Nutritional attributes, bioactive components, antioxidant activity and amino acid profile of fenugreek seed powder supplemented bread

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Roll No: 0219/05

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

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AUGUST 2022

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Dedicated to my beloved family and teachers

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Abbreviation

%	**:** Percentage
ANOVA	**:** Analysis of variance
AOAC	**:** Association of Official Analytical Chemists
°C	**:** Degree Celcius
DPPH	**:** 2,2-diphenyl-1-picrylhydrazyl
et al	**:** Et alii/ et aliae/ et alia
etc	**:** Et cetera
FSP	**:** Fenugreek seed powder
g	**:** Gram
GAE	**:** Gallic acid equivalent
mg	**:** Miligram
ppm	**:** Parts per million
QE	**:** Quercetin equivalents
SD	**:** Standard deviation
TE	**:** Trolox equivalent

Abstract

Fenugreek (*Trigonella foenum-graecum L.*) seeds are nutrient-dense having high protein, fiber and mineral content. But the bitter flavor of fenugreek seeds restricts their widespread use in culinary applications. Therefore, attempts have been made in the current study to develop wheat-fenugreek-based bread by using fenugreek seed powder with wheat flour at 5%, 7.5% and 10% level of supplementation to ascertain the sensorial acceptability and subsequently to analyze the nutritional composition, bioactive compounds, antioxidant capacity and amino acid profile. The ranges of protein, fat, crude fiber, ash and carbohydrate content were determined to be 15.31-16.28%, 3.77-4.99%, 1-1.70%, 2.53-2.72% and 39.62-43.54% respectively. Among the bioactive compounds, total flavonoid content and total phenolic content were higher in the formulated breads, ranging from 37.88-61.34 mg QE/100g and 2.82-3.33 mg GAE/100 ml respectively, compared to the control (18.68 mg QE/100g and 2.71 mg GAE/100ml). However, total anthocyanin content decreased significantly. The antioxidant capacity was increased by 82.39% (1.76 to 3.21 mg TE/100g). Moreover, seven essential and eight non-essential or conditionally essential amino acids were found in fenugreek seed powder supplemented bread from which lysine and glycine were absent in the control bread. The quantity of lysine and glycine in the formulated bread with 7.5% fenugreek seed powder was 51.96±2.69 ppm and 3.99±0.10 ppm respectively. Bread with 7.5% supplementation had the highest acceptance rate in the sensory evaluation.

Keywords: Antioxidant capacity, Amino acid profile, Bread, Bioactive compounds, Fenugreek seed powder

Chapter-1: Introduction

A plant's therapeutic value identifies it as a medicinal plant. Today, it is discovered that medicinal plants are used in a variety of products, including pharmaceuticals, neutraceuticals, cosmetics, and food supplements. Additionally, they are employed as conventional sources of medication. Fenugreek is one of those significant medicinal plants on the list. In many countries across Asia, Africa, and the Mediterranean, the seeds of the plant are consumed on a daily basis as a primary source of nutrition (Basch *et al.,* 2003). Additionally, the seeds are utilized in a variety of other contexts, including medicine, nutrition, beverages, perfumes, cosmetics, and industrial applications (Djeridane *et al.,* 2006). The seeds of the fenugreek plant are ground up and used as a spice in the production of several cheeses, most notably parmesan. The flavor and scent of foods are both improved by the addition of fenugreek seeds. Fenugreek seeds are also used to flavor coffee and vanilla extracts (Szczyglewska, 2000).

In addition to fiber, lipids, vitamins, minerals, and an easily digestible form of lysine (5.7 g/16 g N), fenugreek also has a percentage of protein that hovers around 30 percent (Chaubey *et al.,* 2018). Because of its high protein content, which can range from 250.00 to 386.00 grams of protein per kilogram, fenugreek seeds are a good source of protein derived from plants. Albumins (438.00 g/kg), globulins (272.00 g/kg), glutelins (172.00 g/kg), and prolamines (74.00 g/kg) are the primary protein components, in that order (Leela and Shafeekh, 2008). The seeds of fenugreek include a number of different types of bioactive substances, such as flavonoids (quercetin, rutin, and vetexin), saponins (graecunins, fenugrin B, and fenugreekine), and amino acids (isoleucine, 4-hydroxyisoleucine, histidine, leucine, lysine).

Fenugreek possesses a wide range of pharmacological properties, including those that are antibacterial, anticholesterolemic, carminative, febrifuge, laxative, restorative, uterine tonic, expectoral, galactogogue, anticarcinogenic, antiinflammatory, antiviral, antioxidant, hypotensive, and many others (Moradi-Kor and Moradi, 2013).

Fenugreek has been shown to have a beneficial effect on blood purification, and being a diaphoretic, it also has the ability to induce sweating and contribute to the detoxification of the body. It's possible that the antidiabetic benefits of fenugreek seeds come from the soluble fiber fraction of the seeds, which has a high

concentration of galactomannan. Glycemic control was improved, according to the findings of a clinical examination of a small sample of people with intermediate type-2 diabetes mellitus (Losso *et al.,* 2009). Additionally, mucilage, tannins, pectin, and hemicellulose lower blood levels of low-density lipoprotein (LDL) cholesterol by inhibiting bile salt absorption in the colon. This results in lower overall cholesterol levels (Ahmad *et al.,* 2016). For instance, phenolic components are thought to have the capacity to prevent cancer due to their antioxidant activity (Afzal *et al.,* 2016).

Recent scientific studies have shown that fenugreek extracts are efficient in managing blood sugar levels in both streptozotocin diabetic mice (Swanston-Flatt *et al.,* 1989) and human trials (Sharma *et al.,* 1990). Fenugreek extracts have also shown their potential in the treatment of coronary artery disease (CAD) in patients (Bordia *et al.,* 1997), the alleviation of physiological oxidative stress and inflammation in arthritic mice (Sindhu *et al.,* 2012), and the prevention of hepatotoxicity from the anti-cancer drug Adriamycin in albino rats (Sakr and Abo-El-Yazid, 2012).

Bakery goods including pizza, bread, muffins, and cakes have been made using flour that has been enriched with 8–10% fenugreek fiber and yet have acceptable sensory qualities (Srinivasan, 2006). When fenugreek flour was used to partially replace wheat flour in the amount range of 50-200 g/kg, significant improvements were seen in the protein content, sensory qualities, and rheological properties of foods such as bread, biscuits, noodles, and macaroni (Hooda and Jood, 2003). Studies have also indicated that adding 150 g/kg of germinated fenugreek seed flour to wheat flour enhanced the quantity of lysine, crude fiber, and protein digestibility; however, as a result of the addition, there are issues regarding the bread's volume and consistency (Hooda and Jood, 2005). For availing the health benefits of fenugreek we attempt to prepare fenugreek bread with different proportion of fenugreek seed powder with whole wheat flour which provide high protein and fiber content; possess good amount of amino acid, antioxidant and bioactive compounds.

Aims and objectives:

I. To prepare bread using fenugreek seed powder.
II. To analyze and contrast the formulated bread’s nutritional composition, bioactive components, antioxidant activity and amino acid profile.
III. To assess the palatability and overall acceptability of the developed product.

Chapter-2: Review of literature

2.1 Overview of Fenugreek

Since ancient times, the plant Trigonella foenum-graecum L., also known as fenugreek and more generally referred to as methi, has been used in the kitchen as a spice, as an ingredient in flavoring, and as a medicinal herb. One of the seed spices, fenugreek is a crop that belongs to the leguminous, herbaceous, and rainfed family. It attains a height of 30 to 60 centimeters when fully grown, with leaflets that are around 2 to 2.5 centimeters in length, and axillary sessile blooms that are 1 to 2 centimeters in length. It is cultivated all across the country. Fenugreek is one of the spices that is used in esoteric cooking. It is used to make the dish better in terms of its flavor, color, and taste, as well as to make the texture different (Chatterjee, 2015).

Fenugreek is a leguminous herb that is a member of the fabaceae family and is grown in many different regions of the world, but it is most popular in Asia and North Africa. Distinct languages have different names for it, including Fenugrec (French), Methi (Hindi), Bockshorklee (German), Fienogreco (Italian), Pazhitnik (Russian), Alholva (Spanish), Koroha (Japanese), Hulba (Arabic), Halba (Malaya), and K'u-Tou (China). Fenugreek is mostly produced in India, and its primary uses are in food and medicine.

Scientific classification:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsidaa

Order: Fabales

Family: Fabaceae

Genus: *Trigonella*

Species: *foenum-graecum*

2.2 Nutritional properties

The seeds of fenugreek contain a significant quantity of fatty acids, the majority of which are made up of oleic, linoleic, and linolenic acids. The dried seeds of fenugreek

contain 7.5% total lipids, 84.1% neutral lipids, 5.4% glycolipids, and 10.5% phospholipids, according to studies (Hemavathy and Prabhakar, 1989). Additionally, the seeds are a great source of galactomannans, which make up nearly half of the seed's weight. These galactomannans are an important component of the seed (Raghuram *et al.,* 1994). Galactomannan is a polysaccharide that has hypoglycemic activity. It has a high water binding capacity and is a soluble component of fiber (Srinivasan, 2006). The seeds of this plant are also commercially useful due to the presence of the saponins diosgenin, tigogenin, yamogenin, and gitogenin (Taylor *et al.,* 1997), and the bitter flavor of the seeds is produced by the presence of 4 to 8% saponins together with 1% of the alkaloid 'Trigonelline' (Fatima *et al.,* 2018).

