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College of Dryland Agriculture and Natural Resources

Department of Food Science and Postharvest Technology

Postgraduate Program: Food Processing Technology

Effect of Fenugreek Processing and Blending Ratio with Wheat Flour on Bread's
Nutritional and Sensory Quality.

By:

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A Thesis Submitted to the Department of Food Science and Postharvest
Technology in Partial Fulfillment of the Requirements for the Master of Science
Degree in Food Processing Technology

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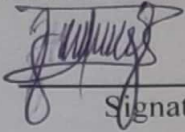
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DECLARATION

I, the undersigned student, hereby present my thesis entitled “Effect of fenugreek processing and blending ratio with wheat flour on bread's nutritional and sensory quality” for consideration by the **Food Science and Postharvest Technology** Department within the College of Dry land Agriculture and Natural Resources at Mekelle University, my thesis in partial fulfillment of the requirement for the degree of Masters in Food processing technology. I sincerely declare that this thesis is the product of my own efforts. No other person has published a similar study, which I might have copied, and at no stage will be published without my consent and that of the **Food Science and Postharvest Technology** department.

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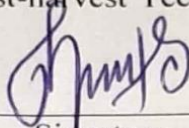

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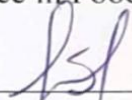
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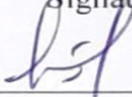
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DEDICATION

This work is dedicated to my beloved family!!

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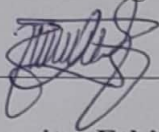
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STATEMENT OF THE AUTHOR

I declare that this thesis is my work and all sources and materials used in this thesis have been properly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc. in Food Processing Technology. I confidently declared that the thesis is not submitted to other institutions for any award of an academic degree or diploma. Brief quotations from this thesis are allowed without any special permission provided that an accurate acknowledgment of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the Head of the Department of Food Science and Post-harvest Technology or the Dean of the College of Dryland Agriculture and Natural Resources when the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

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LIST OF ABBREVIATIONS/ACRONYMS

- AACC----- America Association of Cereal Chemists
- ANOVA-----Analysis Of Variance
- CRD-----Complete Randomized Design
- FBB-----Fenugreek Blended Bread
- GM-----Galactomannas
- OAC-----Oil Absorption Capacity
- OVA -----Overall Acceptability
- PAGE-----Poly Acrylamide Gel Electrophoresis
- PED-----Protein Energy Deficiency
- RH-----Relative Humidity
- SDS-----Sodium Dodecyl Sulphate
- WAC-----Water Absorption Capacity
- WB-----Wheat Bread

ABSTRACT

*Bread is the most commonly consumed product made from wheat, which provides a carbohydrate-rich source of starch and dietary calories. Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume used as an ingredient for wheat flour in bread making and as a condiment to improve the quality of cereal-based foods. This study was conducted to evaluate the effect of processing fenugreek and blending ratio (w/w) with wheat flour on the nutritional and sensory quality of bread. The experiment was planned with a single factor and flour blends were formulated in respective percentages of 95:5, 90:10, and 85:15% from wheat flour and fenugreek flour and laid out in CRD with three replications. The fenugreek flour blending ratio had a significant ($P < 0.05$) effect on ash, protein, crude fat, crude fiber, and carbohydrate contents. The bread blended with 15% germinated fenugreek resulted in higher moisture content ($10.67 \pm 0.005\%$), crude protein ($15.88 \pm 0.05\%$), and crude fiber content ($1.04 \pm 0.05\%$) as compared to 10, and 5% blended and the control bread. But the higher ash content ($1.38 \pm 0.01\%$), fat content ($1.80 \pm 0.01\%$), and carbohydrate content ($74.69 \pm 0.02\%$) were exhibited in the 15% roasted fenugreek blended bread. Also, 15% roasted fenugreek-blended bread had the highest Ca (66.03 ± 0.057 mg/100g) and Fe (8.21 ± 0.005 mg/100g). But, the Zn content was higher (2.54 ± 0.05 mg/100g in 15% germinated fenugreek blended bread. Moreover, composite flours blended with 15% roasted fenugreek flour revealed the highest in water absorption capacity ($1.56 \pm 0.05\%$), and oil absorption capacity ($1.30 \pm 0.00\%$), whereas the bulk density ($1.11 \pm 0.005\%$) was higher in the composite flour blended with 15% germinated fenugreek flour. In physical properties, blending ratio had a significant ($p < 0.05$) effect on the loaf weight, loaf volume, and specific loaf volume. The highest loaf weight (94.36 ± 0.05 g) was obtained in 15% roasted blended bread. But, 15% germinated blended bread had the lowest reduction in loaf volume (363.68 ± 0.006 ml, but the highest loaf volume (412.67 ± 0.57) was observed in control bread (100%) wheat flour. Also, 15% germinated fenugreek blended bread had lower (3.90 ± 0.00 ml/g) specific loaf volume but higher (4.45 ± 0.00 ml/g) in 5% roasted fenugreek blended bread. In sensory evaluation, blending ratio and processing had a significant ($p < 0.05$) effect on the sensory attributes (colour, texture, taste, aroma, including overall acceptability). In conclusion, blending of germinated & roasted fenugreek flour with wheat flour revealed more improvement in nutritional, mineral content, and functional properties.*

Key words: *Wheat bread, fenugreek seed, fenugreek processing, blending ratio, nutritional quality, sensory acceptability.*

CHAPTER ONE: INTRODUCTION

1.1. Background and Justification

Wheat is a vital cereal crop globally regarding production and consumption. It serves as a significant source of energy, protein, and dietary fiber in human nutrition and animal feed (Khalid & Hameed, 2019; Žilić et al., 2011). Numerous appetizing and filling dishes are made with wheat. In the majority of the world, it is cultivated and used for industrial, feed, and food applications (Shevkani et al., 2024). Its agronomic adaptability, nutritional value, ease of storage, and ability to create a variety of enticing and satisfying recipes make it an essential part of the world's diet (Kaur et al., 2016). A vital component in many diets, wheat bread has a significant effect on people's health (Parenti et al., 2020). The most important crop for making bread is *Triticum aestivum*, one of the best varieties of wheat, because it bakes better than any other cereal (Johansson et al., 2018). Due to increasing consumer demand, wheat is enjoyed by consumers worldwide in a variety of delicious products, including bread, biscuits, cookies, pasta, cakes, and noodles (Goel et al., 2021). Bread is a staple food product made from wheat, which is a carbohydrate-rich source of starch and dietary calories. It is readily available and takes less time to prepare than other traditional meal items (Abera et al., 2017). The growing industrial demand for bread has made the manufacturing of bread that is both excellent in quality and extremely nutritious a constant source of concern (Ibidapo et al., 2019). As a result, new and more sophisticated technologies are being used to enhance and enrich bread, making it more nutrient-enriched, extremely stable, and long-lasting (Goel et al., 2021).

Fenugreek (*Trigonella foenum-graecum L.*) is an annual plant belonging to the legume family used to improve the quality of cereal-based foods, functioning as a condiment and as an ingredient to wheat flour for bread making (Birhane, 2012). The species name "*foenum-graecum*" means "Greek hay," indicating its use as a forage crop in the past (Petropoulos, 2002). This crop is native to an area extending from Iran to northern India, but is now widely cultivated in China, North and East Africa, Ukraine, and Greece (Petropoulos, 2002). It is locally used as a pulse, spice, and medicinal plant, and has a long history in Ethiopia (Alevtina & Zerihun, 2009). Fenugreek seed can be utilized for value addition of cereal-based food products to attain multiple benefits (Ahmad et al., 2023). It is used as a condiment and as a supplement to wheat and maize flour for bread making and as a constituent of the daily diet of the general population in the

Indian subcontinent (Kasaye & Jha, 2015). It can be incorporated into food products, such as cookies, cereals, crackers, doughnuts, bagels, biscuits, pizza dough, pasta, bread, juices, salads, sauces, and candies (Kandekar et al., 2022). Its leaves are widely consumed in India as a green, leafy vegetable and are a rich source of calcium, iron, β -carotene, and other vitamins (Sharma et al., 1990). Currently, fenugreek seeds are incorporated into wheat flour to enhance its functionality in producing a variety of food items. However, its bitter and peculiar flavor makes it difficult to utilize (Kandekar et al., 2022). Through the germination and roasting processes, the fenugreek seed becomes able to overcome this challenge by reducing its bitterness and enhancing its flavor (Pandey et al., 2015). Dwivedi et al. (2019b) reported that it has been possible to debitter fenugreek seeds by employing various processing methods such as soaking, germination, and roasting. Therefore, it is evident from this study that fenugreek can be incorporated into baked products in acceptable limits, which may reduce insulin resistance and benefit diabetes patients as well (Losso et al., 2009). The nutritional and sensory qualities of wheat bread can be significantly enhanced by adding fenugreek flour during the baking process (Roy, 2022). Due to the aforementioned factors, the present study has been planned to evaluate how processing methods for fenugreek and the ratio of concentration with wheat flour influence the nutritional value and sensory quality of bread. It was also proposed to investigate the bread's physical properties, chemical composition, functional properties, and sensory quality. In some parts of Ethiopia, especially in the Tigray region, people do not use processed fenugreek-incorporated bread, usually because of a lack of knowledge about the importance of utilizing processed (roasting and germination) fenugreek incorporation into wheat flour to make bread. Also, in our society, there is no exact acceptable blending level of processed fenugreek in bread making. Therefore, this study is intended to evaluate fenugreek (roasted and germinated) blended bread for its nutritional composition and the acceptable sensory quality of fenugreek in bread making.

1.2. Statement of the Problem

The effects of various processing methods on fenugreek and its concentration ratio with wheat flour, and their influence on the final bread's nutritional value and sensory qualities, are not well studied. Furthermore, the nutritional makeup, processing properties, and potential for creating value-added products from fenugreek have not been fully explored. The traditional and historical use of fenugreek in Ethiopia, especially in Tigray, has not been supported by academic research

on its chemical and nutritional composition, processing quality, functional properties, and technological advancement (Birhane, 2012). Due to the lack of vital amino acids, dietary fiber, and minerals, wheat flour is considered less nutrient-dense than fenugreek flour (Dhingra & Jood, 2002; Gupta et al., 1999). People with inadequate nutrition can benefit from a rich source of nutrients that may help address malnutrition. Fenugreek can achieve this by being added to staple foods like wheat flour. In places like the Tigray region, this is particularly advantageous. This study aims to raise awareness of the nutritional and functional benefits of fenugreek for improving bread quality and promoting its use.

1.3. Objective

1.3.1. General Objective

- To investigate the effect of different processing methods of fenugreek and the blending ratio on the quality of wheat bread.

1.3.2. Specific Objective

- To evaluate the effect of roasting and germination of fenugreek on the quality of wheat-fenugreek blended bread.
- To determine the effect of blending ratios of fenugreek to wheat flour on the nutritional composition of their bread.
- To evaluate the sensory acceptability of wheat-fenugreek blended bread.

1.4. Research Hypothesis

Null Hypothesis:

- The processing method of fenugreek and its blending has no significant effect on the overall nutritional & sensory quality of wheat bread.

1.5. Significance of the study:

This study offers valuable insights into enhancing the use of fenugreek flour in bread making, creating nutritious and tasty foods through different processing methods that enhance bread quality. The findings of this study may help develop practical household applications

for fenugreek and provide opportunities to promote and support its use in various traditional recipes, particularly bread made with wheat-fenugreek blends.

1.6. Scope and limitations of the study

The study focused on the effect of processing fenugreek and blending ratios with wheat flour on the nutritional and sensory quality of the bread. The study is limited thematically to examining the nutritional, functional, and physical characteristics, as well as sensory qualities, of wheat-fenugreek composite bread. In the next study, the microbial load, antinutritional qualities, and shelf life stability of wheat-fenugreek composite bread remain to be investigated by other researchers.

CHAPTER TWO: LITERATURE REVIEW

2.1. Bread wheat (*Triticum aestivum*) & its Historical Background

About 10,000 years ago, wheat was first cultivated during the Neolithic Revolution, marking a transition from hunting and gathering to organized agricultural production (Iqbal et al., 2022). Wheat is thought to have first been cultivated in the Fertile Crescent, an area in the Middle East spreading from Jordan, Palestine, and Lebanon to Syria, Turkey, Iraq, and Iran (Arzani & Ashraf, 2017). Wheat-based products such as bread, baked goods, pasta, and noodles have been staple foods for thousands of years and are essential for food security worldwide. Bread wheat (*Triticum aestivum*) is the most common wheat and accounts for up to 95 % of global bread production (Fuad et al., 2010). Common wheat (*Triticum aestivum* L.), also known as bread wheat, is the most widespread wheat species and comprises around 95% of the wheat cultivated. Most of the remaining 5% consists of durum wheat (*Triticum durum* L.), known for its use in pasta. Other wheat species, such as einkorn (*Triticum monococcum* L.), emmer (*Triticum dicoccum* (Schrank) Schübler), and spelled (*Triticum spelta* L.), play a minor role in terms of utilization and are processed into specialty products. Wheat is regarded as unique among cereals due to its flour's capacity to form a viscoelastic dough when mixed with water, allowing for the preparation of a variety of palatable and satisfying foods (Wieser et al., 2020). Thousands of wheat cultivars and varieties, each with different grain and flour properties, are cultivated. They are categorized based on planting season (winter or spring), color (white, red, or pigmented), and endosperm characteristics (vitreous or mealy, soft or hard). However, wheat is primarily classified according to its grain hardness or softness, which correlates with the quality of the finished product (Shewry, 2023). Hard wheat grains typically yield strong flour with higher protein content, making them suitable for bread making, whereas soft wheat varieties produce flour with lower protein and damaged starch, ideal for crafting high-quality cookies, biscuits, and pastries (Quayson et al., 2016). Wheat is an important staple food globally, providing a significant contribution to daily energy, fiber, and micronutrient intake (Shewry, 2019).

