soaking and germination.
4. Germination significantly increased polyphenol content from 80.85±0.85 mg GAE/g to 117.70±0.39 mg GAE/g, 85.70±0.54 mg GAE/g during roasting and 83.38±0.31 mg GAE/g during soaking.
5. Phytate significantly reduced from 85.09±6.69 mg/100gm to 54.15±6.69 mg/100gm during roasting, 73.48±6.69 mg/100gm during soaking and 38.67±6.69 mg/100gm during germination.
6. Tannin significantly reduced from 674.84±5.76 mg/100gm to 481.13±3.77 mg/100gm during roasting, 435.84±3.77 mg/100gm during soaking and 224.52±3.77 mg/100gm during germination.
7. The highest amount of antioxidant activity was found to be in germinated and soaking sample i.e., 75.66% (1.28 mg dm/ml) and 62.28% (1.54 mg dm/ml).
8. Germination for 72 hours seems to be a promising and effective method for reduction of antinutrients and increasing nutritional composition, phytochemicals and antioxidant.

5.2 Recommendations

Based on the present study, the following suggestions are recommended for future work in the field of fenugreek seeds:

1. Fenugreek has medicinal importance especially for controlling sugar for diabetic patient, treating hypercholesterolemic problems thus the research can be studied.
2. According to my research, the antinutritional factors (phytate and tannin) during germination were significantly reduced as compared to soaking and roasting which results in decrement of bitterness and so further research can be done in fortification of treated fenugreek with other food materials.
3. Similarly, phytochemicals like polyphenol and antioxidants were significantly increased during germination followed by soaking and roasting. Thus, germinated fenugreek seeds can be consumed more as it has many beneficial roles for a healthy life.
4. Effects of different combined treatments (de-hulling and cooking, pressure cooking, fermentation) in nutritional and anti-nutritional factors can be studied.
5. Effect of different processing techniques in other anti-nutritional factors like trypsin inhibitors, saponin which are abundant in fenugreek can be studied along with which preparation and quality evaluation of fenugreek incorporated product can be done.
6. Above treatments reduces the antinutritional components which shows the low resistivity of nutrient absorption and aids digestion for all the consumers. Thus, these treatments especially germination of fenugreek seeds must be practiced in our daily life.

Part VI

Summary

Fenugreek (*Trigonella foenum-graecum L.)* is an annual herb of Leguminosae, is being used as spice with its seeds and as vegetable with its leaves and has been recognized and utilized for its medicinal attributes since times immemorial as evidenced by its use in traditional ayurvedic and Chinese medicines for alleviating high cholesterol plasma levels, diabetes oxidative damage. Rich in vitamins and minerals, and because it is a seed and a legume, it is high in protein (Altuntas. *et al.*, 2005). Fenugreek seeds was brought from Biratnagar, Morang district, Nepal and the effect of various treatment on nutritional, antinutritional and phytochemical composition of fenugreek was studied.

In the present study, three types of treatments were used to investigate reduction of anti-nutrient level i.e., phytate and tannin, increment of antioxidant, bioactive components like polyphenol. The treatments included roasting (130ºC for 7 mins), soaking (12 hours) and germination (72 hours). Tannin, polyphenol and antioxidant were analyzed spectrophotometrically while phytate was determined by titration using ammonium thiocyanate. The crude extracts of samples were prepared using 80% methanol by maceration technique for analysis. Proximate analysis was calculated as percentage dry basis (db) except moisture was calculated as % wet basis. Soaking contains highest amount of moisture whereas significantly decreased during roasting. During treatment there was no significant change ($p>0.05$) found in crude fiber, ash and iron content but found significant change ($p<0.05$) in protein, fat and calcium content. Highest amount of Protein, Fat, Crude fiber and Ash was found in germination i.e., 31.47% db, 4.33% db, 9.34% db, 4.58% db in germination. Highest amount of antinutrients was reduced during germination i.e., phytate from 85.09±6.69 mg/100gm to 38.67±6.69 mg/100gm and tannin from 674.84±5.76 mg/100gm to 224.52±3.77 mg/100gm. Germinated sample increased the bioactive compounds i.e., polyphenol (from 80.85±0.85 mg GAE/g to 117.70±0.39 mg GAE/g) more than that of roasted and soaked sample. The lowest IC_{50} concentration was found to be 1.28 mg dm/ml in germination. It means that 1.28 mg fenugreek extract is needed to 50% inhibit the 1 ml DPPH. Germinating fenugreek seeds for 72 hours was the best processing as majority of nutritional components (protein, crude fiber, ash), bioactive compound (polyphenol) and antioxidant was significantly increased and maximum reduction of anti-nutritional compounds (tannin, phytate) was determined.