When the chemical makeup of fenugreek extract was examined, it was shown that raw fenugreek seeds had more dietary fiber (46.50%) than soaked seeds (42.12%) or germinated seeds (32.50%). Soaking reduced the amount of dietary fiber, reducing sugars, non-reducing sugars, and total soluble sugars while increasing the amount of protein, starch digestibility, and mineral availability. Furthermore, it was shown that fenugreek seeds that had been treated contained considerably more protein and lysine than raw seeds. However, fenugreek seeds' starch and dietary fiber content decreased during germination, whereas their sugar level increased (Hooda and Jood, 2003).

Vitamins such as vitamin A, B1, B2, C, nicotinic acid, and niacin are abundant in fenugreek seed. Biotin, calcium pantothenate, pyridoxine, vitamin C, and cyanocobalamine are all present in germination seeds. However, according to Leela and Shafeekh (2008), exposure to radiations B and C considerably lowers the vitamin contents. Fenugreek seeds also contain a decent quantity of phosphorus, sulfur (Nasri and Tinay, 2007) and calcium (Jani *et al.,* 2009).

Diosgenin, the main steroidal sapogenin found in fenugreek seeds, is in quite high demand in the pharmaceutical business for the manufacture of steroidal medicines, sex hormones, and oral contraceptives (Jayadev *et al.,* 2004). Because of the seeds' capacity to prevent dandruff, its commercial formulations are particularly well-liked, for instance, it may be used as a hair conditioner.

Fenugreek was added to bread by Losso *et al.* (2009) who also illustrated that fenugreek in diet lowers blood sugar levels. Additionally, according to Hussein *et al.* (2011), raw, soaking, and germinated fenugreek seed significantly reduced total

lipids, serum total cholesterol, and LDL cholesterol. On the other hand, triglycerides and serum HDL-cholesterol did not demonstrate any significant changes. When fenugreek leaves, seeds (dry and germinated), and wheat flour enriched with germinated fenugreek powder at levels of 5–10% were added to basal diets, the total protein, fiber, iron, zinc, calcium, vitamin B2, carotene, vitamin E, and vitamin C contents were raised. In addition, the vitamin B2 content was increased (Wani and Kumar, 2018).

2.3 Bioactive components

2.3.1 Flavonoids

Flavonoids are an important class of natural products. More specifically, flavonoids are a class of secondary plant metabolites with a polyphenolic structure. They are typically found in fruits, vegetables, grains, bark, roots, stems, flowers, and tea. Flavonoids have been shown to have anti-inflammatory and antioxidant properties and play important roles in a diverse assortment of biological activities that occur in plants, animals, and microorganisms. Flavonoids in plants are responsible for a variety of biological processes, including the color and smell of flowers, the ability of fruits to attract pollinators, and, as a result, fruit dispersion, which helps in seed and spore germination, as well as the growth and development of seedlings. Flavonoids also play a role in the formation of seedlings (Griesbach, 2005). Flavonoids have several functions, including those of signal molecules, allopathic compounds, phytoalexins, detoxifying agents, and antimicrobial defense chemicals (Takahashi and Ohnishi, 2004). Flavonoids may also act as unique UV filters. In addition to this, they shield plants against the effects of a wide range of biotic and abiotic stressors (Samanta *et al.*, 2011).

Flavonoids can be chemically distinguished into six structural subgroups: flavones, flavonols, flavanones, flavanonols, flavan-3-ols (catechins), and anthocyanidins. These substances (aglycones) are typically glycosylated (at one or more sites with a variety of sugars) and may also be alkoxylated or esterified. This has led to the identification of approximately 5000 distinct flavonoids in plant materials (Harborne and Williams, 1992). The analytical processes used to determine the flavonoid content in various plants are based on the formation of an aluminum chloride complex, which is employed in most documented methods for the measurement of flavonoids

(Grubesic *et al.,* 2007). Literature survey reveals the presence of three classes of flavonoids in *Trigonella foenum-graecum*: flavones, flavonols and anthocyanins (Benayad *et al.*, 2014).

2.3.2 Phenolic compounds

Phenolic compounds are the most extensively dispersed secondary metabolites and are found across the plant kingdom, even if the specific sort of phenolic compound that is present varies depending on the phylum under consideration. Accounting for around 40% of the organic carbon that circulates in the biosphere, phenolic compounds are produced biosynthetically by the malonate/acetate system, also known as the polyketide pathway, or the shikimic acid pathway (Chapman and Regan, 1980). Phenolics found in food material can be divided into three major groups: simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids (Ho, 1992).

Plant phenolics have been scientifically demonstrated to protect a wide range of chronic illnesses associated with oxidative stress, including cancer, cardiovascular, and neurological disorders (Dai and Mumper, 2010).

Muhson and Mashkor (2014) examined the effect of extraction solvents on total phenolic content (TPC) of fenugreek seeds and results showed that the highest content of TPC were found in 50% acetone extracts (15.45 to 25.90 mg GAE/100 g DW). However, Kenny *et al.,* (2013) reported that the fenugreek ethyl acetate crude extract contained the highest phenolic content (106.316 ± 0.377 mg GAE/g).

2.3.3 Anthocyanins

Anthocyanins are glycosides of anthocyanidins that are water-soluble. Anthocyanins are a subclass of the phenolic chemicals. They are primarily responsible for the attractive coloring that can be found in a variety of plant tissues, such as flowers, leaves, fruits, as well as storage organs, roots, tubers, stems, and grains. The colors light yellow, orange, red, magenta, violet, and blue can be found in these plant tissues (Martin *et al.,* 2017). In addition to their function in improving plant tolerance to a number of abiotic conditions such as salinity, drought, excessive light, UV radiation, and cold stress, these compounds have attracted a lot of interest in recent years as food colorants that replace artificial dyes. This is in addition to their function in

improving plant tolerance to a number of abiotic conditions such as salinity, drought, excessive light, UV radiation, and cold stress. In addition, past studies demonstrated the importance of anthocyanins to human health as well as their potential to defend against the development of chronic diseases (Nassour *et al.,* 2020).

Anthocyanins and anthocyanidins have a higher antioxidant activity compared to other flavonoids because of the distinctive chemical composition of these pigments. The capacity of these compounds to bind metal ions is what gives them the power to combat the production of free radicals and reduce the amount of peroxidation caused by metals (Dai *et al.,* 2012; Martin *et al.,* 2017). Anthocyanins are also highly powerful donors of hydrogen to reactive oxygen species (ROS) and free radicals, which detoxifies them and prevents further creation of radicals. This is because of their positive charge, the quantity of hydroxyl and methoxyl groups they contain and their positions within the molecule, as well as the existence of electron-donating and electron-drawing substituents. This function protects vital biomolecules, including as DNA, proteins, and lipids, from the oxidative damage that has been linked to a variety of disorders and the acceleration of the aging process (Pojer *et al.,* 2013; Martin *et al.,* 2017). The anthocyanidins and anthocyanins that are most usually discovered have higher radical-scavenging activity than the well-known and potent antioxidants; for instance, cyanidin has an antioxidant capacity that is up to 4.4 times bigger than that of ascorbic acid and the vitamin E equivalent (Gould *et al.,* 2002).

2.4 Antioxidant activity

Antioxidants are in charge of neutralizing free radicals and defending our bodies against numerous ailments linked to free radicals. The initiation, propagation, and termination of the mechanism are all related to the oxidative process that is mediated by free radicals. Antioxidant production can take place both within the body and naturally in a variety of foods (Alam *et al.,* 2020).

Studies have demonstrated that fenugreek seeds are a rich source of polyphenols, which has led to the quantification of several compounds by HPLC. These compounds include apigenin and a number of kaempferol and quercitin gylcosides (Chatterjee *et al.,* 2009), as well as the flavonoids; vitexin, tricin, naringenin, quercetin, and tricin 7-O-β-D-glucopyranoside (Shang *et al.,* 1998). Additionally,

Chatterjee *et al.* (2009) hypothesized a connection between these polyphenols and the antioxidant activity of fenugreek.

2.5 Amino acid profile

Amino acids are an important class of nutrients. They are utilized by the human body as substrates in several metabolic processes as well as for protein synthesis, cell signaling, and the synthesis of low-molecular weight nitrogenous compounds. Because amino acids are involved in the regulation of certain physiological processes, it is possible to use them to assist in the treatment of a variety of illnesses. Supplementing with methionine, for example, has been shown to be beneficial for people with multiple sclerosis (Singhal *et al.,* 2018); arginine therapy has been shown to have a neuroprotective effect after brain ischemia injury (Chen *et al.,* 2020); supplementing with histidine has been shown to improve insulin sensitivity and reduce hyper-insulinemia; supplementing with glycine has been shown to lessen the effects of liver and lung injury; and tryptophan is administered to patients suffering from depression as well as sleep disturbances (Wu, 2013).

Although wheat is a significant source of calories and other nutrients, it is regarded as a poor source of nutrition because the cereal proteins lack essential amino acids like lysine and threonine. The demands of individuals in underdeveloped nations for protein, minerals, and B-complex vitamins are considerably aided by grain legumes. In addition to being high in calcium, iron, and beta-carotene, fenugreek contain a high quantity of protein (25%), lysine (5.7 g/16 g N), soluble dietary fiber (20%), and insoluble dietary fiber (28%) as well (Sharma, 1986; NIN, 1987).

2.6 Beneficial aspects of fenugreek

Fenugreek seeds are rich in Vitamin E. Fresh fenugreek leaves are helpful for treating a sluggish liver, flatulence, and indigestion. Nursing moms are given fenugreek seeds in gruel to boost milk production and decrease the proportion of calcium oxalate that builds up in the kidneys and causes kidney stones. Fenugreek appears to reduce the risk of colon cancer in animal tests by obstructing the activity of certain enzymes (Yadav *et al.*, 2011).