2.1.1. Proximate Composition of Bread Wheat (*Triticum Aestivum*) /Nutritional attributes of wheat

Wheat (*Triticum aestivum* L.), belonging to one of the most diverse and substantial families, *Poaceae*, is the principal cereal crop for the majority of the world's population. This cereal is

polyploid and is domestically grown worldwide (Arzani & Ashraf, 2017). Wheat is the source of approximately half of the food calories consumed worldwide and is rich in proteins (gluten), minerals (Cu, Mg, Zn, P, and Fe), vitamins (B-group and E), riboflavin, niacin, thiamine, and dietary fiber (Belitz et al., 2009). Wheat seed-storage proteins represent an important source of food and energy and play a major role in the determination of bread-making quality. The two groups of wheat grain proteins, i.e., gliadins and glutenins, have been widely studied using SDS-PAGE and other techniques (Khalid et al., 2023a)

2.1.1.1. *Wheat Proteins*

Protein is regarded as the most significant nutrient for animals and humans, as the name of its origin indicates (“proteins,” meaning primary in Greek). The protein content varies from 10–18% of the entire dry mass of the wheat grain (Iqbal et al., 2022). Proteins determine the capability of wheat flour, which can be dispensed into diverse foodstuffs. Wheat proteins have an important role in carbon dioxide retention, dough development, and baking quality due to their qualitative and quantitative attributes (Muhammad Irshad et al., 2013). Wheat contributes a larger percentage of protein than energy to the nutritional requirement of an adult male (Khalid et al., 2023b). The percentage of protein in wheat can be influenced to a certain extent by the time of fertilizer application and fertilizer type (Iqbal et al., 2022). During human digestion, protein is broken down into its constituents, absorbed by the bloodstream, and then assembled again to form different types of protein required by the body for growth, maintenance, and repair (De Sousa et al., 2021). The biological significance of wheat is determined by limiting essential amino acids. These amino acids become deficient due to the body’s increased requirements. Lysine is the deficient amino acid in wheat (Poutanen et al., 2022). During the process of milling, one-third of the total protein is removed along with lysine, as the majority of the protein and lysine are present in the bran and the germ (Siddiqi et al., 2020). Mature wheat grains contain 8 to 20% protein. The proteins in wheat exhibit great complexity and diverse interactions with one another, making them difficult to describe (Žilić et al., 2011). Hard wheat classes tend to have higher protein contents (~10-14%), making them more suitable for pan bread, while soft wheat classes have lower protein levels (~8-10%), rendering them unsuitable for bread production (Delcour & Hosney, 2010).

Characteristics/Classification and Function of Wheat Proteins

The proteins in wheat display great intricacy and diverse collaboration with one another, rendering them hard to describe. Wheat proteins have been classified (Figure 1) (Chinuki & Morita, 2012) by their enforceability and solubility in various diluents.

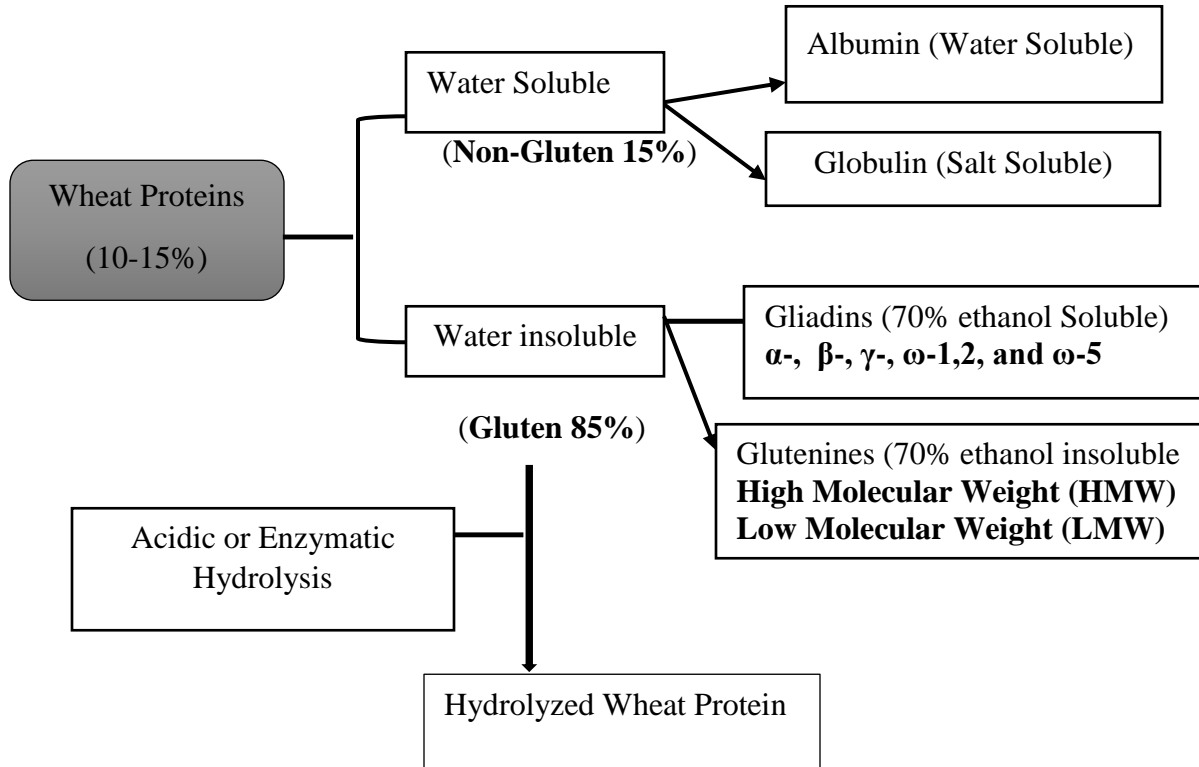


Figure 1: Types of wheat proteins (Shewry, 2023)

Cataloging was conducted on the basis of Thomas. D. Osborne's work from the shift of the previous era (Shewry, 2019). According to his method, serial withdrawal of crushed wheat kernels gives rise to protein properties as follows:

- Water soluble albumins
- Globulins, not soluble in natural water but soluble in diluted solution of sodium chloride while insoluble at high NaCl concentrations
- Gliadins, soluble in 70% ethanol
- Glutenins, soluble in diluted NaOH or acid solutions

2.1.1.2. Wheat carbohydrates

The wheat grain, at maturity, consists of 85% carbohydrates, most (80%) of which are starch of the endosperm. The non-starch carbohydrate is made up of mono-, di-, and oligo-saccharides and fructans (7%), plus cell-wall polysaccharides (12%) (Mares & Stone, 1973). The main wheat carbohydrate (starch) is the predominant carbohydrate source for human diets, the substrate for producing alcoholic beverages and fuel ethanol by fermentation, and the raw material for many other industries (Tosi et al., 2018).

Starch

Starch is the most abundant storage carbohydrate in wheat, comprising about 60–75 % of the grain and 70–80 % of the flour. The content and characteristics of starch in flour affect the quality of wheat-based products. Starch occurs as semi-crystalline granules in wheat kernels. The major components of starch are the glucose polymers amylose and amylopectin. Normal starch, such as normal maize, rice, wheat, and potato, contains about 70–80% amylopectin and 20–30% amylose (Pérez & Bertoft, 2010). Amylopectin and amylose are two distinct polysaccharide molecules that make up starch, differing in both shape and size. Amylopectin (69-73%) is highly branched and large (107 to 109 Da), whereas amylose (27-31%) has minimal branching and is smaller (105 to 106 Da) in nature (Avramenko et al., 2018).

2.1.1.3. Wheat lipids

Wheat lipids make up 3–4% of the weight of the whole grain and about 1–2.5% of straight-run milled flour. Even though lipids are a minor constituent in wheat, they play an important role in dough mixing, the baking process, and consumer acceptance of the finished products. The lipids interact with the gluten proteins to form complexes, which contribute to the stabilization of the gas-cell structure, thus having a significant effect on loaf volume and on the final texture of the baked product. Wheat flour contains approximately 2% lipids, and the lipid content determined depends not only on the genetic and environmental characteristics but also on the milling and lipid extraction method (Pareyt et al., 2011).

2.2. Production and Utilization of Fenugreek

A significant part of the healthcare system in many nations worldwide has been played by herbal medicines (Khan et al., 2015). A member of the legume family, fenugreek (*Trigonella foenum-*

graecum L.) is an annual crop (Dwivedi et al., 2019a). It is widely used as a spice and condiment to add flavor to various foods. Although it was originally grown in a region that stretched from Iran to northern India, this crop is currently commonly grown in China, north and east Africa, Ukraine, and Greece (Ahmad et al., 2016). Fenugreek is now cultivated in all habitable continents of the world. Greek hayseed called fenugreek comes from the Mediterranean, Southern Europe, and Western Asia (Singh et al., 2020). *Trigonella*, *Trifolium*, and *Medicago* species originated in the Near East, extending from Israel to Syria and Southern Turkey into Iran and Iraq, and the Mediterranean area includes Spain, Morocco, and Turkey (Serbessa et al., 2019). Although its value as a food source is widely acknowledged (Al-Asadi, 2014), it is presently attracting interest as a therapeutic plant. Fenugreek is now being used extensively by the pharmaceutical and nutritional sectors to create a variety of pharmacological products. Throughout several fields, including medicine, nutrition, drinks, perfumes, cosmetics, and industrial uses, plant seeds are employed in Asia, Africa, and Mediterranean nations (Legesse et al., 2020).



Figure 2: Fenugreek plant and seed (Chowdhury et al., 2014)

2.2.1. Uses of Fenugreek

2.2.1.1. As Human Food

Some researchers have recently considered adding additional legumes (such as beans, lentils, peas, chickpeas, and Fabaceae) to increase the nutritional content of extruded meals. It has been suggested that nutrient-dense extruded multi-legume snacks combined with whey protein concentrate, honey, and palm oil can reduce malnutrition in underdeveloped nations (Martineau-Côté et al., 2022; Pasqualone et al., 2020). The excellent nutritional standards of fenugreek, which are present in the leaves and seeds and include macro- and micro elements as well as dietary fiber (Thomas et al., 2011), make it one of the extraordinary plants. The entire plant is utilized as food and fodder. In contrast, the seeds are used for food, spice, dyeing, flavoring,

industrial applications, and medicine (whole, powdered, in flour, or roasted). In India and Egypt, young plants and fresh fenugreek leaves are delicious, used as a salad or cooked, and often function as a condiment. This is because fresh plants are high in vitamin C.(Wani & Kumar, 2018). Fenugreek seeds are used to make beverages in Sudan (Naeem et al., 2021). In Yemen, it is regarded as a staple food and is used to make bread as an alternative to wheat and maize in Egypt. In some countries, roasted seeds are used as a coffee substitute, probably because of the alkaloid Trigonella content, which is a basic constituent of the coffee seed. In Ethiopia, fenugreek seed is commonly used for preserving Vienna, for infant food preparation, and for infant feeding by boiling the whole seed, as food blending, as a beverage, and for the preparation of staple food called lasso (Beriso et al., 2016). In North Africa, it is mixed with breadstuff (Birhane, 2012).

2.2.1.2. As a food supplement

Excellent protein sources include green vegetables and legumes, often used in meals and dietary supplements. Only soybean pods are now used as a protein source. Given their high protein content (20–30%), fenugreek seeds can potentially be utilized as a source of protein. Plant proteins need to meet several criteria to be used as food ingredients. The proximate composition and physicochemical characteristics of a protein concentrate made from *Trigonella foenum* were investigated by Allaoui et al. (2019). In comparison to neutral pH, they discovered that *Trigonella foenum* was more soluble at acidic and alkaline pH. Additionally, it possesses a great capacity for absorbing water and oil (Rehman et al., 2018). Fenugreek seeds are food preservatives because they are high in vitamin E, particularly in pickles (Srivastava et al., 2022). Fenugreek seeds are nourishing and can be consumed while recovering to help with weight gain (in anorexia). Fenugreek seeds are useful for treating stomach ulcers and gastritis because they are calming. Fenugreek seeds are used to treat cervical cancer in China (Srivastava et al., 2022)

2.2.1.3. Fenugreek as spices

Herbs and spices are among the most versatile and frequently used ingredients in food preparation. They are increasingly utilized as natural preservatives and for their potential health benefits, such as their antioxidants, as well as their traditional roles as flavorings that add scent, texture, and color (Öztürk et al., 2018). Spices consist of fiber, fats, sugars, proteins, gum, ash, volatile compounds (essential oils), and various nonvolatile ingredients. There are historical, chemical, and physiological connections between spices and food condiments. Fenugreek seeds

are used to flavor soups and curries while also providing nutritional benefits (Żuk-Gołaszewska & Wierzbowska, 2017). Fenugreek seed is often used as a flavoring agent and as an ingredient in sauces and curry powder (Ozturk et al., 2018). The significance of the secondary effects of spices is growing as the public increasingly prefers natural or organic foods and non-pharmaceutical approaches to health. Spices have long been used to stimulate appetite, improve digestion, reduce stress, and increase hunger.