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Appendices

Appendix A

Table A.1 List of chemicals used

Chemicals	Supplier/Manufacturer	Other specifications
Sodium hydroxide (NaOH)	Thermo Fisher scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Hydrochloric acid (HCL)	Thermo Fisher scientific India Pvt. Ltd.	36%, LR grade
Sulphuric acid (H_2SO_4)	Thermo Fisher scientific India Pvt. Ltd.	97%, LR grade
Boric acid	Merck, Powder	99.5% Assay
Oxalic acid	Merck (India) Limited	Crystal
Sodium Carbonate (Na_2CO_3)	Qualigens fine Chemicals	99.5%, LR grade
L-ascorbic acid	S.D. fine chemicals Ltd.	99% Assay
Tannic acid	Avarice Laboratories Pvt. Ltd.	Analytical Reagent
Phosphoric acid	Loba chemicals	56-60%
Potassium Persulfate	GlaxoSmithKline Pharmaceuticals Ltd.	98% Assay
Sodium acetate	Qualigens fine chemicals	99-102% Assay
Ammonium oxalate	Qualigens fine chemicals	99% Assay
Aluminum chloride ($AlCl_3$)	S.D fine-chem Ltd.	98% hygroscopic
Nitric acid	Fisher Scientific India Pvt. Ltd.	68-75% Assay
Sulphuric acid	Fisher Scientific India Pvt. Ltd.	97% Assay

Ammonium thiocyanate	Qualigens	99% Assay
Methanol	Merck life science Pvt. Ltd	99% Liquid
Ethanol	Merck life science Pvt. Ltd	99% Liquid
Ferric chloride ($FeCl_3$)	Thermo Fischer scientific India, Pvt. Ltd.	96% anhydrous
Folin-Ciocalteu Reagent	Thermo Fischer scientific India, Pvt. Ltd.	Liquid
Folin-Denis Reagent	Thermo Fischer scientific India, Pvt. Ltd.	Liquid
Acetic acid	Thermo Fischer scientific India, Pvt. Ltd.	99% Liquid
DPPH (2,2diphenyl-1-picrylhydrazyl	Himedia laboratories (India) Pvt. Ltd.	Amorphous
Phenolphthalein indicator	Merck life Pvt. Ltd.	pH 8.2-9.8
Sodium Sulphite	Thermo Fisher Scientific India Pvt. Ltd.	99%, Fused flakes
Sodium nitrate ($NaNO_2$)	Thermo Fischer scientific India, Pvt. Ltd.	98%
Dil. Ammonia	Fisher Scientific	25% NH_3
Potassium Permanganate	Avantor Performance Materials Ltd.	99% Assay
Potassium thiocyanate	Thermo Fisher Scientific India Pvt. Ltd.	97% Assay
Metaphosphoric acid	S.D. fine chemicals Ltd.	66 % HPO_3, 40 % $NaPO_3$

Table A.2 List of Equipments used

Physical apparatus	Specifications
Electric balance	Phoenix instruments, India
Spectrophotometer	Labtronics, India
Soxhlet apparatus	Y.P. Scientific glass work
Hot air oven	Victolab, India
Incubator	Y.P. scientific glass work
Centrifuge	Y.P. scientific glass work
Muffle furnace	Accumax, India
Cabinet dryer	AIset YDL-2000
Colorimeter	Jenway Ltd., UK
Heating mantle	Y.P. scientific glass work
Thermometer, Desiccator, Water bath	Y.P. scientific glass work