Cyclophosphamide (CP), a medicine that is often used in the treatment of cancer, is known to have negative effects on the reactive metabolites it produces. The toxicity

that was generated by exposing mice to fenugreek extract together with CP and L-buthionine-SR-sulfoximine (BSO) was evaluated by measuring the levels of lipid peroxidation (LPO) and antioxidants in the urine bladder of the mice. Fenugreek had protective effects on enzymatic antioxidants as well as LPO. Glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GP), and catalase (CAT) activity all significantly decreased in cyclophosphamide-treated mice compared to the controls. All of the enzymes' activities were restored by the pre-treated fenugreek extract, which demonstrated a general protective effect on the synergistic impact of CP and BSO (Bhatia *et al.,* 2005).

The second leading cause of mortality in the world is cancer. When administered at a dose of 200 mg/kg to rats, fenugreek seed extract shown possible preventive action against 7, 12-dimethylbenz (a) anthracene (DMBA)-induced breast cancer. Fenugreek seed extract significantly inhibited the DMBA-induced mammary hyperplasia and decreased its incidence. Apoptosis was mentioned as a potential mediator of the antibreast cancer properties of fenugreek in an epidemiological investigation (Amin *et al.,* 2005).

The process of cell death is known as apoptosis. The ability of flavonoids and catechins to trigger apoptosis in human cancer cells was first discovered (Ahmad *et al.*, 2000). Since that time, the same observation has been made regarding cancer cells of the lung (Valette *et al.,* 1984), colon, breast, prostate (Hannan *et al.,* 2003), stomach (Zia *et al.,* 2001), brain, head and neck and cervical regions of the body. It has also been published that additional dietary flavonoids suppress the development of cancer in animal models (Puri *et al.,* 2002; Devi *et al.,* 2003; Thakran *et al.,* 2003). It has also been demonstrated that fenugreek extract stimulates macrophages. According to the current study, fenugreek has significant anti-cancer efficacy. Although the most potent anti-cancer component of fenugreek is currently unknown, on the basis of research that has been made public, flavonoids appear to be the most efficient in inducing an anti-tumorigenic effect.

Dietary fiber has long been known to provide significant advantages for those with diabetes mellitus. According to animal research, dietary fiber can prolong stomach emptying and prevent the release of insulinotropic hormones and gastric inhibitory peptides. According to several studies, fenugreek seed decoction therapy alleviated

diabetes, controlled glycosuria in people with moderate diabetes, and improved their severe diabetic state (Srinivasan *et al.,* 2005). Fenugreek contains fiber to an extent of 51.7%, 19.2% of which is mucilaginous and 32.5% of which is neutral. Additionally, it includes the trigonelline alkaloid, which affects glycosuria.

Fenugreek increases fecal bile acid and cholesterol excretion. Too big of micelles develop in the digestive system for absorption due to an interaction between bile acid and saponin generated from fenugreek. Another explanation for the decrease of cholesterol is that the seed's fiber-rich gum component slows the production of cholesterol in the liver. Both pathways most likely contribute to the overall impact (Yadav *et al.,* 2011).

Researchers found that fenugreek seed extract, which is high in polyphenols, may have a protective effect against hydrogen peroxide (H2O2)-induced oxidation in both healthy and diabetic human erythrocytes (RBCs) (Yadav *et al.,* 2011).

It was discovered that the leaf and seed extract, when combined with a variety of organic solvents, was efficient against a variety of bacteria, including *Escherichia coli, Salmonella typhi, Staphylococcus aureus*, and fungi like *F. oxysporum f. sp. lycopersici* (FOL) and *F. oxysporum f. sp. radicis-lycopersici* (FORL) etc (Yadav *et al.,*2011). According to a research evaluating the quality of honey with different pollen contents, heterofloral honey was shown to be the most nutrient-dense variety, while samples that had the most fenugreek pollen were the ones that showed the most effective antibacterial action against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* (Mercan *et al.,* 2007). The tested honey was more efficient in eliminating the mentioned bacteria than antibiotics.

When the aqueous and methanolic extracts of fenugreek seed were compared to diclofenac potassium, a well-known analgesic medicine, the tail flick test revealed that the fenugreek seed extracts may have a possible analgesic impact (Yadav *et al.,* 2011).

Except that fenugreek may lower potassium levels in the blood, raise the risk of bleeding, induce loose stools in certain women, cause face puffiness, and make breathing difficult. Additionally, it might cause uterine contractions, hypoglycemia in some mothers and other side effects (Yadav *et al.,* 2011).

2.7 Bread as whole wheat bakery product

Bread is a common food that is closely associated with people's everyday life. It is made by baking dough consisting of flour, water, and leavening agents. Bread is one of the oldest dishes consumed all across the world. Typically, bread is regarded as a significant source of carbs in dietary guidelines. The primary component of bread is flour, and its attributes greatly rely on the characteristics of the grain from which flour is made. The amount of milling affects the flour's chemical makeup. Increased milling increases the amount of inorganic compounds, insoluble fiber, and vitamins in the bark while decreasing the amount of starch (Belitz *et al.*, 2004; Hui, 2006). The components of flour are protein, starch and other carbohydrates, lipids, fiber, water, and ash. In addition to this, it contains a meager amount of vitamins, minerals, and enzymes (Giannou *et al.*, 2003). The protein content determines the quantity of gluten in the flour to be formed, which in turn affects the degree of strength, dough's form as well as structure. Since hard wheat has a greater protein level than soft, it is utilized to make bread (Andrikopoulos, 2010). Along with the proteins that make up 10-12% of the flour, starch makes up 70-75%, while about 14% is water. It also contains non-starch polysaccharides ranging from 2% to 3%, with arabinoxylans and lipids in the same proportion. These elements, while having minimal quantities, are crucial to the baking process and the final product's quality (Bushuk, 2001).

The bread serves as a fantastic source of complex carbohydrates, which provide the body the energy it requires and are crucial to maintaining blood glucose (Psaltakis, 2002). Almost all bread varieties come with low-calorie, low-fat plant-based protein. Moreover, they include B vitamins, vitamin E, trace elements including iron, potassium, and calcium (Andrikopoulos, 2010). Bread is considered to be a product that is rich in fiber and, as a result, has a positive effect on the physiology of the body when it contains at least 6 grams of fiber for every 100 grams of product, as stipulated by European legislation (Regulation (EC) 1924/2006, 2006) (Pereira and Ludwig, 2001).

2.8 Conclusion

Due to rising consumer health consciousness, there has been a significant surge in interest in adding active substances like dietary fiber and phenolic antioxidants to popular foods like bread. Consuming this kind of food might improve health

performance and illness prevention in people. When creating functional bakery items like bread, it's crucial to create something that will be both physiologically useful and acceptable to consumers in terms of appearance, flavor, and texture. Therefore, using natural components in bread while maintaining essential bread quality characteristics including hardness, surface color, texture, and flavor can give various benefits in the promotion of human health.

Chapter-3: Materials and methods

3.1 Study area

The experiment was carried out in the lab of the Department of Applied Food Science and Nutrition, Department of Food Processing and Engineering of Chattogram Veterinary and Animal Sciences University, Chattogram. The experiment was conducted for a period of six months from 1 April 2022 to 15 September 2022.

3.2 Collection of sample

Fenugreek seed was collected from the Pahartoli local market of Chattogram district. Other components necessary for the production of bread, including wheat flour, sugar, salt, butter, skim milk powder, and yeast, were acquired from the neighborhood market and super shop. Other materials that were necessary for the experiment were obtained from the laboratory stockpiles.

3.3 Preparation of fenugreek seed powder (FSP)

Fenugreek seeds were subjected to extensive water washing in order to clear them of any dirt or debris. Then they were spread on a tray and dried in a cabinet dryer at 60°C for 8 hours. To make powder, dried seeds were ground in a grinder and sieved to get fine powder. Thereafter, the powder was placed in an airtight container after being packaged in a zipper bag and stored until use.

3.4 Preparation of bread

Table 3.1 Formulations of bread

Ingredient	Control bread	Bread with 5% FSP (Sample A)	Bread with 7.5% FSP (Sample B)	Bread with 10% FSP (Sample C)
Wheat flour	56%	51%	48.5%	46%
FSP	-	5%	7.5%	10%
Sugar	3.4%	3.4%	3.4%	3.4%
Salt	1%	1%	1%	1%
Butter	1.6%	1.6%	1.6%	1.6%
Skim milk powder	1%	1%	1%	1%
Yeast	1%	1%	1%	1%
Water	36%	36%	36%	36%

Procedure

The bread formulations were baked using the straight dough method (Edwards, 2007) by following the recipe of Olaoye *et al.* (2006). At first, the ingredients were weighed according to the formulation given in the table 3.1. Then weighted wheat flour, FSP, salt, skim milk powder and melted butter were taken in a bowl and mixed. After that, the water was warmed; sugar was added in half of the warm water and stirred until the sugar dissolved. Then the yeast was added to the sugar solution, stirred and let it sit for 5 minutes. When the yeast puffed up and covered the entire surface of the water, it was added to the bowl of mixed ingredients. The remaining water was also added to the mixture and mixed well for about 2 minutes. To make the dough, the mixture was kneaded by hand for about 10 minutes. Kneading the dough helped it to rise better, be lighter and fluffier. Then the dough was covered by a clean wet towel and allowed to rise in an incubator at 30°C for about 1 hour with occasional sprinkle of water. After taking out the dough from the incubator, it was kneaded to release the air pockets that have developed. Then the dough was placed in a greased bread pan, covered with the wet towel and again placed in the incubator for 1 hour at 30°C for second rise. Thereafter, the pan was taken out from the incubator; the towel was removed and placed the pan in the preheated oven. Then it was baked at 200°C for 25-30 minutes. After baking, the bread was cooled, removed from the pan, cut into slices and stored in an air tight container until tested.