2.2.2. Possible side effects of fenugreek seed consumption

Fenugreek seed is considered safe and easy to use based on folklore and anecdotal evidence. While fenugreek is generally regarded as "Possibly Safe" when used in moderation, studies with high doses have shown some minor side effects, including nausea, diarrhea, stomach upset, bloating, gas, and a urine odor like maple syrup. Fenugreek seed can induce labor because it can promote uterine contractions and may have estrogenic and oxytocin effects. Pregnant women should avoid taking fenugreek unless directed by their doctor (Snehlata & Payal, 2012). Additionally, inhaling fenugreek seed powder can cause hypersensitivity symptoms such as dizziness, a runny nose, and wheezing. Its high fiber content is slippery and may delay the ingestion of oral medications (Kandhare et al., 2019; Öztürk et al., 2018).

2.2.3. Nutritional Composition of Fenugreek

Nutritional characteristics of fenugreek seeds are composed of 20 - 30% proteins, 45-60% carbohydrates (primarily mucilaginous galactomannan fibers in the cell walls), and 5-10% lipids (Naeem et al., 2021; Yao et al., 2020). Other important components include saponins, glycosides that hydrolyze into steroidal saponins like diosgenin, pyridine-type alkaloids mainly trigonelline, and free amino acids, especially 4-hydroxyisoleucine. According to Singh et al. (2020), fenugreek leaves contain various vitamins and minerals. They are particularly abundant in choline. Seeds have antibacterial, carminative, bitter, aromatic, and carminative qualities. The leaves constitute 50% unavailable carbohydrates (fiber), thus making them the richest natural source of fiber. The fiber portion consists of an insoluble (30%) and soluble (20%) fraction, which is mostly galactomannan (Nagulapalli Venkata et al., 2017). Total lipids extracted from fenugreek seeds amounted to 7.5% of the dry seeds and comprised 84.1% neutral lipids, 5.4% glycolipids, and 10.5% phospholipids. Fenugreek contains approximately 4 to 8% saponins and about 1% alkaloids, contributing to its bitterness. Fenugreek seeds contain significant amounts of

Fe, P, Ca, Zn, and Mn, and they are abundant in vitamins A, B1, C, and nicotinic acid. Due to their unique anti-diabetic properties, both leaves and seeds are consumed as a remedy for various health conditions. Well-known that fenugreek has a high content of free amino acids, mainly isoleucine and histidine, which may stimulate insulin secretion. Fenugreek is also abundant in lysine, whose quality is comparable to soybean lysine, so fenugreek. Seeds are consumed as a dietary supplement (Birhane, 2012). Mabrouki et al. (2015) found significant Glu, Asp, Lue, Thr, and Arg concentrations in fenugreek seeds. They established that fenugreek protein isolate is a basis of protein with significant functional properties. The grain of durum wheat was richer in leucine, phenylalanine, and valine. Unlike cereal grain proteins, fenugreek proteins are rich in Lys and low in His and Met. Therefore, they could enhance the nutritional value of cereals and snack foods such as bread, biscuits, and cakes (Żuk-Gołaszewska & Wierzbowska, 2017).

2.2.4. Functional food aspects of fenugreek as an ingredient

Fenugreek is valued for its culinary and medical benefits. The plant is frequently used as a spice to enhance food flavor and support healthy metabolism and general well-being. The maximum amount of polyphenols was found in biscuits supplemented with 10% germinated fenugreek, which also had a high nutritional value. Vitamin B2 and carotene concentrations in biscuits were improved by supplementing wheat flour with 5% and 10% fenugreek flour (Afzal et al., 2016; Żuk-Gołaszewska & Wierzbowska, 2017). The mentioned study claims that fenugreek products have restorative qualities and may help those with iron-deficient anemia. Fenugreek seeds are a spice used to flavor some cheeses, primarily Parmesan. In salad dressings and cottage cheese spreads, seeds are powdered or crushed. They also used a typical component of the Chubritza spice from Bulgaria in the curry sauce. Coffee and vanilla extracts can also be flavored with fenugreek seeds. Fenugreek seeds and extracts improve nutritional absorption, particularly of amino acids, and assist with digestion. They offer to nourish and energize qualities and aid in building muscle and body mass. Fenugreek-based food supplements are advised for diabetic patients due to their hypoglycemic effects. For direct ingestion, fenugreek seeds are roasted and put into broth and tea. Fenugreek leaves are used as a spice and are powdered and cooked in butter (Żuk-Gołaszewska & Wierzbowska, 2017). They are also used for salads.

2.2.5. Important chemical Constituents of Fenugreek

Galactomannans, isoleucine, and steroidal sapogenins are three significant chemical components in fenugreek that have medical potential. Fenugreek is now one of the most well-known "Nutraceuticals" or health foods because of these components (Syed et al., 2020a).

2.2.5.1. Steroidal sapogenins

Steroid sapogenins from fenugreek seeds, such as diosgenin, are widely employed in the pharmaceutical and nutraceutical sectors (Ozturk et al., 2018). Diosgenin is frequently employed as a raw precursor in the synthesis of steroidal medications and hormones, including progesterone, glucocorticoids, and testosterone (Syed et al., 2020a). According to Dhull et al. (2020), steroidal sapogenins are efficient medications for treating hypocholesterolemia, a condition frequently linked to diabetes.

2.2.5.2. Galactomannans

Galactomannans, also known as mucous fibers, are polysaccharides that include hemicellulose and are found in the endosperm cell walls (Ozturk et al., 2018). They are utilized to thicken the surface of these cells (Syed et al., 2020a). Galactomannans have promise for usage as a gelling agent or gum due to their strong water-binding ability and superior enzymatic breakdown compared to cellulosic microfibrils (Khorshidian et al., 2016a). By lowering these people's hyperglycemia, fenugreek galactomannans seem to help manage type 2 diabetes in both laboratory animals and humans. They may generate very viscous solutions at relatively low concentrations due to their great propensity to bind water, which appears to limit the absorption of glucose in the digestive system (Ozturk et al., 2018).

2.2.5.3. Isoleucine

As a precursor to 4-hydroxyisoleucine, which is known to control insulin production in animals, isoleucine (a branch-chain amino acid, or BCAA) is a kind of amino acid (Ozturk et al., 2018). Most of the fenugreek's hypoglycaemic and anti-hyperglycaemic benefits are attributed to the seed's digestive effects from dietary fiber and its systemic effects from amino acids like 4-hydroxy isoleucine (Syed et al., 2020a).

2.2.6. Effect of fenugreek processing on nutritional composition, functional, and phytochemicals.

2.2.6.1. *Roasting*

Legumes are commonly processed for use in cuisine via roasting. According to Dwivedi et al. (2019a), fenugreek seeds undergo processing such as roasting, which raises the contents of crude protein, crude fiber, and total ash while lowering those of fat and dietary fiber. Roasted flour is a common food component used to make weaning, supplementary, and therapeutic meals (Raigar & Mishra, 2021). It is well known that roasting can change the physicochemical (color, texture, composition, and flavor) and sensory (taste and aroma) characteristics of roasted grains in both desirable and occasionally undesirable ways. The roasting treatment's duration and temperature are essential to get the desired qualities of the legumes and flour. Therefore, it is crucial to understand how roasting factors affect product quality to achieve the desired characteristics (Raigar & Mishra, 2021). Moisture and fat levels decrease during roasting, and ash content increases. The loss of volatile oils during dry heating of fenugreek seeds may cause a reduction in fat content after roasting (Pandey & Awasthi, 2015). Fenugreek seeds are processed using heat in a variety of ways for a variety of reasons. It has been commonly observed that roasting can aid in the release of improved flavor (Dwivedi et al., 2019b). During roasting, several studies noticed reduced moisture content and changes in texture and color (Pandey & Awasthi, 2015). The majority of earlier studies on roasting fenugreek concentrated on food product composition, generating supplementary protein and energy sources for cattle and young animals. The ideal roasting parameters (temperature and duration) weren't disclosed, though. Studying the roasted fenugreek's qualitative traits under these model processing settings is necessary (Usmael et al., 2022).

2.2.6.2. *Germination*

Because of legumes' high protein and fiber content, low glycemic index, antioxidant levels, and functional qualities like protein solubility and foaming abilities, the food processing industry is becoming more and more interested in incorporating these ingredients into food products. According to Dwivedi et al. (2019a), fenugreek seeds undergo processing such as germination, which raises the contents of crude protein, crude fiber, and total ash while lowering those of fat and dietary fiber. Germination of the grain has important effects on the chemical composition, nutritive value, and acceptability characteristics of products for human consumption. Ojha et al.

(2018b) evaluated the moisture content, total ash, crude protein, crude fat, and total carbohydrates of germinated fenugreek seeds. The crude protein content of fenugreek seeds increased with germination time. The highest crude protein was recorded with 72 h germination time, and the lowest was 24 h germination time. This is because germination is a biotechnological process in which metabolic enzymes, such as proteinases, are activated (Rai, 2021). 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 (Ojha et al., 2018b). Pandey and Awasthi (2015) reported that upon germination, the amount of total fat decreases along with the amount of free fatty acids, monoglycerides, and polar lipids, while the crude fiber significantly increases. When fenugreek seeds germinate, the ash content decreases by a significant amount. According to Ojha et al. (2018b), the carbohydrate contents of fenugreek flour decrease as germination time increases. He said that the highest carbohydrate for germinated fenugreek flour after 24 h, and then decreased as germination time increased. 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 the breakdown and synthesis of seed carbohydrates. The decreased carbohydrate content in the sprouted fenugreek flour might be attributed to the use of the nutrient as a readily available energy source during sprouting. The production of structural carbohydrates during germination, such as cellulose and hemicelluloses, may be the cause of a rise in crude fiber content (Ojha et al., 2018b). As the time of germination increases the crude fiber content slightly decreases. This decrease is accompanied by a drop in galactan content. The ash content of fenugreek flours decreased as the germination time increased. Iron concentration in the minerals also reduces upon germination. However, calcium and phosphorus levels dramatically increase (Pandey & Awasthi, 2015). According to Birhane (2012), phenolics are initially antioxidants in nature because they exhibit higher antioxidant activity during early germination, which correlates with higher phenolic content.

2.2.7. Functional Properties of Fenugreek Blended Bread

Growing rates of protein-energy deficits, micronutrient malnutrition, and food insecurity are the main causes of the illness burden among vulnerable population groups in developing and rising countries. Creating innovative therapies is crucial to addressing these grave health concerns. A great source of proteins, dietary fiber, and bioactive phytonutrients, fenugreek seed powder can help avoid a variety of illnesses and nutritional health problems (Ahmad et al., 2023). According to Roy (2022), adding fenugreek seed powder to wheat flour improves its antioxidant and nutritional content without sacrificing the bread's acceptability. The bread's crude protein, crude fiber, and ash contents increase with the addition of fenugreek powder. Similarly, when supplementation levels increased, so did the polyphenol, flavonoid content, and antioxidant activity of bread. The results of this study suggest that adding fenugreek seed powder to bread mixtures up to a 7.5% concentration won't have a detrimental effect on the bread's sensory quality. Producers and consumers can use this strategy to provide healthier bread options because it is inexpensive and simple to prepare (Roy, 2022). Furthermore, it was observed that adding fenugreek flour to wheat flour at varying percentages (5–20%) raises the bread's protein, lysine, mineral, and fiber contents (Hooda & Jood, 2005). Fenugreek (raw, soaked, and germinated) additions up to 15% result in bread with a satisfactory loaf volume and other sensory quality attributes (crumb color, crumb texture, taste, etc.); in contrast, fenugreek supplementations up to 20% cause a depression effect in loaf volume and bread that is found to taste bitter (Hooda et al., 2005). Nonetheless, out of all the bread supplemented with flour, the bread supplemented with 15% germinated fenugreek flour exhibits satisfactory organoleptic and baking qualities and contains a respectable amount of minerals, dietary fiber (12.04%), total lysine (3.02 g/100 g protein), and protein (24%) (Hooda & Jood, 2005).