Appendix B

Table B.1 Mean value of moisture content (%)

Parameter	Mean ± Std. Deviation
Control	$9.39^{a} \pm 0.01$
Roasted	$5.56^{b} \pm 0.67$
Soaked	$9.64^{a} \pm 0.15$
Germinated	$8.15^{c} \pm 0.18$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 0.677

Table B.2 Mean value of protein content (% on dry basis)

Parameter	Mean ± Std. Deviation
Control	$22.05^{a} \pm 0.82$
Roasted	$23.07^{a} \pm 0.66$
Soaked	$24.95^{b} \pm 0.31$
Germinated	$31.47^{c} \pm 0.72$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 1.241

Table B.3 Mean value of Fat content (% on dry basis)

Parameter	Mean ± Std. Deviation
Control	$4.33^{a} \pm 0.26$
Roasted	$4.15^{a} \pm 0.15$
Soaked	$4.19^{a} \pm 0.10$
Germinated	$2.90^{b} \pm 0.10$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$. LSD between samples = 0.3199

Table B.4 Mean value of Crude fiber content (% on dry basis)

Parameter	Mean ± Std. deviation
Control	$8.51^{ab} \pm 0.46$
Roasted	$8.31^{a} \pm 0.32$
Soaked	$8.50^{ab} \pm 0.27$
Germinated	$9.34^{b} \pm 0.76$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 0.930

Table B.5 Mean value of Ash content (% on dry basis)

Parameter	Mean ± Std. Deviation
Control	$3.89^{a} \pm 0.01$
Roasted	$3.94^{a} \pm 0.19$
Soaked	$4.12^{a} \pm 0.13$
Germinated	$4.58^{a} \pm 0.85$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 0.838

Table B.6 Mean value of Carbohydrate content (% on dry basis)

Parameter	Mean ± Std. Deviation
Control	$51.81^{a} \pm 0.39$
Roasted	$54.95^{b} \pm 0.37$
Soaked	$48.58^{c} \pm 0.52$
Germinated	$43.54^{d} \pm 0.89$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 1.106

Table B.7 Mean value of Iron content (mg/100g db)

Parameter	Mean ± Std. deviation
Control	$11.45^{a} \pm 0.70$
Roasted	$12.79^{b} \pm 0.60$
Soaked	$10.85^{a} \pm 0.37$
Germinated	$11.18^{a} \pm 0.98$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 1.326

Table B.8 Mean value of Calcium content (mg/100g db)

Parameter	Mean ± Std. deviation
Control	$78.29^{a} \pm 1.01$
Roasted	$79.07^{a} \pm 0.97$
Soaked	$74.97^{b} \pm 1.02$
Germinated	$72.01^{c} \pm 1.00$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 1.895

Table B.9 Mean value of Polyphenol content (mg GAE/g db)

Parameter	Mean ± Std. Deviation
Control	$80.85^{a} \pm 0.85$
Roasted	$85.70^{b} \pm 0.54$
Soaked	$83.38^{c} \pm 0.31$
Germinated	$117.70^{d} \pm 0.39$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 1.063

Table B.10 Mean value of Phytate content (mg/100gm)

Parameter	Mean ± Std. deviation
Control	$85.09^{a} \pm 6.69$
Roasted	$54.15^{b} \pm 6.69$
Soaked	$73.482^{a} \pm 6.69$
Germinated	$38.67^{c} \pm 6.69$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 12.61

Table B.11 Mean value of Tannin content (mg/100gm)

Parameter	Mean ± Std. Deviation
Control	$674.84^{a} \pm 5.76$
Roasted	$481.13^{b} \pm 3.77$
Soaked	$435.84^{c} \pm 3.77$
Germinated	$224.52^{d} \pm 3.77$

*The values are mean values of triplicate determination ± standard deviation. Mean values within a column and a row with different superscripts are significantly different at $p < 0.05$.