3.5 Nutritional analysis

Fenugreek seed powder and bread samples consisting of FSP were examined according to the procedures of AOAC 2016 for the quantity of moisture, protein, fat, fiber and ash.

3.5.1 Moisture content

Determining moisture is the most important and commonly utilized metric in the production and testing of meals. Since the percentage of dry matter in a serving of food is negatively correlated with the quantity of moisture it contains, the moisture content is directly relevant economically for both the processor and the consumer. However, the effect of moisture on the stability and quality of food is far greater. The water level was measured using a technique based on the Association of Official Analytical Chemists (AOAC, 2016) standard. An empty crucible was weighed, and

then a sample of 10 grams was placed inside the clean crucible. The weight of the crucible with sample was noted. After that the crucible was placed in a hot air oven at 105°C and dried for a period of 48 to 72 hours. After being taken from the furnace, the crucible was put into a desiccator so that it could cool down and be weighed. In this manner, a number of repetitions were carried out until a consistent result was found. The moisture percentage was calculated as follows

$$\text{Moisture\%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Sample weight}} \times 100$$

3.5.2 Crude protein

Using the Kjeldahl method, the nitrogen concentration of samples, both organic and inorganic, is assessed. Kjeldahl nitrogen is measured in foods and beverages, meat, feeds, cereals, and forages in order to determine the protein level. It is a standard operating procedure that may be found outlined in a variety of normative sources, like as (AOAC, 2016). This technique's fundamental premise is to digest the sample using a digestion mixture (sodium sulphate and mercuric oxide) and concentrated sulfuric acid (H_2SO_4). This results in the oxidation and destruction of protein as well as the conversion of organic nitrogen into ammonia, which subsequently remains as ammonium bisulphate in the acid mixture. In order to figure out the amount of ammonia nitrogen the digest must be made alkaline, then the discharged ammonia be distilled into a standard acid solution, and thereafter titrated the solution to measure the quantity.

A clean and dry 100 ml Kjeldahl flask was used to collect a 0.5 gram sample that was wrapped in an ash-free piece of filter paper. Following the addition of 10 ml of concentrated H_2SO_4 and a digestion combination consisting of sodium sulphate and mercuric oxide in a ratio of 1:1 gram, the content of the digestion chamber was cooked for a period of six hours until it was free of contaminants. Following the completion of the digestion procedure, the beaker was let to cool before the liquid that had been digested was transferred to a 100 ml volumetric flask and diluted with distilled water until it reached the mark. This process was repeated three times. After being infused with 5 ml of 50% NaOH and 2.5 ml of 15% $Na_2S_2O_3$, 10 ml of that solution was placed inside of a micro Kjeldahl distillation unit. The unit was then filled with that solution. The solution went through a ten-minute steam distillation

process. The distillate was first collected in solutions containing 2% boric acid with an indicator, and then the solutions were titrated with 0.02N hydrochloric acid. At the same time, a blank digestion was carried out in which none of the chemicals were included. Estimates for the amount of nitrogen and protein must be adjusted accordingly, taking into account both the nature of the solution that will be receiving the product and any dilution factors that will be applied during the distillation process. The term "normality" is denoted by the letter "N" in the equation that follows. If standard acid is the receiving solution, "ml blank" refers to the milliliters of base required to back titrate a reagent blank; if boric acid is the receiving solution, "ml blank" refers to the milliliters of standard acid required to titrate a reagent blank. When boric acid is serving as the receiving solution, the equation reads as follows:

$$\text{Nitrogen\%} = \frac{(\text{ml standard acid} - \text{ml blank}) \times \text{N of acid} \times 1.4007}{\text{Weight of sample in gram}}$$

3.5.3 Crude fat

It is possible to quantify the amount of fat in food by dissolving a sample of the meal in an organic solvent such as chloroform or methanol and then separating the filtrate using filtration. After separating the filtrate into numerous funnels, drying the mixture, and then calculating the approximate fat content, the extract is quantified. In accordance with the recommendations provided by AOAC (2016), the crude fat content of the samples was calculated with the use of a soxhlet apparatus. To indicate the percentage of crude fat, the following equation was used:

$$\text{Fat\%} = \frac{\text{Weight of the extract}}{\text{Weight of the sample}} \times 100$$

3.5.4 Crude fiber

A moisture and fat free sample that weighed around 2-5 grams was placed into a beaker with a capacity of 500 milliliters, and then 200 milliliters of boiling sulfuric acid with a concentration of 1.255% weight-per-volume was added to the mixture. The mixture was brought to a boil and kept at the same volume during the cooking process by continuously adding water at regular intervals. At the conclusion of this time period, the boiling process may be made more manageable by inserting a glass rod into the beaker. After passing the mixture through a filter made of shirting fabric,

the residue was rinsed with hot water until it no longer contained any acid. After that, the residue was moved to the same beaker, and 200 milliliters of boiling 0.313N (1.25%) sodium hydroxide was added. The mixture was strained through shirting fabric after being subjected to boiling for thirty minutes with the volume being maintained throughout. The residue was rinsed with hot water until it was clear of alkali, and then it was cleaned with an equal quantity of ether and alcohol. After that, it was put into a crucible, heated between 80 and 100 degrees Celsius for a whole 24 hours, and then weighed. After being heated in a muffle furnace at 650 degrees Celsius for two to three hours, the crucible was allowed to cool before being weighed once again. The weight of the crude fiber is equal to the difference in weight between the two quantities. Crude fiber percentage was calculated by using the following formula,

$$\% \text{ Crude fiber} = \frac{Weight\ of\ crude\ fiber}{\text{Weight of sample taken}} \times 100$$

3.5.5 Ash content

AOAC (2016) procedures were used to determine the ash content. The inorganic residue that is left behind following the destruction of organic material is referred to as the ash content. After removing the empty crucible, it was placed in a desiccator for one hour, heated to 105°C to speed up the drying process, and finally, it was weighed until it reached a consistent value. The sample weighed roughly one gram, and it was placed inside of the weighted but empty crucible. After adding one drop of nitric acid to the crucible, the specimen was combusted over a low flame before being examined. After that, the crucible was put inside of a muffle furnace, and the temperature was raised to 650°C before being kept at that level for three hours. After that, it was taken out of the crucible, allowed to cool, and placed in a desiccator while the weight of the crucible containing the ash was determined. For the calculation of the ash content, the following formula was used,

$$\text{Ash\%} = \frac{\text{The amount of ash in supplied sample}}{\text{Sample weight}} \times 100$$

3.5.6 Carbohydrate

The total percentage of carbohydrate was determined by the difference method as described by Edeogu *et al.,* (2007). The content of the available carbohydrate is determined by subtracting the sum of the values (per 100 gm) for moisture, ash, protein and fat from 100. The formula used for calculating carbohydrate content was as follows

$$\% \text{ Carbohydrate} = 100 - (\text{Moisture} + \text{Ash} + \text{Protein} + \text{Fat})$$

3.6 Energy estimation

The energy content of each sample was calculated using the following equation (Baer *et al.,* 1997),

$$\text{Energy} = (\text{Protein} \times 4.1) + (\text{Fat} \times 9.2) + (\text{Carbohydrate} \times 4.1)$$

3.7 Determination of bioactive compounds

Extract preparation:

In Felcon tubes, 5 grams of sample were acquired for the total anthocyanin content (TAC), whereas only 1 gram of sample was taken for each of the other total phenolic content (TPC) and total flavonoid content (TFC) tests. After that, 10 ml of ethanol that was 100 percent was added, and the combination was let to sit for three days. After taking a rest for four hours, constant straining was carried out. After waiting for 72 hours, a filtration sample was taken, and an ethanol extract was found.

3.7.1 Total Flavonoid content (TFC)

The total flavonoid content (TFC) of the samples was determined by using a technique that was significantly altered from the aluminum chloride colorimetric approach that was reported by Chang *et al.* (2002). Following the preparation of a stock solution of extracts at a concentration of 1 mg/ml, aliquots of 0.5 mL of the diluted extract were further diluted in a cuvette using 1.5 mL of 95% C_2H_5OH. After that, 0.1 mL of 10% $AlCl_3$ was added to the immixture that was contained in the cuvette, along with 0.1 mL of 1 mol/L potassium acetate and 2.8 mL of distilled water. The mixture was allowed to reach room temperature for a period of thirty minutes. The absorbance was determined at a wavelength of 415 nm using a UV-

visible spectrophotometer manufactured by Shimadzu Corporation in the United States (UV2600). The blank for the experiment was 10% aluminum chloride that had the same amount of D.H_2O added to it. The total quantity of flavonoids present in the sample was determined by analyzing the absorbance of the sample extracts and comparing it to a quercetin standard curve. TFC was calculated and presented in the form of milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g).