According to Chaubey et al. (2018), adding 10% (wt/wt) of debittered and germinated fenugreek seed to wheat flour fortification resulted in improved protein, fiber, total polyphenol, and flavonoid contents compared to the control wheat bread. Additionally, the bread showed a lower glycemic index and a decreased rate of in vitro starch digestibility. However, out of all the enriched loaves, the one made with germinated fenugreek flour had acceptable organoleptic and baking qualities and had notable levels of minerals, dietary fiber, protein, and total lysine (Hooda & Jood, 2005).

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study Area:

The study was conducted at Mekelle University's Food Science and Post-Harvest Technology laboratory to investigate the effect of roasting, germination, and blending ratios of fenugreek with wheat flour on the bread's physical characteristics, proximate composition, functional properties, and sensory evaluation. All of the experimental items, including commercial wheat flour and raw fenugreek, were gathered from Mekelle City, which is situated in the Tigray Region of Northern Ethiopia at a latitude of about 13.49° N and an elevation of about 2,084 meters (6,837 feet) above sea level. Analysis for the proximate composition (moisture, ash content, crude protein, crude fat, crude fiber), functional properties, and sensory analysis was conducted at the Department of Food Science & Post-harvest Technology laboratory, and the minerals (iron, zinc, and calcium) were analysed at the Geology laboratory, Mekelle University.

3.2. Experimental Materials:

The materials, such as commercial bread wheat flour and fenugreek seed (*Trigonella foenum-graecum L.*), were collected from a market in Adihaki, and other materials such as distilled water, aluminium foil, Olive oil, laboratory glove, and labeling paper were bought from Kedamay Weyane.

3.3. Experimental Design

The experiment was planned with a single factor to investigate the effect of the processing of fenugreek (Roasted and Germinated) and blending ratio with wheat flour on bread's nutritional and sensory quality. The factor consists of three blending ratios of roasted and germinated fenugreek flour to wheat flour (5:95, 10:90, and 15:85). The effect of roasting and germinating fenugreek on the bread was analyzed independently. The treatments were laid out in a completely randomized design (CRD) to see the effect of the blending ratio of roasting and germination of fenugreek, and the comparison between each of these factors on the nutritional and sensory quality of wheat bread. Bread made of 100 % wheat flour was prepared as a control treatment for comparison purposes. To ensure statistical reliability, each blending level was replicated three (3) times, resulting in a total of 21 samples (6 treatments × 3 replicates) and 1×3 control sample (100 % refined wheat flour).

Table 1: Experimental plan

Fenugreek Blending Levels To Wheat Flour	Processing Methods of Fenugreek	
	Roasted (Ro)	Germinated (Gr)
B1	RoB1	GrB1
B2	RoB2	GrB2
B3	RoB3	GrB3
Control	100 % Wheat Flour	

Where: *B1: 5 % Fenugreek Powder + 95 % Bread Wheat Flour; B2: 10 % Fenugreek Flour + 90 % Bread Wheat Flour; B3: 15 % Fenugreek Flour + 85 % Bread Wheat Flour; Ro: Roasted Fenugreek Seed Powder at 130°C for 7 minutes; Gr: Germinated Fenugreek Seed Powder for 72 hrs. at room temperature.*

3.4. Raw Material Preparation

3.4.1. Sample Preparation

Commercial wheat flour, which was bought from the local market and commonly used in bread-making on a 14 % moisture basis, was utilized for analysis. Before analysis, the commercial wheat flour procured from the local market was sealed in an airtight container and kept in a cool and dry place. The fenugreek was subjected to different processing techniques, viz., roasting and germination. The roasted and germinated dry fenugreek was ground to a fine powder using a laboratory miller or grinder and passed through a standard test sieve of 0.425mm size. Flour was collected and stored in air-tight food-grade containers separately for further analysis at ambient temperature. The following steps were taken in the fenugreek processing methods:

3.4.2. Processing of fenugreek seed:

3.4.2.1. Germination

The fenugreek seeds were cleaned, sorted, and washed using potable water. Then, the seeds were soaked in potable water for 24 hrs at room temperature with a seeds-water ratio of 1:5 (w/v) (Ojha et al., 2018a). The unimpeded water was discarded, and the soaked seed was rinsed twice with boiled, cooled water to avoid post-contamination during germination. The soaked seed was germinated by covering (tying) it with a muslin cloth at room temperature for 72 hrs. (Ojha et al.,

2018b). The germinated seed was dried in an oven at 60 °C for 24 hrs. till the moisture content was below 14 % (Dwivedi et al., 2019a)

3.4.2.2. Roasting

Roasting can be done in various ways, including an open pan and a hot-air oven. In this study, 50 g of fenugreek was roasted in an open pan at 130°C for 7 minutes. It was stirred with a ladle to maintain a proper and uniform roasting until it became slightly brown and left a peculiar aroma. A stopwatch was used to monitor the roasting time, ensuring the maintenance of nutritional and sensory quality in fenugreek.

Table 2: Chemical composition of wheat flour, raw fenugreek seed, and germinated fenugreek flour.

Raw materials	Parameters					
	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Carb (%)
Wheat Flour	12.01±0.005 ^a	0.6±0.005 ^d	10.95±0.005 ^d	1.58±0.005 ^d	0.49±0.005 ^d	74.34±0.01 ^a
Raw FS	7.12±0.005 ^c	3.58±0.00 ^b	21.79±0.005 ^c	4.27±0.005 ^a	8.41±0.005 ^b	54.81±0.01 ^c
Roasted FF	5.64±0.005 ^d	3.96±0.00 ^a	22.11±0.005 ^b	4.02±0.005 ^b	7.93±0.005 ^c	56.31±0.01 ^b
Germinated FF	9.87±0.00 ^b	2.94±0.00 ^c	31.01±0.00 ^a	2.95±0.005 ^c	10.25±0.005 ^a	42.97±0.005 ^d
CV (%)	5.72	1.86	9.57	1.75	5.64	3.23

Note: Values are the mean of triplicate determinations with standard deviation. The different letters in the same column mean significant differences at (P<0.05), and the same letters indicate no significant differences with the raw materials. Carb- Carbohydrate, FS- Fenugreek Seed, FF- Fenugreek Flour CV- Coefficient of Variation.

3.4.3. Preparation of blends

Fenugreek seed powder (roasted & germinated) was blended separately with wheat flour at different ratios, viz., 5:95, 10:90, 15:85% (Godebo et al., 2019).

3.5. Bread Making Procedure

The bread-making performances of the treatments (control and blends) were determined using the straight dough (AACC., 2000) No. 10-09 method. The bread formula for each loaf included wheat flour 300 g (on 14% moisture basis) with 5, 10, and 15 % of germinated fenugreek (GF) and roasted fenugreek (RF) separately was blended well. All the ingredients (salt 1.5%, instant yeast 3%), and water (~60%) were exactly weighed and mixed thoroughly, followed by

hydration, mixing, dough development, leavening, baking, cooling at room temperature, and storing (Avramenko et al., 2018). Once the dough becomes wet sticky, and extremely extensible, it was kept for proofing under anaerobic conditions at 30°C for 120 min (Chaubey et al., 2018). Then, the loaves were kneaded and sheeted for equal distribution of gas and molded into the proper shape. The loaf was baked at 240 °C for 20 min. The bread was cooled, packed, and stored at room temperature (Chaubey et al., 2018). Wheat-Fenugreek blended bread was prepared as follows, with the picture and diagram below.



Control (100%)



GB1 (5%)



GB2 (10%)



GB3 (15%)



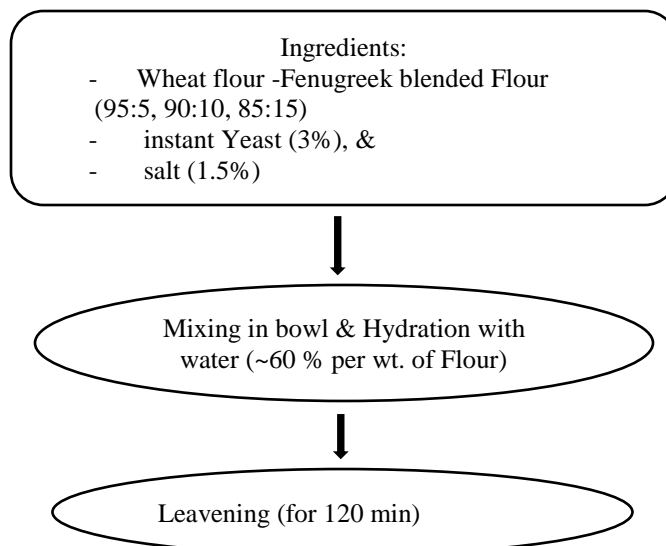
RB1 (5%)



RB2 (10%)



RB3 (15%)



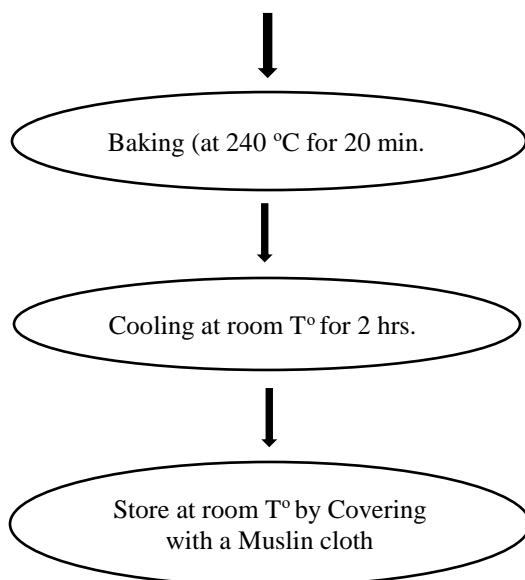


Figure 3: Flow diagram of bread making

3.6. Determination of Chemical Composition

Samples of commercial wheat flour, fenugreek flour, and a dried powder of composite bread for proximate analysis were carried out to determine overall quality parameters such as moisture content, crude protein, crude fat, carbohydrate, Ash, and fiber content, Mineral composition such as Calcium, Iron, and Zinc. All the parameters, such as the proximate composition, functional properties, bread physical characteristics, and sensory analysis, were analyzed at Mekelle University Food Science and Post-harvest Technology laboratory, and the minerals (iron, zinc, and calcium) were analyzed at the Geology laboratory, Mekelle University.

3.6.1. Proximate Composition

3.6.1.1. Moisture Content

Moisture content was determined according to the AACC. (2000) method No. 44-15A. Aluminum weighing dishes were numbered, dried in the oven for 30 minutes, cooled in a desiccator, and weighed again. A 2 g sample was weighed out and repeated in triplicate to get an accurate value of the sample. The Temperature of the hot air oven was set at 130 °C. After the hot air oven was reached at a temperature of 130 °C, the aluminum weighing dishes containing the samples were placed in a hot air oven for 1 hour. The samples were removed and placed in

desiccators to cool for 30 minutes and reweighed again. The moisture content was calculated according to the following equation:

Equation 1: % Moisture content determination

$$(\text{Moisture \%}) = \frac{W2 - W3}{W2 - W1} \times 100\%$$

Where: **W1** = weight of the dish (g),

W2 = initial weight of the sample and dish before drying (g) and

W3 = weight of sample and dish after drying (g)

3.6.1.2. *Crude protein*

The crude protein content of all samples was determined according to the AACC. (2000) method No.46-10. The ground samples were analyzed using the Kjeldahl method. A sample of 1.0 g was weighed and added into a Kjeldahl digestion flask. A catalyst mixture (Na₂SO₄) mixed with anhydrous CuSO₄ in the ratio of 10:1 of 1 g was added. After adding 5 mL of concentrated H₂SO₄, the digestion flask was placed in the digester and the temperature was brought to 350°C allowed to digest for over 2 hrs until digestion was completed. The flask was removed from the digester and allowed to cool. After it had cooled, the content in the flask was diluted with 30 mL of distilled water, followed by 25 mL (40% NaOH) was added into the digestion flask to neutralize the acid and to make the solution slightly alkaline. The contents were distilled immediately, inserting the digestion tube line into the receiver flask that contains 25 mL of 4% boric acid solution, and about 150 mL of distillate was collected. Finally, the distillate was titrated with a standard 0.1N HCl. The % of nitrogen was converted to % of protein by using an appropriate conversion factor (protein = 6.25×%N). Each sample was repeated in triplicate.

Equation 2: % Nitrogen determination

Nitrogen Determination

$$N(\%) = \left(\frac{V_{HCl} \times N_{HCl} \times 14}{m \times 1000} \right) \times 100$$

Equation 3: % Protein determination

Protein Determination

$$\% \text{ protein} = F \times \%N$$

Where: V HCl = Volume of HCl in litter consumed to the endpoint of the titration, N HCl =Normality of HCl (used often is 0.1 N), m = the sample weight on a dry matter basis, 14= Molecular Weight of Nitrogen, N=is Nitrogen (%), F=Conversion factor 6.25, and P=is protein (%).