LSD between samples = 8.20

Appendix C

Standard Curve of Gallic acid for Polyphenol

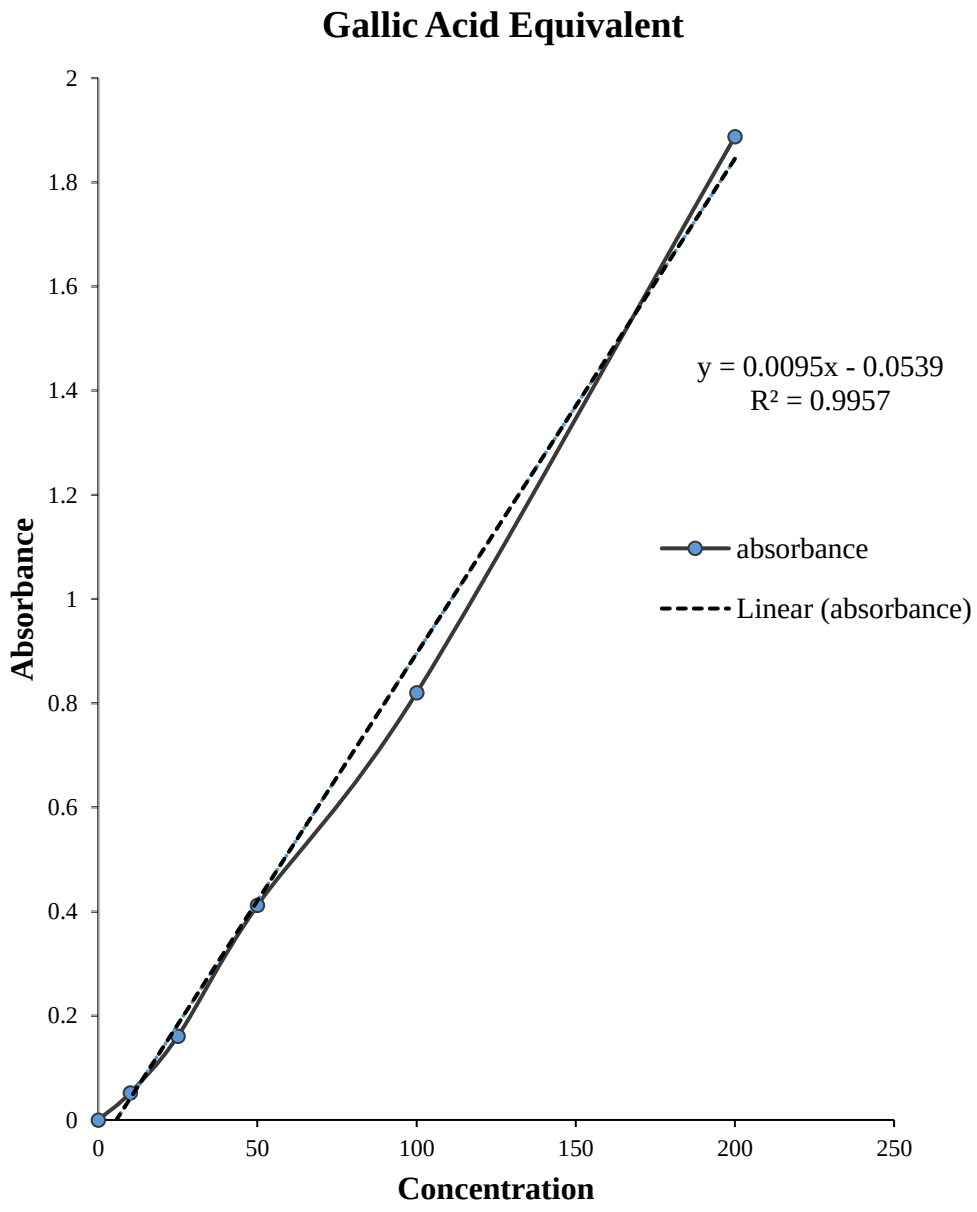

Fig C.1 Gallic Acid Standard Curve

Standard Curve of Tannic acid for Tannin

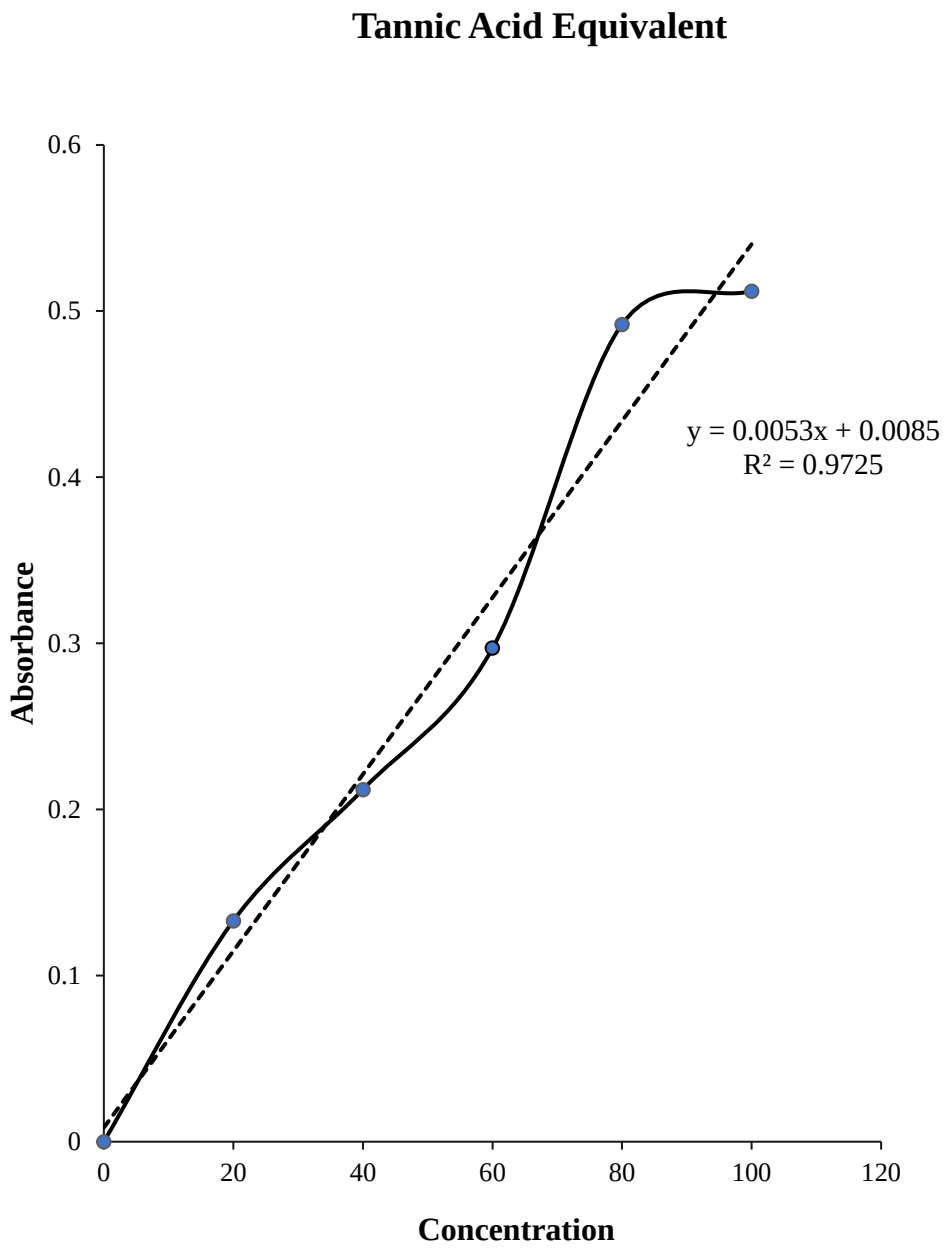

Fig C.2 Tannic Acid Standard Curve

Standard curve of Ascorbic Acid

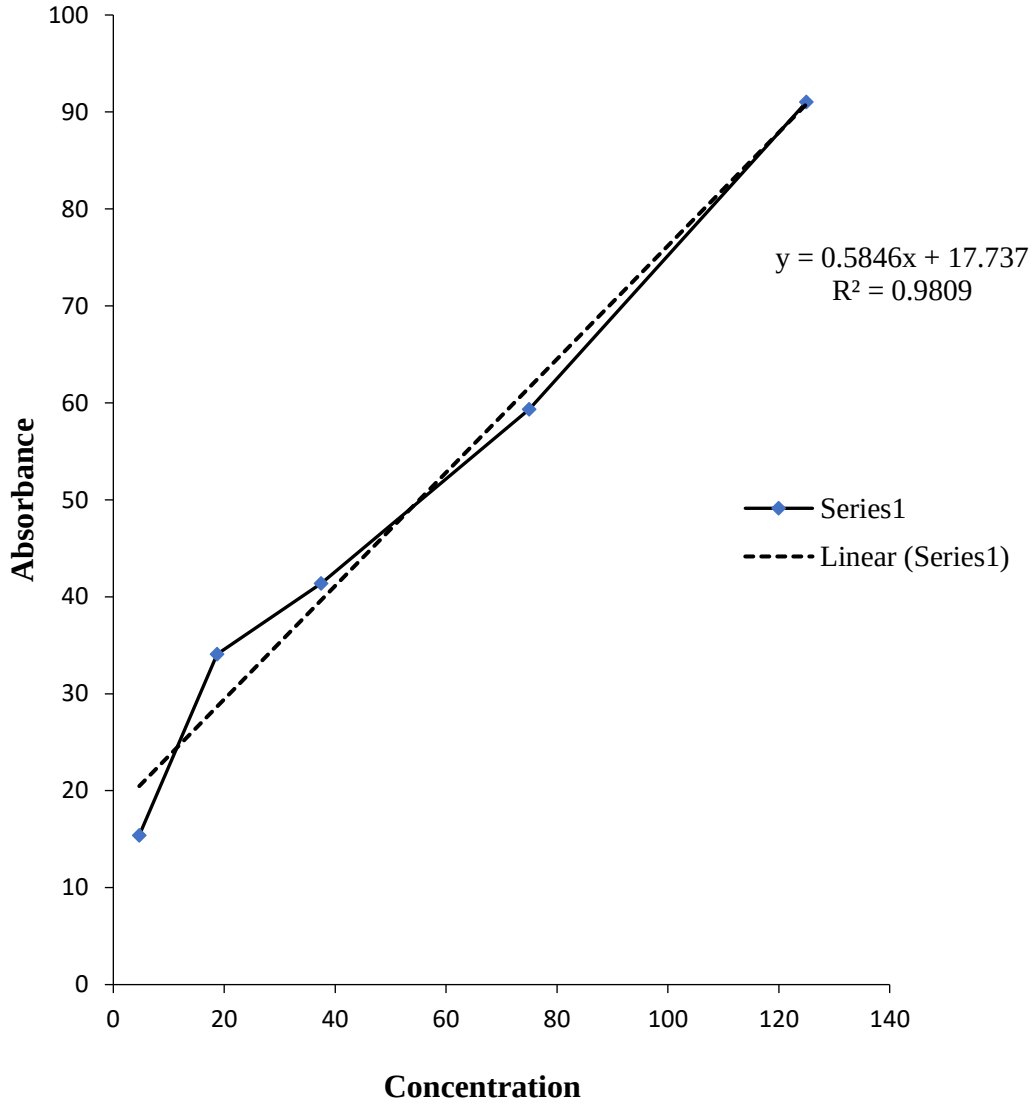

Fig C.3 Ascorbic Acid curve

Appendix D

ANOVA Results

Table D.1 One Way ANOVA table for Moisture

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Treatment	3	31.3908	10.4636	80.86	<.001
Residual	8	1.0352	0.1294		
Total	11	32.4261			

Since F pr <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.2 One Way ANOVA table for Protein

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Treatment	3	161.0898	53.6966	123.55	<.001
Residual	8	3.4770	0.4346		
Total	11	164.5668			

Since F pr<0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.3 One Way ANOVA table for Fat

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Treatment	3	3.99773	1.33258	46.18	<.001
Residual	8	0.23087	0.02886		
Total	11	4.22860			

Since F pr <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.4 One Way ANOVA table for Crude Fiber

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Treatment	3	1.9067	0.6356	2.60	0.124
Residual	8	1.9530	0.2441		
Total	11	3.8596			

Since F pr >0.05, there is no significant difference between the samples. So, LSD testing is not necessary.