3.7.2 Total Phenolic Content (TPC)

For the purpose of determining the TPC of the extracts, the Folin-Ciocalteu reagent technique was used. After the addition of 1.5 ml of FC reagent to 1 ml of ethanoic extract in a falcon tube, the mixture was allowed to sit undisturbed at room temperature for three minutes. After that, 1.5 milliliters of 7.5% sodium carbonate was added to the mixture, and it was let to rest for an hour. At a wavelength of 765 nm, the absorbance was measured using a UV-VIS Spectrophotometer (model number UV2600, manufactured by Shimadzu Corporation in the United States) using C_2H_5OH as the blank. The theoretical potency concentration (TPC) was calculated to be mg of gallic acid equivalents per gram of extracts (mg GAE/g).

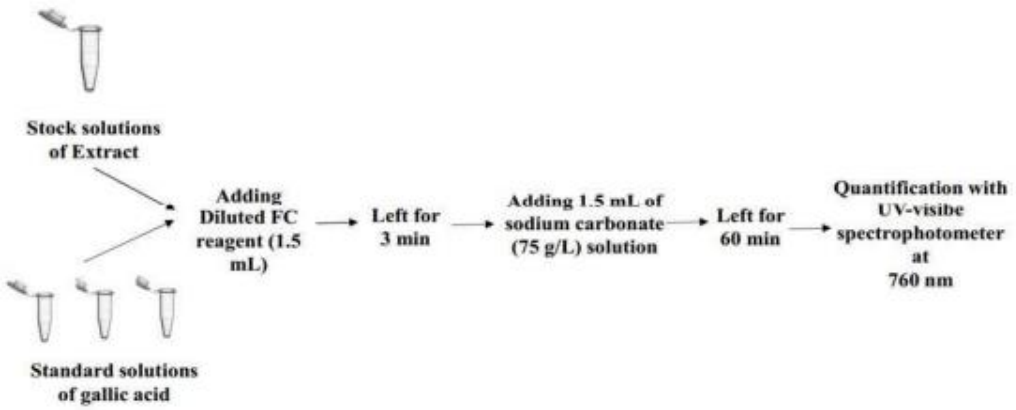

Figure 3.1: Determination of total phenolic content (TPC)

3.7.3 Total Anthocyanin content (TAC)

Stock solutions of 10 mg/ml of extracts were prepared. Extract solution (3 ml) was pipetted into a cuvette. The intensity of the extract color was measured at wavelength 520 nm using UV-VIS spectrophotometer. Ethanol was used as blank.

TAC was calculated and expressed as mg/100 ml using the following equation:

TAC = Absorbance of sample × DF × 100/M × E

Where, DF stands for dilution factor, M means weight of sample used to make stock solution, E refers to extinction co-efficient (55.9) (Giusti and Wrolstad, 2001).

3.8 Determination of Antioxidant capacity by DPPH scavenging method

Extract preparation:

A sample weighing one gram was taken and placed in a felcon tube. After that, 10 ml of methanol that was diluted to 100% was added, and the mixture was let to sit for three days. Following a break of four hours, repeated bending and reaching were carried out. After waiting for 72 hours, the methanoic extract was found in the supernatant that had been collected.

Procedure

The DPPH test was used to evaluate the antioxidant permeability of the extracts, with a few minor adjustments made to the protocol that was published by Azlim *et al.* (2010). In order to prepare a methanoic DPPH solution, 6 mg of DPPH was first dissolved in 100 mL of pure methanol. After that, 1 ml of methanoic extract and 2 ml of DPPH solution were mixed together. After giving the ingredients a little stir, they were allowed to sit undisturbed for half an hour at room temperature in the dark. The absorbance was determined by using a UV-VIS spectrophotometer and setting it to the wavelength of 517nm (UV-2600, Shimadzu Corporation, and USA). A control was created by combining 1 milliliter of methanol with 2 milliliters of DPPH solution. Methanol was utilized as the blank in this experiment. Both methanol and trolox were used in this experiment; the control was methanol and the reference was trolox. As a point of reference for the scavenging mobility, the DPPH standard solution's lowered intensity was compared to the samples' decreased intensity. The antioxidant capacity of the extracts was calculated with the use of the following equation, which was based on their ability to neutralize DPPH free radicals:

$$\% \text{ of inhibition} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100$$

For the purpose of drawing the calibration standard curve, the TEAC composite (Trolox equivalent antioxidant mobility) was used. This composite was also employed as the standard. The readings were given as mg/100 g of Trolox equivalents (TE) per gram of powder on a dry weight (DW) basis.

3.9 Sensory analysis

3.9.1 Affective test

The results of this test will show how acceptable "FSP incorporated Bread" is. It was carried out by 10 panelists, equally split between men and women, on the grounds of CVASU. A panel of untrained individuals administered the test. The panelists were asked to rank the three bread compositions according to their level of approval. The characteristics of the sample were evaluated using a 5-point grading system, with scores ranging from -1 (dislike extremely) to -5. (like extremely). Color, smell, texture, taste, and overall acceptance were the sensory attributes that the panelists had evaluated.

Table 3.2 Grading system for sensory assessment

Ranks	Score
Like very much	5
Like slightly	4
Neither like nor dislike	3
Dislike slightly	2
Dislike very much	1

3.10 Amino acid profile

Amino acid profile of the most preferable bread formulation and the control one was done by applying the Waters AccQ.Tag Amino Acid Analysis Method using High Performance Liquid Chromatography (Waters Corporation, USA, Model: e2695).

Sample preparation

The procedure for analyzing amino acids was applied to three replications of each sample. 2 grams of each sample was taken into a 50 ml volumetric flask and labeled. Then 20 ml of 6N HCl was added to each conical flask. After that the caps of the volumetric flasks were closed properly so that they become airtight. The hydrolysis was performed by keeping each flask in the oven at 105°C for 24 hours. When the hydrolysis was finished the flasks were cooled and each solution was mixed with 20 ml of water. The solution was mixed properly and filtered with whatman filter paper. The filtrate was collected and the pH of the filtrate was adjusted to 7-10 by using 0.1N NaOH. Then each solution was further filtered with 0.2 µm filter syringe and taken into sample vial of 2 ml as stock solution. For each stock solution about 50 µl sample was pipetted to another vial (reagent + sample) and labeled according to the replications. In this vial about 350 µl of AccQ-Fluor Borate Buffer was pipetted and vortexed for 10 seconds. After that 50 µl of reagent diluent and 50 µl reagent powder were pipetted into each vial and vortexed for 10 seconds. Finally, the solutions were heated at 55°C for 10 minutes. From each vial (reagent + sample) about 150 µl of the solution was pipetted into the HPLC vials containing the inner tube. Amino acid analysis was done by High Performance Liquid Chromatography System (Model: Waters e2695, Waters Corporation, USA) with Fluorescence detector (FLR 2475) with the C8 column (3.9 × 150 mm). The instrumental parameters were as follows: Column temperature 37°C, injection volume 10 µl, detection= Fluorescence at 250-395 nm and flow rate 1 ml/min. The method was done using the mobile phases: Aqueous Buffer, Acetonitrile, Deionized water.

3.11 Cost analysis

The overall cost of the items that were used to manufacture the bread was taken into account when determining how much the bread with fenugreek seed powder added would cost. The total was shown in taka and compared to the price of per package of bread.

3.12 Statistical analysis

Minitab 14.0 software was used to conduct statistical analyses. On the gathered data, a one-way analysis of variance was carried out (ANOVA). To determine whether there

were any statistically significant differences between them, Fisher's LSD test was employed following statistical software; level of significance is ($P<0.05$). Ten members of the panel decided to take part in the study, the results of which are detailed below.

Chapter-4: Results

4.1 Nutritional attributes

Nutritional characteristics of wheat flour and fenugreek seed powder was assessed by determining moisture, protein, fat, crude fiber, ash and carbohydrate content.

4.1.1 Nutritional composition of Fenugreek Seed Powder and wheat flour

Table 4.1 represents the nutritional composition of FSP and wheat flour that is used in this experiment. It was found that FSP contained significantly higher amount of protein (31.68±0.50), fat (4.97±0.31), crude fiber (13.55±0.24), ash (4.86±0.21) and lower amount of moisture (9.26±0.02) and carbohydrate (49.23±0.26) compared to wheat flour.

Table 4.1 Nutritional composition of FSP and wheat flour

Variables	Moisture (%)	Protein (%)	Fat (%)	Crude fiber (%)	Ash (%)	CHO (%)
FSP	9.26±0.02	31.68±0.50	4.97±0.31	13.55±0.24	4.86±0.21	49.23±0.26
Wheat flour	12.01±0.02	10.07±0.50	1.08±0.02	1.12±0.05	0.88±0.05	74.84±0.25

4.1.2 Nutritional composition of bread formulations

Nutritional properties of bread incorporated with FSP are shown in table 4.2; nearly all of the samples are significantly different from one another. Sample C that is bread formulated with 10% FSP contained the highest percentage of protein (16.28±0.11), fat (4.99±0.25), crude fiber (1.70±0.01) and ash (2.72±0.21). The lowest percentage of protein (15.05±0.06), fat (3.26±0.24), crude fiber (0.33±0.05) and ash (2.33±0.16) was found in the control bread.

Table 4.2 Nutritional composition of bread formulations

Variables	Moisture (%)	Protein (%)	Fat (%)	Crude fiber (%)	Ash (%)	CHO (%)
Control	34.05±0.02^{d}	15.05±0.06^{d}	3.26±0.24^{b}	0.33±0.05^{d}	2.33±0.16^{b}	45.31±0.28^{a}
Sample A (5% FSP)	34.85±0.01^{b}	15.31±0.12^{c}	3.77±0.20^{b}	1.00±0.02^{c}	2.53±0.07^{ab}	43.54±0.17^{b}
Sample B (7.5% FSP)	34.57±0.02^{c}	15.75±0.15^{b}	4.65±0.37^{a}	1.17±0.02^{b}	2.59±0.12^{ab}	42.44±0.35^{c}
Sample C (10% FSP)	36.39±0.05^{a}	16.28±0.11^{a}	4.99±0.25^{a}	1.70±0.01^{a}	2.72±0.21^{a}	39.62±0.50^{d}
P value	0.000	0.000	0.001	0.000	0.002	0.000

Legends: Means ± SD and values in the same column with different superscripts are statistically significant (P<0.05)

4.1.3 Energy content

From the figure 4.1, energy content was calculated in the highest amount (281.36 kcal/100gm) in sample B and lowest (275.10 kcal/100gm) in sample C.