3.6.1.3. *Crude fat*

The crude fat analysis was determined using the Soxhlet extraction method, according to the AACC. (2000) No. 30-10. The ground sample (3 g) was weighed and added into a thimble. The thimble with the sample was placed in a 50 ml beaker and dried in an oven for 2 hr. at 110 °C. A 150- 250 ml dried beaker was weighed and rinsed several times with petroleum ether. The sample contained in the thimble was extracted with petroleum ether in a Soxhlet extraction apparatus for 6-8 hr. After the extraction, the extracted fat was transferred into a pre-weighed beaker (mi). The beaker with extracted fat was placed in a fume hood to evaporate the solvent on a steam bath until no solvent odor was detectable. Then the beaker with content was dried in an oven for 30 minutes at 100 °C. Finally, the beaker with its contents was removed, cooled in a desiccator, and weighed (mf). The amount of fat in flour was calculated by using the following formula:

Equation 4: % Fat determination

$$\text{Fat (\%)} = \frac{(Mf - Mi)}{M} \times 100$$

Where:

Mf is a dried mass of fat with a beaker (g),

Mi is the Mass of the beaker (g) and

M is Sample mass (g, db.)

3.6.1.4. *Ash Content*

According to the AACC. (2000), the total ash content of the sample was determined by the gravimetric method No. 08-01. The crucible was cleaned, dried, and ignited at 550 °C for 1 hr. and weighed (m1). Ground sample (3 g) was weighed (m2). The sample was dried at 120 °C for 1 hour. Then the dried sample was carbonized over a blue flame and ignited in a muffle furnace at 550 °C until ashing is complete (over 12 hr.). After being ignited, the sample was cooled to ambient temperature and weighed (m3). Finally, the total ash content was calculated as follows:

Equation 5: % Ash determination

$$\text{Ash (\%)} = \left(\frac{M3 - M1}{M2 - M1} \right) \times 100$$

Where;

M1 is the mass of the crucible (g),

M2 is the sampled mass with the crucible (g) and

M3 is the final mass of the sample with the crucible (g)

3.6.1.5. *Crude fiber*

The crude fiber was analyzed according to the AACC. (2000) No. 32-10. The ground sample (3 g) was weighed (m1) and placed in a 500 ml beaker. This was digested with 1.25% sulfuric acid, washed with water, and further digested with 1.25% sodium hydroxide, filtered in a coarse porous (75 μm) crucible in an apparatus at a vacuum of about 25 mm. The residue left after refluxing was washed again with 1.25% sulfuric acid at near boiling point. Then the residue was dried at 110 °C overnight, cooled in a desiccator, and weighed (m2). After being dried, the sample was ashed at 550 °C until ashing is completed, cooled in a desiccator, and weighed again (m3). The total crude fiber was expressed in percentages as follows:

Equation 6: % Fiber determination

$$\text{Fiber (\%)} = \left(\frac{M2 - m1}{M1} \right) \times 100$$

Where;

F is total crude fiber (%),

M1 is the mass of the sample (g, db.),

M2 is the mass of the sample before ashing (g), and

M3 is the mass of the sample after ashing (g, db.)

3.6.1.6. *Utilizable Carbohydrate*

This will be determined by subtracting the sum of other constituents from 100.

Equation 7: % Carbohydrate determination

$$\% \text{ carbohydrate} = 100 - (\% \text{ MC} + \% \text{ C. Protein} + \% \text{ C. Fiber} + \% \text{ C. Fat} + \% \text{ Ash})$$

3.6.2. Mineral Analysis

Mineral analysis such as determination of calcium, iron and zink was carried out at the department of Geology laboratory, Mekelle University.

3.6.2.1. Calcium

The calcium content was determined by an atomic absorption spectrophotometer (AACC., 2000). A sample of 2.0 g was weighed and placed in an ashing vessel (that has been pre-ignited at 550 °C and cooled in a desiccator). The sample was carbonized over a blue flame of a Bunsen burner and put in the muffle furnace at 550 °C until ashing was completed. Then, the ash was dissolved in 10 mL of dilute 3M HCl. The solution was boiled and evaporated nearly to dryness in the steam bath. The residue was re-dissolved quantitatively in 20 mL of 2M HCl and was filtered through coarse porosity filter paper into a 100 mL volumetric flask. The paper was washed. Then, dilute thoroughly with water to a 100 mL mark.

The standard solution (25µg Ca /ml) was prepared from analytical grade calcium wire by dissolving 25µg in 30 ml HCl and 50 ml distilled water and then diluted to 1 L. Finally, calcium was measured by adding enough La stock solution to make the final dilution 1% La (i.e., 5 mL Lantanium solution to a 25 mL flask), and this was added to the sample and final standard solution. The absorbance of the sample was read with an Atomic Absorption Spectrophotometer at 422.7 nm. Calcium content was calculated with the following formula.

Equation 8: Calcium determination

$$\text{Ca (mg/100g)} = \frac{\text{C} \times 100}{\text{Sample mass (g)(db)}}$$

Where;

C is the concentration of the sample from the plot of absorption in (µg/ml);

S is Sample mass (g)

3.6.2.2. Iron

Iron content was determined by Atomic Absorption Spectrophotometry (AACC., 2000). A sample of 2.0 g was taken into the ashing vessel (that has been pre-weighed at 550 °C and cooled in desiccators). Ashing was at 550 °C and the ashed was dissolved in a volume of HCL-H₂O (1:1); 20 ml of this solution was added and evaporated to dryness in a steam bath. After cooling to ambient temperature, absorbance was read at 248.3 nm using air-acetylene as a source of flame for atomization with Atomic absorption standard solution (1000 µg Fe/mL) was prepared from pure iron wire by dissolving 1 g Fe into 30 ml 6 M HCL with boiling and dilute to 1 L.

Equation 9: Iron determination

$$\text{Fe (mg/100g)} = \frac{(\text{mg/ml}) \times 100}{\text{Sample mass (g)}(\text{db})}$$

Where $\mu\text{g/ml}$ is the absorbance reading concentration.

3.6.2.3. Zinc

Zinc content was determined by an Atomic Absorption Spectrophotometer (AACC., 2000). The sample 2.0 g was taken into the ashing vessel (which was pre-weighed/Pre-ignited at 550 °C) and cooled in a desiccator. Ashing was done at 500 °C, and then the ash was dissolved in a volume of HCL-H₂O (1:1); 20 mL of this solution was added and evaporated to dryness on a steam bath. After cooling to ambient temperature, absorbance was read at 213.8 nm using air-acetylene as a source of flame ionization with atomic absorption standard solution (10 μm Zn/mL) was prepared from analytical grade ZnO by dissolving 1.380 g into 10 ml 6M HCL and diluted to 100 mL and 5 mL of the solution was taken and diluted to 500 mL mark with distilled water and a series of standard solution construct the calibration curve. The following formula was used to calculate zinc content.

Equation 10: Zinc determination

$$\text{Zn (mg/100g)} = \frac{(\text{mg/ml}) \times 100}{\text{Sample mass (g)}(\text{db})}$$

Where $\mu\text{g/ml}$ is the absorbance reading concentration.

3.7. Functional Properties of Fenugreek-Wheat Flour

3.7.1. Water Absorption Capacity

The water absorption capacity of flour samples was determined according to the method described by Chandra et al. (2015). The sample of 1.0 g was mixed with 10 mL of distilled water and transferred to centrifuge tubes for water absorption. Then, the dispersion was stirred periodically, held for 30 min, followed by centrifugation (model EBA 8S Hettich, Germany) for 25 min at 2310 g. The water absorption capacity was expressed as a gram of water bound per gram of the sample on a dry basis. The water absorption capacity was calculated as follows:

Equation 11: Water Absorption Capacity Determination

Water absorption capacity (WAC)

$$= \text{Volume of water absorbed (ml)} / \text{weight of sample (Fenugreek or wheat flour) (g)}$$

3.7.2. Oil Absorption Capacity

The oil absorption capacity of flour samples was determined using the method described by Chandra et al. (2015). To measure oil absorption capacity, a 0.5 g sample was mixed with 6 mL of olive oil and centrifuged at 2310 g for 25 minutes. The separated oil was removed from the tubes and left inverted for 25 minutes to drain before reweighing. The oil absorption capacity was expressed as grams of oil bound per gram of dry sample. It was calculated using Equation 12.

Equation 12: Oil Absorption Capacity Determination

$$\text{OAC (g/g)} = \frac{\text{weight of centrifuge tube after drawing oil} - (\text{centrifuge tube weight}) - \text{weight of sample}}{\text{weight of the sample}}$$

3.7.3. Bulk Density

Bulk density was determined using the method described by Oladele and Aina (2007). Fifty grams (50g) of the sample wheat-fenugreek blended flour was placed into a 1000 mL graduated measuring cylinder. The graduated cylinder was then tapped continuously on a laboratory table until a constant volume was obtained. The bulk density (g/mL) was calculated by dividing the weight of the flour (g) by the volume of the flour (mL).

Equation 13: Bulk Density Determination

$$\text{BD} \left(\frac{\text{wt}}{\text{Volume}} \right) = \frac{\text{weight of sample}}{\text{volume of sample}}$$

3.8. Physical Characteristics

Breads from control and blended flours were baked in replicates (thrice).

3.8.1. Loaf volume:

The loaf volume was determined using the seed displacement method, as described in AOAC. (2010) No. 10.11 loading millet grains into an empty, calibrated box to the marked level and then unloading. The bread sample was then placed in the box, and the measured millet was loaded

again. The volume of the leftover grains from the box was taken, using a measuring cylinder, and recorded as the loaf volume in mL.

3.8.2. Loaf weight:

The weight of bread samples were determined after sufficient cooling using a digital balance (0.01 g accuracy). After being removed from the oven, loaves were immediately weighed and then placed on a wire grid for 2 h before volumes were determined (Dhingra & Jood, 2002).

3.8.3. Specific Loaf Volume:

Specific loaf volumes were calculated by dividing the loaf volume by the loaf weight and expressing the results as milliliter per gram (Dhingra & Jood, 2002). The specific volume (volume per unit weight) in ml/g was thereafter calculated as:

Equation 14: Specific loaf volume.

$$\text{Specific Loaf Volume} = \frac{\text{Loaf Volume (ml)}}{\text{Loaf weight (g)}}$$

3.9. Sensory evaluation:

A panel of 20 members were selected from staff, undergraduate, and graduating class students of the Food Science and Post-Harvest Technology program at Mekelle University. The wheat flour-fenugreek composite bread products were evaluated for their acceptability in terms of sensory attributes after 2 hours of baking. Sensory parameters like crust colour, texture such as (softness, chewiness, crumble), taste such as (saltiness, sourness, sweetness), appearance, aroma, and overall general acceptability were evaluated using a 7-point hedonic scale with scores ranging from -1 (dislike extremely) to 7 (like extremely) according to the method described by Tsegay et al. (2024). Samples of bread (100% wheat bread (control) and a composite of commercial wheat flour and fenugreek flour bread were kept on a plate covered by muslin cloth. The evaluation was conducted at room temperature. Coded samples from each selected product were arranged in random order on white plates and served to the sensory judges. Before each test session, orientation was given to the judges on the procedures of the sensory evaluation sheet. A glass of drinking water was provided to wash the oral cavity after tasting each sample.

Table 3: Grading system for sensory assessment.

Ranks	Score
-------	-------

Like Extremely	7
Like Moderately	6
Like Slightly	5
Neither like nor dislike	4
Dislike Slightly	3
Dislike Moderately	2
Dislike Extremely	1

3.10. Statistical Analysis

The data analysis was carried out by Analysis of variance (ANOVA) to investigate the effect of processing methods of fenugreek and blending ratios on the quality of fenugreek-wheat bread using Minitab statistical software (version 21.2). Mean values were considered at a 95% significance level ($p < 0.05$). The hedonic scores for the sensory evaluation were analyzed by ANOVA. Fisher's LSD tests were used to identify significant differences among mean main effects for processing methods and blending ratios. A 7-point hedonic scale ranging from 1 (dislike extremely) to 7 (like extremely) was used to express whether subjects liked the look, smell, aroma, taste, and mouthfeel.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1. Effect of processing fenugreek and blending ratio on proximate composition of wheat bread.

The nutritional composition of processed fenugreek blended wheat bread is presented in Table 5. Proximate analysis of the composite breads indicates that samples containing roasted and germinated fenugreek flour had higher ash, crude protein, crude fat, and crude fiber than those without it (Table 5). A similar observation was noted earlier in these parameters between flours obtained from processed fenugreek seeds and wheat (Table 5).

4.1.1. Moisture Content

From the results shown in Table 5, it is clear that adding processed fenugreek significantly affected the compositional features of wheat bread. Based on the data in the table, processed fenugreek at levels of 5, 10, and 15% showed significant ($p < 0.05$) differences in the bread's moisture content. The highest (10.67) moisture content was observed on bread developed from a 15% germinated fenugreek composite flour, and the lowest one (8.48%) was seen on bread developed from wheat flour (100%) control. It can be observed that bread baked from wheat-fenugreek flours had a high moisture content compared to wheat bread as a control. Likewise, Ismail (2007) found that the presence of legume flour increased the water required for the optimum bread-making absorption. Similar observations have also been previously reported by Kasaye et al. (2015) and Afzal et al. (2016).