Table D.5 One Way ANOVA table for Ash

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Treatment	3	0.8761	0.2920	1.48	0.293
Residual	8	1.5832	0.1979		
Total	11	2.4593			

Since F pr >0.05, there is no significant difference between the samples. So, LSD testing is not necessary.

Table D.6 One Way ANOVA table for Carbohydrate

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Treatment	3	213.4754	71.1585	206.41	<.001
Residual	8	2.7580	0.3447		
Total	11	216.2334			

Since F pr <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.7 One Way ANOVA table for Iron

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Treatment	3	6.5241	2.1747	4.38	0.042
Residual	8	3.9684	0.4960		
Total	11	10.4925			

Since F pr < 0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.8 One Way ANOVA table for Calcium

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Treatment	3	94.950	31.650	31.23	<.001
Residual	8	8.107	1.013		
Total	11	103.057			

Since F pr <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.9 One Way ANOVA table for Polyphenol

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
Treatment	3	2695.7172	898.5724	2817.76	<.001
Residual	8	2.5512	0.3189		
Total	11	2698.2683			

Since F pr <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.10 One Way ANOVA table for Phytate

Source of variation	Degree of freedom	Sum of square	Mean square	Variance	F probability ratio
Treatment	3	3802.96	1267.65	28.25	<.001
Residual	8	358.98	44.87		
Total	11	4161.94			

Since F pr <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.11 One Way ANOVA table for Tannin

Source of variation	Degree of freedom	Sum of square	Mean square	Variance	F probability ratio
Treatment	3	307483.09	102494.36	5398.25	<.001
Residual	8	151.89	18.99		
Total	11	307634.98			

Since F pr <0.05, there is significant difference between the samples so LSD testing is necessary.

Table D.12 One Way ANOVA table for Antioxidant

Source of variation	Degree of freedom	Sum of square	Mean square	Variance	F probability ratio
Treatment	3	7048.869	2349.623	1094.90	<.001
Residual	8	17.168	2.146		
Total	11	7066.037			

Since F pr <0.05, there is significant difference between the samples so LSD testing is necessary.

Appendix E

Photo Gallery

Plate 1: Germinated fenugreek seeds

Plate 2: Soaked fenugreek seeds

Plate 3: Roasted fenugreek seeds

Plate 4: Crude fiber determination

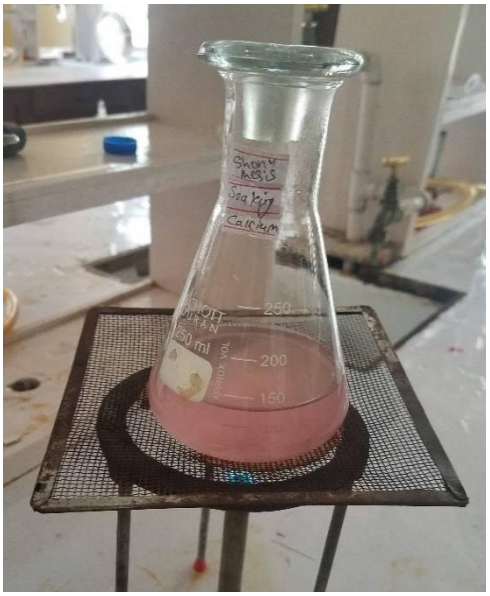

Plate 5: Calcium determination

Plate 6: Determination of Phenol

Plate 7: Determination of Phenol

Plate 8: Distillation in Kjeldahl's distillation set

Plate 9: Determination of DPPH radical scavenging activity

Plate 10: Study of free radical scavenging activity of fenugreek seed using spectrophotometer