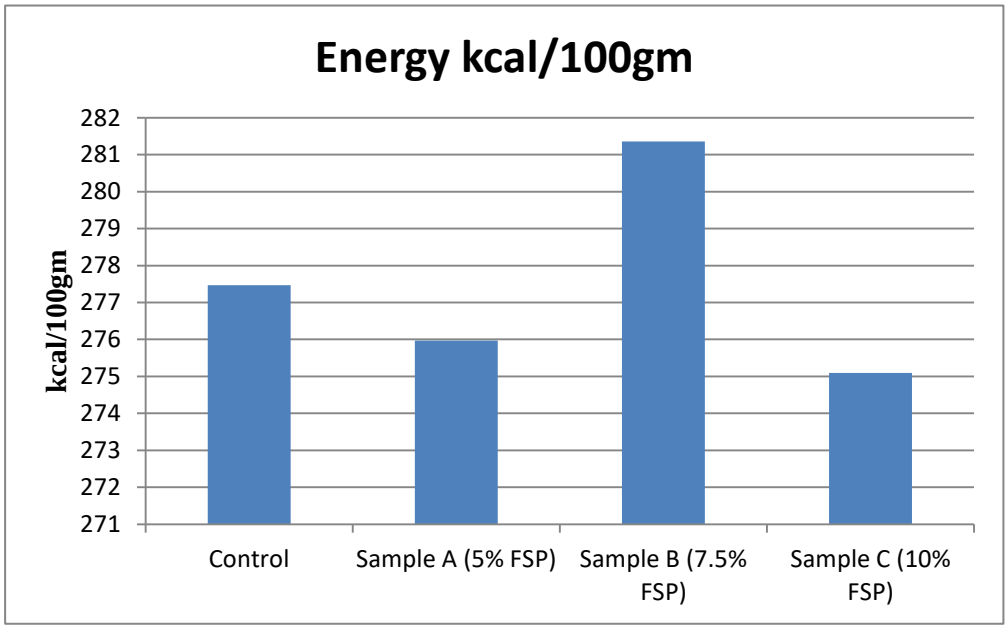

Figure 4.1: Comparison of energy content among bread formulations

4.2 Bioactive components

4.2.1 Bioactive components of FSP

Table 4.3 shows the results of the bioactive components (TFC, TPC, and TAC) of fenugreek seed powder.

Table 4.3 Bioactive components of FSP

Total Flavonoid Content (TFC) (mg QE/100g)	242.42±0.48
Total Phenolic Content (TPC) (mg GAE/100 ml)	3.97±0.01
Total Anthocyanin Content (TAC) (mg TA/100 ml)	229.38±1.96

4.2.2 Bioactive components of bread formulations

The quantity of TFC, TPC and TAC of breads are presented in table 4.4. There are significant differences in the values. The maximum quantity of total flavonoid content (61.34±0.05 mg QE/100g) and total phenolic content (3.33±0.010 mg GAE/100 ml) was found in sample C while sample A contained the highest value of total anthocyanin content (16.77±0.56 mg TA/100 ml). The lowest quantity of total anthocyanin content (8.57±0.32 mg TA/100 ml) was found in sample C whereas the value with the lowest amount of total flavonoid content (18.68±0.04 mg QE/100g) and total phenolic content (2.71±0.001 mg GAE/100 ml) was reported in the control bread.

Table 4.4 Bioactive components of bread formulations

Variables	Total Flavonoid Content (TFC) (mg QE/100g)	Total Phenolic Content (TPC) (mg GAE/100 ml)	Total Anthocyanin Content (TAC) (mg TA/100 ml)
Control	18.68 ± 0.04^{d}	2.71 ± 0.001^{d}	15.47 ± 1.71^{a}
Sample A	37.88 ± 0.04^{c}	2.82 ± 0.007^{c}	16.77 ± 0.56^{a}
Sample B	53.13 ± 0.02^{b}	2.87 ± 0.006^{b}	10.06 ± 0.56^{b}
Sample C	61.34 ± 0.05^{a}	3.33 ± 0.010^{a}	8.57 ± 0.32^{b}
P value	0.000	0.000	0.010

Legends: Means ± SD and values in the same column with different superscripts are statistically significant ($P<0.05$)

4.3 Antioxidant capacity

The antioxidant capacity of fenugreek seed powder was found to be 18.03±0.007 mg TE/100g. It is possible to deduce from table 4.5 that the antioxidant capacity was substantially greatest (3.21±0.001 mg TE/100g) in sample C and substantially lowest (1.76±0.005 mg TE/100g) in the control bread.

Table 4.5 Antioxidant capacity of bread formulations

Variables	Antioxidant capacity (mg TE/100g)
Control	1.76 ± 0.005^{d}
Sample A	2.76 ± 0.005^{c}
Sample B	3.14 ± 0.002^{b}
Sample C	3.21 ± 0.001^{a}
P value	0.000

Legends: Means ± SD and values in the same column with different superscripts are statistically significant (P<0.05)

4.4 Sensory evaluation

Sensory attributes of formulated breads are represented in table 4.6, where it can be seen that there were no significant differences between sample A and B although there were significant differences for sample C with sample A and B in terms of color, smell, taste and overall acceptability. In terms of texture, no significant variation was seen between sample A and C while sample B got the highest score. Overall sample B had the highest acceptance rate.

Table 4.6 Sensory score of bread formulations

Formulation	Color	Smell	Texture	Taste	Overall acceptability
Sample A	3.67 ± 0.52^{a}	3.50 ± 0.55^{a}	3.17 ± 0.75^{b}	3.17 ± 0.98^{a}	3.33 ± 0.52^{a}
Sample B	4.00 ± 0.01^{a}	4.00 ± 0.01^{a}	4.50 ± 0.55^{a}	3.00 ± 0.02^{a}	3.83 ± 0.41^{a}
Sample C	2.50 ± 0.55^{b}	2.33 ± 0.52^{b}	2.83 ± 0.41^{b}	1.00 ± 0.01^{b}	1.50 ± 0.55^{b}
P value	0.000	0.000	0.005	0.000	0.001

Legends: Means ± SD and values in the same column with different superscripts are statistically significant (P<0.05)

4.5 Amino acid profile

Sample B and control bread were analyzed to determine the essential and non-essential or conditionally essential amino acids which are Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Valine, Alanine, Asparagine, Cystine, Glutamine, Glycine, Proline, Serine and Tyrosine. The results are shown in table 4.7 and 4.8.

Table 4.7 Essential amino acid profile of breads

Amino acid	Control bread (ppm)	Bread with 7.5% FSP (ppm)	P value
Arginine	95.07 ± 8.46^{b}	149.01 ± 1.49^{a}	0.000
Histidine	-	-	-
Isoleucine	33.34 ± 2.46^{a}	9.24 ± 0.16^{b}	0.000
Leucine	4.59 ± 0.34^{b}	7.22 ± 0.17^{a}	0.000
Lysine	-	51.96 ± 2.69	-
Methionine	160.67 ± 7.08^{a}	135.00 ± 115.3^{a}	0.719
Phenylalanine	93.23 ± 8.23^{a}	108.53 ± 10.37^{a}	0.116
Threonine	-	-	-
Valine	1.71 ± 0.02^{b}	3.04 ± 0.07^{a}	0.000

Legends: Means ± SD and values in the same row with different superscripts are statistically significant ($P<0.05$)

Among the essential amino acids, histidine and threonine were not found in the samples. Sample B contained higher amount of arginine, leucine and valine compared to the control bread and isoleucine was higher in the control bread. When comparing the levels of methionine and phenylalanine in each of the samples, there was no discernible difference between them.

Table 4.8 Non-essential or conditionally essential amino acid profile of breads

Amino acid	Control bread (ppm)	Bread with 7.5% FSP (ppm)	P value
Alanine	2.68 ± 2.27^{a}	0.15 ± 0.01^{a}	0.126
Asparagine	74.82 ± 5.13^{b}	227.59 ± 2.40^{a}	0.000
Cystine	39.85 ± 1.53^{a}	31.53 ± 0.30^{b}	0.001
Glutamine	716 ± 562^{a}	858 ± 939^{a}	0.833
Glycine	-	3.99 ± 0.10	-
Proline	187.10 ± 7.06^{b}	240.30 ± 4.67^{a}	0.000
Serine	2735 ± 36.37^{a}	1165.7 ± 51.6^{a}	0.496
Tyrosine	14.9 ± 22.7^{a}	1.90 ± 0.09^{b}	0.037

Legends: Means ± SD and values in the same row with different superscripts are statistically significant ($P<0.05$)

Among the non-essential or conditionally essential amino acids, the amount of alanine, glutamine and serine showed no significant difference for both of the samples. Asparagine and proline was higher in sample B while the content of cysteine and tyrosine was higher in control bread.

Lysine and glycine were present in sample B but not found in the control bread.