The use of different types of processing and blending ratios influenced the bread's moisture content. Bread with germinated fenugreek flour tends to retain more moisture due to its dietary fibers and proteins, which can bind water in a higher capacity. The same study was reported by Man et al. (2019) and Awulachew et al. (2023). The moisture content for wheat bread as well as processed fenugreek blended bread was in an acceptable limit for safe storage (Dhull et al., 2018).

4.1.2. Ash Content

The ash content of all fenugreek blended bread samples and wheat bread (control) had significant ($p < 0.05$) differences. The ash content was higher (1.38%) in bread developed with 15% roasted fenugreek composite flour and the lowest ash content (1.02%) followed by 0.97% which was

found in bread developed with 5% germinated fenugreek composite flour, and 100% control, respectively. The ash content also showed an increment as the ratio of fenugreek flour increased. This was because of the higher ash content in the fenugreek flour than in the wheat flour, according to Table 5. The ash content was somewhat influenced by the ratio of sorghum-fenugreek, which was previously supported by Terefe (2017). The study was also illustrated by Man et al. (2019). Furthermore, ash content was higher in a bread sample that contained roasted fenugreek blends than in germinated fenugreek-blended bread. The observed increase of ash attributed to the addition of macro and micro minerals may be due to the breaking down of bonds between antinutritional factors (tannin, phytates, and oxalates) upon heating (Sharara & Sciences, 2017). On the other hand, the increase in ash content of bread made from wheat-fenugreek composite flour may be due to the higher ash content of legume flours than wheat flour. Similar findings were reported previously by Ahmed and Technology (2014) and Kasaye et al. (2015).

Table 4: Effect of processing fenugreek and blending ratio on proximate composition of wheat bread.

Sample Test	MC%	Ash%	Protein%	Fat%	Fiber%	Carb%
C	8.48±0.01 ^g	0.97±0.005 ^g	10.82±0.005 ^g	1.22±0.005 ^g	0.64±0.01 ^f	73.75±0.18 ^a
GB1	9.78±0.005 ^c	1.02±0.001 ^f	14.05±0.005 ^c	1.34±0.005 ^f	0.95±0.005 ^c	72.84±0.01 ^e
GB2	10.23±0.005 ^b	1.12±0.001 ^d	14.78±0.005 ^b	1.54±0.01 ^d	1.02±0.01 ^b	71.30±0.01 ^f
GB3	10.67±0.005 ^a	1.18±0.01 ^c	15.88±0.005 ^a	1.65±0.01 ^c	1.04±0.005 ^a	69.56±0.02 ^g
RB1	9.44±0.005 ^d	1.09±0.01 ^e	12.41±0.005 ^f	1.47±0.01 ^e	0.88±0.005 ^e	74.69±0.02 ^b
RB2	9.23±0.005 ^e	1.24±0.01 ^b	13.15±0.005 ^e	1.68±0.01 ^b	0.92±0.005 ^d	73.76±0.005 ^c
RB3	9.13±0.01 ^f	1.38±0.01 ^a	13.85±0.005 ^d	1.80±0.01 ^a	0.92±0.01 ^d	73.01±0.03 ^d
CV (%)	7.27	1.41	11.48	0.51	0.86	3.35

Note: Values are the mean of triplicate determinations with standard deviation. The different letters in the same column mean significant differences at ($P<0.05$), and the same letters indicate no significant differences with the sample codes. C- Control, GB1,2,3 indicates (Bread with 5,10,&15% Germinated Fenugreek blend) RB1,2,3 indicates-(Bread with 5,10,&15% Roasted Fenugreek blend. CV- Coefficient of Variation.

4.1.3. Protein content

As the obtained data (Table 5) revealed, the content of protein in all bread treatments increased significantly ($p<0.05$) with the amount of substitution of fenugreek flour. The crude protein of all fenugreek blended bread was significantly ($p<0.05$) higher than that of the control. The lowest (10.82%) protein content was recorded for the control (100%), and the highest protein content was observed (15.88%) for the bread enriched with 15% germinated fenugreek

composite flour. This result is in agreement with a previous report that shows improvement of bread protein by the addition of fenugreek flour (Terefe, 2017). There was also a significant ($p < 0.05$) difference among the treatments of germinated and roasted with 5, 10, and 15% blending levels, respectively. The highest crude protein was obtained from germinated (15%), and the lowest was in the roasted (5%) blend. This shows there was an outstanding increase in crude protein content of the bread as the ratio of germinated fenugreek seed flour. This may be due to higher protein content in fenugreek seed rather than wheat flour, due to the synthesis of new proteins, some amino acids and peptides release, due to proteinases enzymes activation during germination of fenugreek (Godebo et al., 2019; Rai, 2021). Improvement in protein digestibility during germination may be attributed to the 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 (Rai, 2021). The increase of crude protein in roasted fenugreek substituted bread was also in agreement with that reported by Sharara (2017) for the crude protein composition of fenugreek seed flour.

4.1.4. Fat Content

According to the study, the fat content of all the bread substituted with fenugreek flour was significantly ($p < 0.05$) higher than that of the control. The highest (1.80%) fat content was obtained from the roasted blend (15%), and the lowest (1.22%) was observed in the control (100%). There was also a significant ($p < 0.05$) difference among the treatments of germinated and roasted with 5, 10, and 15% blending levels, respectively. The highest fat content was observed in the roasted blend with 15% fenugreek flour at 1.80%, and the lowest was in the germinated blend with 5% fenugreek flour at 1.34%. The results of fat content, as given in Table 5, were decreased significantly ($p < 0.05$) in breads of germinated substitute. The loss of fat during germination may be due to its consumption as an energy source at the time of germination (Saini et al., 2016).

4.1.5. Fiber content

The obtained data revealed that all the fenugreek-blended bread was significantly ($p < 0.05$) higher in fiber content as compared to the control. The highest crude fiber was observed in the germinated blend (GB) (15%), and the lowest fiber content was recorded in the control (C) (100%). The fiber contents of the germinated and roasted blend with 5%, germinated blend with 10%, and 15% treatments were also significantly ($p < 0.05$) different from each other. However,

the roasted blends with 10 and 15% were not significant ($p>0.05$). An increase in fiber content was observed upon roasted and germinated fenugreek constituted bread. This could be due to a major constituent of cell walls, which might be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicelluloses, during germination (Ojha et al., 2018b) and roasting of fenugreek (Mathur & Choudhry, 2009). Similarly, the amount of crude fiber of bread was increasing while the amount of fenugreek flour increased. This could be due to the higher content of fiber in fenugreek flour as compared to wheat flour (Terefe, 2017). According to Tadesse et al. (2015), fiber contents of bread were important from a nutritional point of view, which facilitates the digestion and absorption process in the human body systems.

4.1.6. Carbohydrate content

As indicated from the obtained result, the total carbohydrate of all the fenugreek blended breads was significantly ($p<0.05$) decreased as compared to the control. This could be due to wheat having a higher carbohydrate content than that of fenugreek. The highest (77.75%) carbohydrate was obtained from the control (C), and the lowest (69.56%) was recorded in (15%) germinated fenugreek blended bread. Fenugreek blended bread significantly ($p<0.05$) differed among the treatments of germinated and roasted fenugreek with 5, 10, and 15% blending levels. As shown from the results, the highest (74.69%) carbohydrate was observed in roasted fenugreek with 5% blended bread, and the lowest (69.56%) was observed in germinated fenugreek with 15% blended bread. This indicates that the carbohydrate content of bread reduced as the percentage of fenugreek seed flour increased. The same trend was reported by Godebo et al. (2019). Finally, the bread blended with germinated fenugreek resulted in higher moisture content, crude protein, and crude fiber content as compared to roasted blended bread. Higher ash content, fat content, and carbohydrate content were exhibited in roasted fenugreek blended bread.

4.2. Effect of fenugreek processing and blending ratio on the mineral content of wheat bread

The results obtained in Table 6 illustrate the mineral composition of 100% wheat bread and those supplemented with fenugreek flour at different blending levels. All blends of bread were significantly ($p<0.05$) varied in Ca^{+2} , Fe^{+3} , and Zn^{+} contents.

4.2.1. Calcium

The obtained data showed that the calcium levels of all the fenugreek blended breads were significantly ($p<0.05$) increased as compared to the control. The highest (66.03mg/100g)

calcium content was observed in 15% roasted fenugreek blended bread, and the lowest (56.82mg/100g) was obtained from the control (100%) bread. However, Bread blended with 15% germinated fenugreek, 5, and 10% roasted fenugreek was not significantly different. As shown from the results, the calcium content of bread increased as the percentage of fenugreek flour increased. This study was similar to the report studied by Walle et al. (2017) and Godebo et al. (2019). Likewise, roasted fenugreek has a higher calcium (Ca) content than germinated fenugreek. This may be due to the release of bound calcium (Ca) by phytochemicals caused by heat during roasting. Even though not significant, it was observed in all treatments that the addition of roasted and germinated fenugreek can increase the Ca content of bread numerically. This increment may be caused by the higher calcium (Ca) content of fenugreek (Pandey & Awasthi, 2015).

Table 5: Effect of fenugreek processing and blending ratio on mineral composition of bread.

Sample Test	Parameters		
	Cal (mg/100g)	Fr (mg/100g)	Zn (mg/100g)
C	56.82±0.005 ^e	5.71±0.005 ^e	1.65±0.005 ^g
GB1	63.40±0.005 ^d	6.15±0.005 ^d	1.98±0.005 ^e
GB2	64.31±0.005 ^c	7.11±0.57 ^c	2.31±0.005 ^c
GB3	64.91±0.005 ^b	7.01±0.005 ^c	2.54±0.005 ^a
RB1	64.77±0.005 ^b	7.22±0.005 ^c	1.95±0.005 ^f
RB2	65.017±0.005 ^b	7.74±0.005 ^b	2.28±0.005 ^d
RB3	66.03±0.57 ^a	8.21±0.005 ^a	2.45±0.005 ^b
CV (%)	4.63	4.18	1.79

Note: Values followed by different letters within a column indicate a significant difference ($p < 0.05$). C- Control, GB1,2,3 indicates (Bread with 5,10,&15% Germinated blend) RB1,2,3 indicates-(Bread with 5,10,&15% Roasted blend. CV- Coefficient of Variation.

4.2.2. Iron

The data showed that the iron (Fe) content in all bread samples was significantly ($p < 0.05$) different. The iron (Fe) content of roasted fenugreek with 15% blended bread was higher than that of other blends. However, there was no significant ($p > 0.05$) difference among 10, 15% germinated and 5% roasted fenugreek blended bread. Furthermore, bread with germinated fenugreek blended bread had slightly lower iron (Fe) content than bread with roasted fenugreek supplemented. The decrease in iron (Fe) content of germinated fenugreek supplemented bread may be due to the leaching of iron in a soaking medium (Saini et al., 2016). The increase in iron

content of roasted fenugreek blended bread may be due to a decrease in anti-nutritional factors that bind iron during roasting of fenugreek (Pandey & Awasthi, 2015).

4.2.3. Zinc

The results showed that all the bread samples, i.e., control and supplemented with fenugreek flour, were significantly ($p < 0.05$) varied in Zn^{+} content. Significantly ($p < 0.05$) highest zinc content was observed in 15% germinated fenugreek blended bread, and the lowest zinc content was in the control (100%). There was also a significant difference between the germinated and roasted fenugreek with (5, 10, and 15%) blended bread, respectively. A similar trend was reported by Ibrahim and Hegazy (2009) and Godebo et al. (2019). Generally, roasted fenugreek blended bread resulted in an increase in calcium (Ca) and iron (Fe) as compared with germinated fenugreek. Meanwhile, germinated fenugreek blended bread had increased in zinc content as compared to roasted fenugreek blended bread. The results obtained for mineral composition were in line with Bukya et al. (2013), who stated that fenugreek seeds are found to contain high levels of minerals (Ca, Fe, and Zn) in roasted, boiled, and germinated fenugreek seeds.

4.3. Effect of fenugreek processing and blending ratio on the functional properties of composite flour

Functional properties are intrinsic physicochemical attributes that can influence how food systems behave while being processed and stored (Lakshmi Menon et al., 2014). The functional properties determine the application and use of food material for various food products (Adeleke & Odedeji, 2010). The results of the functional properties of wheat and Fenugreek flour composite blends are presented in Table 7.

4.3.1. Water absorption capacity (WAC)

Water absorption is a term chosen by the baking industry to represent the target water-to-flour ratio in bread dough. It is important to determine taste, texture, and dough performance during proofing and baking. In particular, WAC is related to dough consistency (Ritika et al., 2016). The most functional properties determined for composite flours exhibited higher values than those observed for wheat flour alone and showed significant variations at $p < 0.05$. This observation agrees with the results reported by Alu'datt et al. (2012) and El-Kewawy (2014). As shown in Table 7, the water absorption capacity (WAC) of composite flours showed significant ($p < 0.05$) variations among all types of blending ratios. The highest WAC was observed (1.58%)

in 15% blended roasted fenugreek flour, followed by 1.53% in 15% blended germinated fenugreek flour, and the lowest was observed (1.15%) in the control (100% wheat flour).

Table 6: Effect of processing fenugreek and blending ratio on the functional properties of composite flour.

Sample Test	Parameters		
	WAC (%)	OAC (%)	BD (g/ml)
C	1.01±0.005 ^f	0.84±0.005 ^g	0.82±0.005 ^f
GB1	1.31±0.005 ^e	1.08±0.005 ^f	0.95±0.000 ^e
GB2	1.40±0.005 ^c	1.15±0.005 ^c	1.05±0.005 ^d
GB3	1.51±0.005 ^b	1.12±0.000 ^e	1.11±0.005 ^a
RB1	1.32±0.005 ^d	1.14±0.000 ^d	1.08±0.005 ^b
RB2	1.38±0.005 ^c	1.21±0.000 ^b	1.05±0.005 ^d
RB3	1.56±0.005 ^a	1.30±0.000 ^a	1.07±0.005 ^c
CV (%)	0.45	0.95	0.58

Note: Values followed by different letters within a column indicate a significant difference ($p < 0.05$). C- Control, GB1,2,3 indicates (Flour with 5,10,&15% Germinated blend) RB1,2,3 indicates-(Flour with 5,10,&15% Roasted blend. WAC-Water Absorption Capacity, OAC-Oil Absorption Capacity. BD-Bulk Density, CV- Coefficient of Variation.

This shows there was a significant ($p < 0.05$) increment of the WAC of the composite flour as the blending ratio increased from 5 to 15%. An increase in the fenugreek flour blending ratio resulted in an increased water absorption capacity of blended flour. Such an increasing trend in WAC with an increase in fenugreek flour proportion has been reported in earlier studies (Abera et al., 2017; Dhull et al., 2018). However, WAC of 10% germinated and roasted composite flours were not significantly ($p > 0.05$) different. Likewise, there was also significant ($p < 0.05$) variation between the composite blends of roasted fenugreek flours and germinated fenugreek flours. The highest WAC was recorded for composite flour that contained roasted fenugreek flour (1.58%) and followed by the composite flour with germinated fenugreek seed flour (1.53%). A similar study was demonstrated by Terefe (2017). Composite flours exhibited maximum values for the water holding capacity in germinated blend (GB) and roasted blend (RB) is due to the high protein content and galactoamannan presence in the fenugreek flour. The ability of protein in flour to physically bind water is a determinant of its water absorption and binding capacity. The high insoluble fiber, 20-25%, and galactomannan, 20-30%, in fenugreek are responsible for high water absorption and binding capacity (Afzal et al., 2016). According to Harijono et al. (2013), protein has both hydrophilic and hydrophobic properties and can interact with water in foods. Low water absorption capacity is related to low polar amino acids in flour. Therefore, the higher

WAC of flour blends could be attributed to their higher protein content in comparison to wheat flour. Water absorption capacity is important in the development of ready-to-eat foods, and a high water absorption capacity may assure product cohesiveness (Ogunlakin et al., 2012).

4.3.2. Oil absorption capacity (OAC)

The results obtained in Table 7 illustrate significant differences in the oil holding capacity of composite flours were also observed. The mean values showed higher oil holding capacity for 15% wheat flour-roasted fenugreek composite flour (1.30%), followed by 10% wheat flour-roasted fenugreek composite flour (1.21%). Whereas, the lowest (1.08%) was for 5% wheat flour-germinated fenugreek composite flour. There was significant ($p < 0.05$) variation in composite flour flours between the wheat flour-roasted fenugreek flour composite blends and wheat flour-germinated fenugreek flour composite blends. The highest (1.30%) oil absorption capacity (OAC) was recorded for composite flour that contained roasted fenugreek flour, and the lowest (1.08%) for composite flour with germinated fenugreek flour. This shows that an increased ratio of roasted fenugreek flour increased the oil absorption capacity of the composite flours. The mechanism of oil/fat absorption capacity (OAC) is explained by Je (1979) as a physical entrapment of flavour retention. Chau et al. (1997) reported that surface area and hydrophobicity improve oil holding capacity. The OAC also makes the flour suitable for facilitating enhancement in flavor and mouth feel when used in food preparation. Absorption of oil by food products improves mouth feel and flavor retention (Modipuram, 2013). The reason for the increase in oil absorption capacity may be due to an increase in the concentration of total protein, which has a hydrophobic nature and therefore can interact with oil in foods, and is ultimately responsible for the enhancement in oil absorption capacity (Chandra et al., 2015). The oil absorption capacity (OAC) of flour is important as it improves the mouthfeel and retains the flavor (Wani et al., 2014). The OAC also makes the flour suitable for facilitating enhancement in flavor and mouth feel when used in food preparation (Modipuram, 2013).

4.3.3. Bulk density (BD)

The results recorded in Table 7 show that composite flour from wheat flour-roasted and wheat flour-germinated fenugreek flour has shown significant ($p < 0.05$) variations in bulk density (BD) due to the blending ratios of fenugreek flour to wheat flour. According to this study, the highest (1.11 g/ml) bulk density (BD) value was recorded for composite flour with 15% germinated

fenugreek flour, and the lowest (0.82 g/ml) was followed by wheat flour and 0.95 g/ml with 5% germinated fenugreek composite flour, respectively. However, the bulk density of 10% wheat-germinated fenugreek flour, and wheat flour-roasted was not significantly ($p>0.05$) different. The results agree with Terefe (2017), who found that the bulk density of sorghum-germinated fenugreek composite flour was 3.77mg/ml. The bulk density is generally affected by the particle size and the density of flour and is important in determining the packaging requirement, raw material handling, and application in wet processing in the food industry (Ajanaku et al., 2012). Furthermore, the decrease in bulk density is due to the breakdown of complex compounds into simpler ones (Maqbool et al., 2017). Akubor and Obiegbuna (1999) reported that the bulk density of a sample could be used to determine its packaging requirements, as this relates to the load the sample can carry if allowed to rest directly on one another. In contrast, low bulk density would be an advantage in formulating complementary foods (Akubor & Obiegbuna, 1999).

4.4. Effect of fenugreek processing and blending ratio on the physical properties of wheat bread.

The physical characteristics of bread samples prepared from wheat and fenugreek composite flours are presented in Table 8. The loaf volume of breads prepared from wheat flour and from fenugreek blended flour, viz., roasted fenugreek flour and germinated fenugreek flour at 5, 10, and 15% levels, was recorded (Table 8).

4.4.1. Loaf volume

Loaf volume is one of the major quality indicators for bread and is influenced by many factors, including wheat flour composition, additives, and dough fermentation conditions (Rosell et al., 2001). The loaf volume of bread made from control (100% wheat flour) was 412.67 mL. However, a significant ($p<0.05$) reduction in loaf volume was observed as the level of supplementation with processed fenugreek (roasted and germinated) flour increased. Maximum reduction in loaf volume (363.68ml) was in bread made from wheat flour blended with roasted fenugreek flour at a 15% level, whereas germinated fenugreek flour blended bread exhibited 367.36 ml loaf volume at a 15% level. This might be due to the dilution effect on gluten with the addition of non-wheat flour to wheat flour and less retention of CO₂ gas, which caused depression in loaf volume (Dhingra, 2000; Sharma & Chauhan, 2000a).

Table 7: Effect of processing fenugreek and blending ratio on the physical characteristics of bread.

Sample Test	Parameters		
	Loaf volume (ml)	Loaf weight (g)	Specific loaf volume (ml/g)
C	412.67±0.57 ^a	83.133±0.057 ^g	4.96±0.005 ^a
GB1	391.23±0.006 ^b	88.63±0.057 ^e	4.41±0.00 ^b
GB2	379.17±0.006 ^c	91.46±0.057 ^c	4.23±0.15 ^c
GB3	367.36±0.006 ^e	94.36±0.057 ^a	3.90±0.00 ^d
RB1	391.54±0.006 ^b	87.93±0.005 ^f	4.45±0.00 ^b
RB2	372.92±0.006 ^d	90.06±0.00 ^d	4.14±0.00 ^c
RB3	363.68±0.006 ^f	92.38±0.005 ^b	3.93±0.00 ^d
CV (%)	4.24	3.85	8.19

Note: Values followed by different letters within a column indicate a significant difference ($p < 0.05$). C-Control, GB1,2,3 indicates (Bread with 5,10,&15% Germinated fenugreek blend) RB1,2,3 indicates (Bread with 5,10,&15% Roasted fenugreek blend. CV-Coefficient of Variation.

4.4.2. Loaf weight

The loaf weight of the different processed fenugreek blended bread samples is presented in Table 8. The loaf weight of control bread was 83.13g, and it did not change significantly ($p < 0.05$) up to 5% level of substitution of wheat flour with roasted and germinated fenugreek flour. However, a significant ($p < 0.05$) increase in loaf weight was observed with each increment of processed fenugreek flour, indicating that an extra amount of water was retained in breads after baking (Haridas Rao & Malini Rao, 1991). Germinated fenugreek flour supplemented bread at a 15% supplementation level exhibited the maximum loaf weight of 94.36g, followed by roasted fenugreek flour supplemented bread at a 15% with a loaf weight of 92.38 g. The loaf weight was found to increase with increasing levels of roasted and germinated fenugreek flours, which may be due to less retention of gas in the blended doughs, thereby providing a dense texture to the bread. Values of loaf weight observed in the present study were similar to those reported by Sharma and Chauhan (2000b); Dhingra and Jood (2002).

2.1. Specific loaf volume

The specific loaf volume of the different processed fenugreek blended bread samples is presented in Table 8. Specific loaf volume was obtained by dividing the loaf volume by loaf weight (Dhingra & Jood, 2002). The results indicated a decrease in specific loaf volume on increasing the levels of non-wheat flours (i.e., germinated and roasted fenugreek) as compared to

the control (4.96 ml/g). The loaf volume decreased with similar results to 4.41 and 4.45 ml/g, followed by 4.23, 4.14 ml/g, and 3.90 and 3.93 ml/g in both 5, 10, and 15% germinated and roasted fenugreek blended bread. The low specific loaf volume of various cereal-legume blended breads might be due to their poor quality and quantity of gluten, which is responsible for the retention of CO₂ gas in the fermented dough as reported by Dhingra and Jood (2004). Similar observations have also been reported by Indrani and Venkateswara Rao (1992) and Ereifej and Shibli (1993) in breads from wheat flour.

4.5. Effect of fenugreek processing and blending ratio on the sensory acceptability of wheat bread.

From the consumers' point of view, the sensory properties are very important. In the present study, these properties of the bread were evaluated by the panelists who were quite familiar with the product quality. The replacement of wheat flour with different proportions of processed fenugreek flour resulted in considerable changes in the sensory properties of bread, in addition to the changes in the physical properties as discussed above. The blending of wheat flour with roasted and germinated fenugreek flour at different levels altered the organoleptic characteristics of different blended breads. Data on crust color, Texture, taste, appearance, aroma, and overall acceptability are presented in Table 9.

Table 8: Results for sensory acceptability of wheat-fenugreek blended bread.

Sample Test	Parameters					
	Crust Color	Texture	Taste	Appearance	Aroma	Overall Acceptability
C	6.65±0.58 ^a	6.30±0.47 ^a	6.40±0.50 ^a	6.45±0.5 ^a	6.25±0.71 ^a	6.50±0.68 ^a
GB1	6.05±0.51 ^b	6.15±0.67 ^a	5.85±0.58 ^b	6.15±0.67 ^a	5.25±0.91 ^{bc}	6.10±0.44 ^b
GB2	5.75±0.55 ^{bc}	5.90±0.64 ^a	5.40±0.68 ^c	5.15±0.81 ^{bc}	5.45±0.99 ^b	5.60±0.82 ^c
GB3	4.5±0.60 ^d	4.85±0.48 ^{cd}	4.8±0.52 ^d	4.65±0.67 ^{de}	4.65±0.81 ^d	4.85±0.48 ^e
RB1	5.6±0.50 ^c	5.30±0.86 ^b	5.25±0.44 ^c	5.55±0.60 ^b	5.60±0.88 ^b	5.30±0.47 ^{cd}
RB2	4.70±0.65 ^d	5.05±0.60 ^{bc}	4.75±0.78 ^d	4.95±0.68 ^{cd}	4.75±0.85 ^{cd}	4.95±0.39 ^{de}
RB3	4.45±0.68 ^d	4.50±0.68 ^d	4.10±0.44 ^e	4.5±0.60 ^e	4.45±0.75 ^d	3.95±0.88 ^f
CV (%)	18.17	16.54	17.35	17.65	19.60	18.76

Note: Values followed by different letters within a column indicate a significant difference (p<0.05). C- Control, GB1,2,3 indicates (Bread with 5,10,&15% Germinated Fenugreek blend) RB1,2,3 indicates- (Bread with 5,10,&15% Roasted Fenugreek blend. CV- Coefficient of Variation.