4.6 Cost analysis

Table 4.9 Production cost of FSP supplemented bread

Heads	Tk	Quantity used	Total tk (for sample B)	Total tk (for control)
Raw materials				
Wheat flour	75tk/kg	224gm(control) 207.2gm(sample B)	15.54	16.8
Fenugreek seed	200tk/kg	16.8gm	3.4	-
Sugar	90tk/kg	13.6gm	1.3	1.3
Skim milk powder	130tk/200gm	4gm	2.6	2.6
Butter	120tk/100gm	6.4gm	7.7	7.7
Salt	38tk/kg	4gm	0.2	0.2
Yeast	60tk/50gm	4gm	4.8	4.8
Sub total			35.5	33.5
Processing cost @ 15% of the cost of raw materials			5.3	5
Packaging cost			3	3
Total production cost			43.8	41.5

Table 4.5 represents total production cost of control bread and bread with 7.5% FSP i.e. sample B which got the highest score in sensory evaluation. Raw materials were calculated for 400gm for both formulations. After baking, the weight of the control bread was 342gm and that of sample B was 354gm.

For control, total production cost was 41.5tk and for sample B, that was 43.8tk.

Chapter-5: Discussions

5.1 Nutritional attributes

When compared to wheat flour, which had much smaller magnitudes of ash, crude fiber, and protein (that is, 0.9%, 1.1%, and 10.1%, respectively), it was found that FSP had a greater amount of ash (4.9%), crude fiber (13.6%), and protein (31.7%) than wheat flour (Table 4.1). The nutritional composition of wheat flour and fenugreek seed powder reported in a study by Afzal *et al.* (2016) was in lined with the mentioned results.

The data shown in Table 4.2 make it abundantly clear that the incorporation of fenugreek seed powder into wheat flour had a substantial effect on the nutritional profile of the loaves made from wheat flour. The addition of FSP to wheat flour led to an increase in the amount of protein, fat, coarse fiber, and ash while concurrently leading to a decrease in the amount of carbohydrates. The augmentation in nutrient content of FSP incorporated breads was due to their higher contents in fenugreek seed powder compared to wheat flour (Table 4.1).

Moisture content of the breads gradually increased with increasing percentage of FSP ranging from 34.05% in the control bread to 36.39% in sample C. The hydrophilic nature of the protein in fenugreek flour may be responsible for the rise in moisture content (Nasri and Tinay, 2007).

The quantity of FSP replacement notably raised the protein levels of the breads. The bread with 10% FSP had much higher protein content (16.28±0.11%) than control (15.05±0.06%). In terms of fat, crude fiber and ash content of the breads, these parameters tend to increase in a manner that is directly proportional to the amount of FSP incorporation. Fat content increased from 3.26±0.24% in the control bread to 4.99±0.25% in sample C, crude fiber increased from 0.33±0.05% to 1.70±0.01% and ash content increased from 2.33±0.16% to 2.72±0.21%. Similar results were found by Chaubey *et al.* (2018) and Man *et al.* (2019). Total carbohydrate content reduced as the fenugreek seed powder increased because of the high levels of protein, fat, crude fiber and moisture.

5.2 Bioactive components

In addition to supplying necessary nutritients, bioactive compounds are vital for the upkeep of the immune system and the prevention of chronic diseases in humans. That's why the quantification of these compounds is crucial and the results of the bioactive compounds identified in FSP and bread formulations are reported in table 4.3 and table 4.4 respectively.

The results for total flavonoid content considerably ($P<0.05$) varied between formulations (0–10% supplementation), ranging from 18.68–61.34 mg QE/100g. The sample with the greatest flavonoid concentration, sample C (10% supplementation), measured 61.34±0.05 mg QE/100g, whereas the control had the lowest flavonoid level, 18.68±0.04 mg QE/100g. As fenugreek powder is a superior source of flavonoid components than wheat flour, the flavonoid content in wheat-fenugreek supplemented bread was shown to be greater when compared with control bread. Total flavonoid content reported by Afzal *et al.* (2016) in 5%, 10% and 15% fenugreek supplemented bread was 2.50±0.10 mg CE/g, 2.68±0.10 mg CE/g and 2.91±0.12 mg CE/g respectively which is analogous to the present values. The findings showed that the total phenolic content of the breads improved significantly ($P<0.05$) when wheat flour was replaced with FSP at different amounts of supplementation ranging from 5 to 10%. In contrast to the control, which displayed the lowest level of total phenolic compounds (2.71±0.001 mg GAE/100 ml); the results predicted the highest concentration of total phenolics (3.33±0.010 mg GAE/100 ml) at a maximum supplementation of 10%. It is possible that the greater phenolic levels in the FSP-based breads are the result of the existence of naturally existing bioactive components in fenugreek seeds including orientin, apigenins, rutin, tannins, and quercetin (Afzal *et al.*, 2016; Naidu *et al.*, 2012). On the other hand, total anthocyanin content decreased drastically with the increasing supplementation. The highest value for total anthocyanin content was reported in sample A (16.77±0.56 mg TA/100 ml) followed by the control (15.47±1.71 mg TA/100 ml), and the lowest level of total anthocyanin content was found in sample C (8.57±0.32 mg TA/100 ml), that is, at 10% supplementation.

5.3 Antioxidant capacity

The effectiveness of natural antioxidants at scavenging free radicals is related to the DPPH test. The results showed that adding FSP to breads had a substantial (p 0.05) impact on their capacity to scavenge free radicals. It was shown that the bread prepared with wheat flour had a lower antioxidant activity (DPPH) value than the bread made with fenugreek, which showed a linear rise in antioxidant activity.

Sample C was found to have the greatest levels of free radical scavenging activities (3.21±0.001 mg TE/100g) while the control showed the lowest DPPH activities (1.76±0.005 mg TE/100g). There was 82.39% increment in the value of antioxidant capacity in comparison with the control. The increased phenolic content of sample C (supplementation at 10%) compared to the control may be attributed to its higher antioxidant capacity (Ahmad *et al.,* 2022). According to a research by Man *et al.* (2019), in wheat flour-fenugreek treatment blends and fenugreek-supplemented leavened loaves prepared at 2-8% supplementation, DPPH free-radical scavenging capabilities ranged from 33-54% and 29-55%, respectively. In another study with similar findings, Afzal *et al.* (2016) found that the DPPH free-radical scavenging capabilities of leavened breads cooked with a maximum of 15% FSP supplementation levels increased significantly ($P<0.05$), going from 35 to 82%.

5.4 Sensory evaluation

The acceptability of a finished product, as well as its suitability to be consumed, is largely dependent on its sensory attributes, which are considered to be essential quality standards. Color, being a crucial quality factor that attracts customers and increases a product's visual appeal and acceptance, was scored the highest (4.0) for sample B (7.5% supplementation), followed by sample A (3.7). The physicochemical characteristics of the dough (pH, water content, amino acid content, and reducing sugar content), as well as processing factors used during baking, such as relative humidity, temperature, air speed, and modalities of heat transmission, determine the color of the baked good (Zanoni *et al.,* 1995). Likewise, a dark brown hue of the bread was seen when the amount of fenugreek seed flour in wheat flour increased (Chauhan and Sharma, 2000). The sensory score for smell and texture showed that sample B had the highest score, that is, 4.0 and 4.5 respectively followed by sample A which had 3.5 and 3.2 respectively. When FSP was added to breads, the taste sensory

score went down, which may be associated to the inclusion of a variety of astringent substances, including galacato-mannans, sapogenin, trigonelline, choline, tannins, and alkaloids (Chaubey *et al.*, 2018; Rasool *et al.*, 2013). In case of overall acceptability, there was no noticeable differentiation between sample A and B although sample B got the highest score. All sensory characteristics of the breads were seen to be dramatically altered when wheat flour was replaced with FSP up to 10%. Supplementation at 10% had an undesirable sensory appeal compared to other formulations. Similar studies by Afzal *et al.* (2016), Chaubey *et al.* (2018), and Man *et al.* (2019) reported the sensory acceptability of fortified bread, muffins, pizza, biscuits and cakes made at 6% FSP supplementation.

5.5 Amino acid profile

The analysis of amino acid in this study has shown a wide range of results. Total 15 amino acids were detected in fenugreek seed powder supplemented bread- 7 essential amino acids (Arginine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Valine) and 8 non-essential or conditionally essential amino acids (Alanine, Asparagine, Cystine, Glutamine, Glycine, Proline, Serine, Tyrosine). The content of arginine, leucine, valine, asparagine, and proline showed higher value in the 7.5% FSP supplemented bread (149.01 ppm, 7.22 ppm, 3.04 ppm, 227.59 ppm, and 240.30 ppm respectively) than control bread (95.07 ppm, 4.59 ppm, 1.71 ppm, 74.82 ppm, and 187.10 ppm respectively). On the contrary, isoleucine, cysteine, and tyrosine were found higher in the control bread (33.34 ppm, 39.85 ppm, and 14.9 ppm respectively) than sample B (9.24 ppm, 31.53 ppm, and 1.9 ppm respectively). The content of methionine, phenylalanine, alanine, glutamine, and serine showed no significant difference. Bread with no FSP was depleted of two important amino acids: lysine and glycine, whereas the amount of lysine and glycine in FSP supplemented bread were 51.96 ppm and 3.99 ppm respectively. Histidine and threonine were absent in both samples. The study by Yasothai (2021) reported that the amino acids content (g/100g sample) of fenugreek seed were arginine (0.71), glycine (1.21), histidine (2.08), isoleucine (0.76), leucine (1.53), lysine (1.13), methionine (0.61), phenylalanine (0.79), threonine (0.64), tyrosine (0.50), and valine (0.46).

Mahfouz *et al.* (2012) evaluated the protein quality in fenugreek and other legumes, including white lupine. White lupine was found to possess 268.50 g/kg of essential

amino acids, but fenugreek seeds had the greatest concentrations of both essential amino acids (304.80 g/kg protein) and total amino acids (576.00 g/kg protein). Methionine and cysteine concentration in fenugreek were around 128% and 49% greater than in white lupine, respectively. Fenugreek has a high concentration of free amino acids, particularly isoleucine and histidine, which may promote the release of insulin, according to Isikli and Karababa (2005). Fenugreek seeds are used as a dietary supplement because they are rich in lysine, which is equivalent to soybean lysine in quality (Mandal, Deb Mandal 2016). Significant amounts of glutamine, asparagine, leucine, threonine, and arginine were detected in fenugreek seeds, according to Feyzi *et al.* (2015), who also came to the conclusion that fenugreek protein isolate is a source of protein with exceptional functional qualities. Fenugreek proteins, which differ from cereal grain proteins in that they are rich in lysine and low in histidine and methionine, might be employed to improve the nutritional content of cereals and snack items like bread, cookies, and cakes (Feyzi *et al.* 2015).

Chapter-6: Conclusion

The main contributors to the disease burden among vulnerable population groups in undeveloped and emerging nations include increasing levels of protein energy deficiencies, micronutrient malnutrition, and food insecurity. It is essential to develop novel interventions to address these serious health issues. Fenugreek seed powder is an exceptional source of proteins, dietary fiber, and bioactive phytonutrients that can aid in the prevention of a wide range of maladies and nutritional health issues. On the other hand, bread is a common food that is closely related to people's daily lives and regarded as a substantial source of carbohydrates in dietary recommendations. In the current research the addition of fenugreek seed powder to wheat flour increased the nutritional and antioxidant status while maintaining an acceptable bread quality. It was shown that adding fenugreek powder boosted the bread's crude protein, crude fiber, and ash contents. Similarly, bread's polyphenol, flavonoid content and antioxidant activity showed an increasing tendency with increasing level of supplementation. From the present study, it can be concluded that fenugreek seed powder may be used in bread making up to a level of 7.5% without negatively impacting sensory quality. Since it is affordable and easy to prepare, producers and consumers can adopt this approach for making healthier option of bread.

Chapter-7: Recommendations and future perspectives

Nowadays most of the people suffer from various ailments such as type 2 diabetes, hypercholesterolemia, cancer and so on. At the same time, people have started to depend more on the goodness of natural ingredients than drugs to stay healthy. Hence, researches are being done to incorporate these ingredients in food products. On the basis of the current study, the following recommendations and potential for further research effort are made.

a) The present study might be repeated in order to provide independent confirmation of the findings of the experiments.
b) To get better palatability, ways to reduce the bitterness of fenugreek seeds should be researched.
c) As the product is easy to prepare, consumers can make this at home and can be developed commercially.
d) The findings will be beneficial from therapeutic perspective as it has medicinal value.
e) Despite the fact that there was sufficient data for statistical comparisons between the findings of the analyses, the limited number of samples that were tested and the need that the findings be supported by a more extensive research both call for a heaping helping of healthy skepticism toward the conclusions that we have drawn.

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Appendices

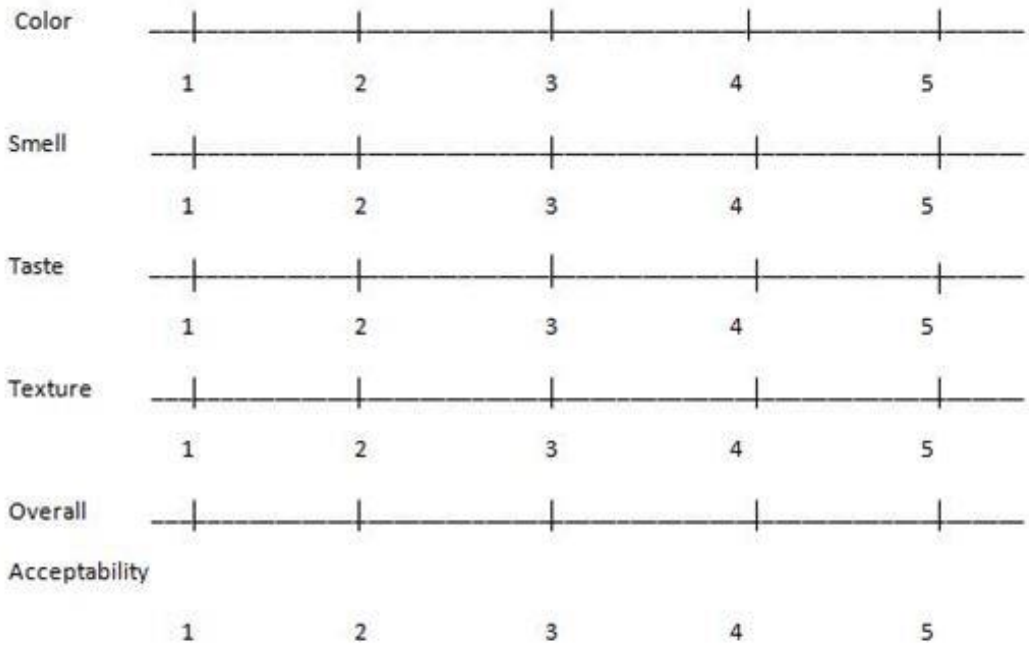
Appendix A: Questionnaire for sensory test of bread

Date: **……**

Name: **……**

Instruction

You are given three samples. Please rinse your mouth with water before starting. Taste each sample from left to right. Rinse your mouth with water between samples. Evaluate the following attributes of the samples of bread. Make a circle on the horizontal line based on your preference.



Note:

5= Like a lot

4= Like a little

3= Neither like nor dislike

2= Dislike a little

1= Dislike a lot

Appendix B: Procedure of making FSP supplemented bread

Fenugreek seed drying

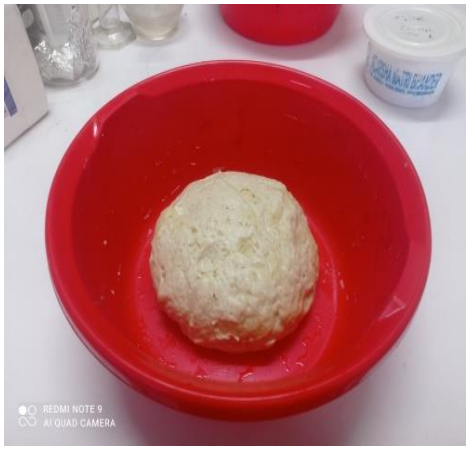
Fenugreek seed powder

Weighed ingredients

Yeast activation

Kneaded dough

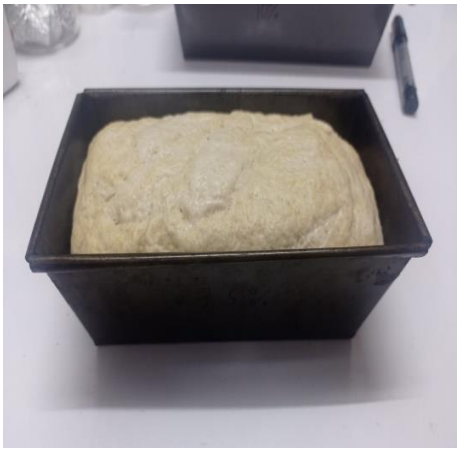
First proofing



Releasing air pockets

Placing dough in greased pan

Second proofing



Ready for baking

Baking

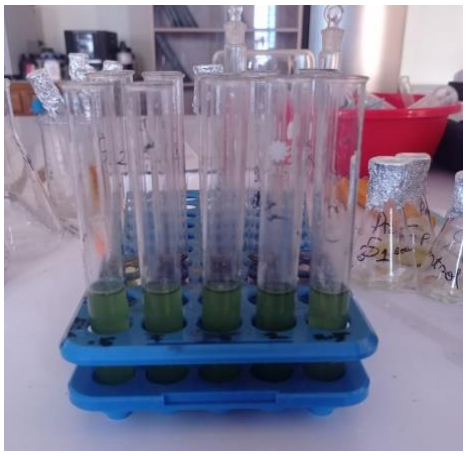
Out of pan after cooling

Appendix C: Pictures of analysis

Protein determination

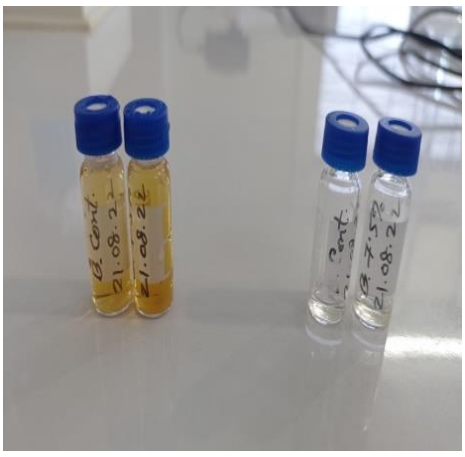
Fat determination

Crude fiber determination



Analysis of bioactive compounds

UV spectrophotometer



Amino acid analysis

Brief biography

Proma Roy passed the Secondary School Certificate Examination in 2011 from Laksam Pilot Girls' High School, and then Higher Secondary Certificate Examination in 2013 from Nawab Faizunnesa Government College, Laksam. She obtained her B.Sc. (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving the health status of people through proper guidance and suggestions and to create awareness among people about food safety and nutrition.