The sensory scores for color are presented in Table 9. The acceptance of color indicated that the control ranked highest due to its excellent color, followed by germinated and roasted blended bread. The lowest color acceptance was observed in roasted fenugreek blended bread. A significant ($p < 0.05$) difference was noted between the control and fenugreek blended bread concerning color. However, no significant ($p > 0.05$) differences were found among the germinated fenugreek blended bread with 5, 10, and 15% and the roasted fenugreek blended bread with 10 and 15%. Similarly, the color acceptance of 10 and 15% roasted fenugreek blended bread was not significantly ($p < 0.05$) different. A decrease was observed, indicating that there was a reduction in color intensity with an increase in the level of blending with fenugreek. This indicates processing method and blending ratio significantly affected the color of the composite bread. A similar trend was reported by Godebo et al. (2019), regarding the effect of incorporating different levels of raw and germinated fenugreek seed flour on the color acceptance evaluation of baladi bread samples. The results from sensory panelists show that the crust color of bread blended with 5% germinated fenugreek was the most acceptable color, which may be due to the activated enzymes of malted fenugreek flour that have their effect during fermentation. It was seen that the dough with germinated fenugreek was pre-fermented than the dough with roasted and raw fenugreek flour. This may be used in the future to decrease fermentation time in addition to using a starter culture in dough making.

The results of the sensory texture scores are presented in Table 9. The crust texture was related to the external appearance of the bread top, i.e., smoothness or roughness of the crust. Noticeable changes were observed in the crust texture of breads. Among the blended loaves of bread, the highest score of crust texture was observed in the control (100%), (5%), and (10%) of germinated fenugreek blended bread. The crust texture of 15% germinated fenugreek blended bread and 10% roasted fenugreek blended bread samples was not significantly ($p > 0.05$) different. However, the crust texture of 5 and 15% roasted fenugreek blended bread was significantly ($p < 0.05$) different. The texture of the crust in bread was observed to decrease significantly with an increase in both germinated and roasted blended breads. A similar study was followed by Hooda et al. (2005)

The results of the sensory taste scores are presented in Table 9. Taste evaluation suggested that control and various supplemented breads had the most satisfactory taste scores at the 5% level.

Bread's taste was associated with sweet, sour, and bitter sensations triggered in the mouth by contact with it (Ghebrehiwot et al., 2016). The taste of control (100%) (6.40), followed by (5%) (5.85) germinated fenugreek blended bread, was significantly ($p < 0.05$) higher. Germinated fenugreek blended bread with (10%) and (15%) and roasted fenugreek blended bread with 5% and 10% samples were not significantly ($p < 0.05$) different. The taste of the germinated and roasted fenugreek blended bread became bland, and the bread was considered slightly bitter, probably due to the dominant taste of the fenugreek flour. However, a 15% level of substitution of roasted fenugreek flour in wheat flour had the poorest taste score, which was considered bitter. A similar study was reported by Hooda et al. (2005).

The results of the sensory appearance scores are presented in Table 9. The appearance score for the control bread decreased significantly upon increasing the blending level to 10 and 15% with fenugreek flour, i.e., roasted and germinated. For Control and 5% germinated fenugreek blended breads, the appearance score was in the category of “like moderately”. Among the blended breads, the highest appearance score was observed for control and germinated fenugreek blended bread at the 5% level, whereas the lowest score was observed for roasted fenugreek blended bread (15% level). However, there was bread significant ($p < 0.05$) difference among the germinated fenugreek blended bread with 10 and 15% and the roasted fenugreek blended bread with 5 and 10%. Gayle et al. (1986) suggested that the appearance of bread was an important sensory characteristic on which the acceptability of bread depends.

The results of the sensory aroma scores are presented in Table 9. The aroma of all the bread added with germinated and roasted fenugreek flour at different levels shows a significant difference ($p \leq 0.05$). The panelists indicated their suggestion that the addition of fenugreek flour improves its flavor (aroma). The highest acceptance of aroma was observed in the control (100% wheat flour bread). There was no significant ($p > 0.05$) difference observed among germinated fenugreek and roasted fenugreek blended bread at 10 and 5% levels, as well as germinated and roasted fenugreek blended bread at 15% levels. However, there were significant ($p < 0.05$) differences between 5 and 10% germinated and roasted fenugreek blended bread, respectively. A similar finding was also reported by Legassa et al. (2022).

The results of overall acceptability scores are presented in Table 9. The overall acceptability rating was the mean score of all the organoleptic characteristics in the present study (Hooda &

Jood, 2005). The overall acceptability of the bread enriched with germinated and roasted fenugreek flour and the control was observed to determine the difference. The results showed that all the bread samples made from wheat flour and processed fenugreek flour were statistically different from the control. The highest score of OVA was observed on control (100% wheat flour bread), followed by 5% germinated fenugreek blended bread, whereas the lowest score was observed on roasted fenugreek blended bread (15% level). This shows that there was a decrease in the overall acceptability score of wheat-fenugreek-supplemented breads as compared to the control bread.

However, loaves of bread containing up to a 5% level of germinated fenugreek flour were rated as good as the control and other fenugreek-supplemented bread samples in terms of acceptability. From the overall acceptability rating, it was concluded that breads containing up to 5% germinated fenugreek could be baked with satisfactory performance. Hence, at 10 and 15% levels, all supplemented breads had poor overall acceptability scores and were considered unacceptable.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In conclusion, nutritional composition, functional properties, physical properties, and sensory properties of processed fenugreek (*Trigonella foenum-graecum L.*) flour blended bread were evaluated. The results of this study revealed that adding fenugreek flour to wheat flour in varying proportions for bread development significantly improved the nutritional composition. The bread blended with processed fenugreek showed more improvement in total ash, protein, fat, fiber, and minerals (Ca, Fe, and Zn) contents as compared to the control. However, the bread blended with germinated fenugreek had higher moisture content, protein, and fiber contents than the bread blended with roasted fenugreek flour. But the bread blended with roasted fenugreek flour was higher in total ash and fat contents. This shows major components like protein, fiber, ash, and fat increased in the bread with the increasing blending ratio of fenugreek flour. In addition, minerals like calcium, iron, and zinc showed an increasing trend when the ratio of fenugreek seed powder

increased. Accordingly, the highest improvement was obtained in germinated fenugreek blended bread. However, bread containing roasted fenugreek seed flour had a lower sensory quality due to the bitterness. Thus, the processing and blending ratio of fenugreek had affected the nutrient status obtained and the sensory acceptability of the bread. It may be concluded from the present study that the nutritional quality of bread could be preferred with the incorporation of germinated fenugreek flour at 5% without adversely affecting the sensory attributes of bread.

5.2. Recommendation

Further investigation should be done, especially on the bakery products making procedures and different processing methods of fenugreek seed flour to enhance consumer acceptability. There should be awareness created about the use of fenugreek seed flour prepared using roasting and germination processing methods, not only in wheat bread but also in different food preparations, bakery products, because of its health benefits.

The use of bread made with processed fenugreek is low among consumers, largely due to limited awareness, understanding, and information regarding its health benefits, nutritional value, shelf life, and economic feasibility compared to regular non-blended bread. Despite the potential indicated by this study, incorporating fenugreek seed flour may alleviate some of these challenges. Further research is required to tackle the limitations of wheat bread by examining various bakery product types and fenugreek seeds.

Generally, this study recommends adding germinated fenugreek flour at a 5% blending level to bread wheat flour to enhance various sensory qualities of bread while simultaneously improving its nutritional quality. But in the case of roasted fenugreek flour, blending below 5% could be improved without affecting the sensory attributes of bread.

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APPENDIX

I. Sensory Evaluation Sheet for wheat flour-fenugreek composite bread

My name is **Teklebirhan Niguse** MSc. Student at Mekelle University, Department of Food Science & Postharvest Technology, Food Processing Technology Program. I'm going to see **“The Effect of Fenugreek Processing and Blending Ratio with Wheat Flour on Bread’s Nutritional and Sensory Quality”**. You are given several servings of bread to taste, and you are asked to express how much you like or dislike it for each of the sensory attributes indicated. Overall general acceptability will be evaluated using a 7-point hedonic scale, with scores ranging from -1 (disliked extremely) to 7 (liked extremely). Use the scales to indicate your attitude by checking the point that best describes your feelings about the bread. Keep in mind that you are the judge. You are the only one who can tell what you like. Nobody knows whether these breads should be considered good, bad, or indifferent. An honest expression of your personal feelings will help us decide. Take a drink of water after you finish each sample and then wait for the next.

Name/Code _____ Sex _____ Age _____ Date _____

Degree of Acceptability

Like Extremely -----7	Dislike Slightly-----3
Like Moderately-----6	Dislike Moderately-----2
Like Slightly -----5	Dislike Extremely-----1
Neither like nor dislike-----4	

Table 9: Sensory evaluation table

Code	Sensory Attributes					
	Crust Color	Texture (Softness, Chewiness, Crumble)	Taste (Saltiness, Sweetness, sourness)	Appearance (uniformity, color, crust quality)	Aroma	Overall Acceptability
C						
RB1						
RB2						
RB3						
GB1						
GB2						
GB3						

Additional Comments-----

II. ANOVA Tables

Table 10: ANOVA of the effect of processing fenugreek and blending ratio with wheat flour on the moisture content of bread.

Sources	DF	Adj Sum Square	Mean Square	F value	Pr>F
BR	6	9.67	1.6	376	0.00
Error	14	0.06	0.04		
Corrected Total	20	9.6			

DF=degree of freedom, BR- Blending Ratio, Level of significant at (P<0.05)

Table 11: ANOVA of the effect of processing fenugreek and blending ratio with wheat flour on the ash content of bread

Sources	DF	Adj Sum Square	Mean Square	F value	Pr>F
BR	6	4.43	0.7	122	<0.00
Error	14	0.08	0.06		
Corrected Total	20	4.44			

DF=degree of freedom, BR- Blending Ratio, Level of significant at (P<0.05)

Table 12: ANOVA of the effect of processing fenugreek and blending ratio with wheat flour on the protein content of bread.

Sources	DF	Adj Sum Square	Mean Square	F value	Pr>F
BR	6	48.53	8.08	242	<0.00
Error	14	0.005	0.03		
Corrected Total	20	48.54			

DF=degree of freedom, BR- Blending Ratio, Level of significant at (P<0.05)

Table 13: ANOVA of the effect of processing fenugreek and blending ratio with wheat flour on the fat content of bread.

Sources	DF	Adj Sum Square	Mean Square	F value	Pr>F
BR	6	24.88	4.14	174	<0.00
Error	14	0.003	0.002		
Corrected Total	20	24.88			

DF=degree of freedom, BR- Blending Ratio, Level of significant at (P<0.05)

Table 14: ANOVA of the effect of processing fenugreek and blending ratio with wheat flour on the fiber content of bread.

Sources	DF	Adj Sum Square	Mean Square	F value	Pr>F
BR	6	18.84	3.14	164	<0.00
Error	14	0.003	0.002		
Corrected Total	20	18.84			

DF=degree of freedom, BR- Blending Ratio, Level of significant at (P<0.05)

Table 15: ANOVA of the effect of processing fenugreek and blending ratio with wheat flour on carbohydrate of bread.

Sources	DF	Adj Sum Square	Mean Square	F value	Pr>F
BR	6	294.76	49.12	2344	<0.00
Error	14	0.003	0.002		
Corrected Total	20	294.76			

DF=degree of freedom, BR- Blending Ratio, Level of significant at (P<0.05)

III. Some images of the laboratory work



C (100%)



GB1 (5%)



GB2 (10%)



GB3 (15%)



RB1 (5%)



RB2 (10%)



RB3 (15%)





Figure 4 Product Sensory Analysis



Figure 5 Fenugreek Germination Process



Figure 6 Germinated Fenugreek Seed Flour

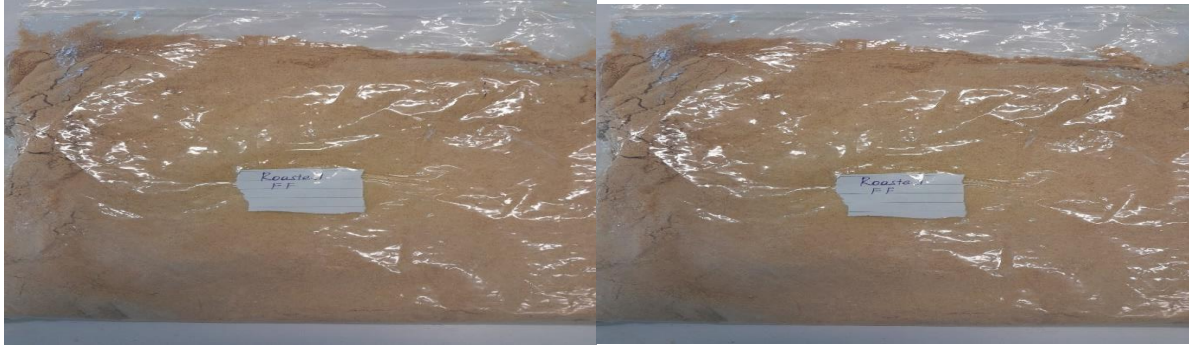


Figure 7 Roasted Fenugreek Flour



Figure 8 Roasted Fenugreek Seed

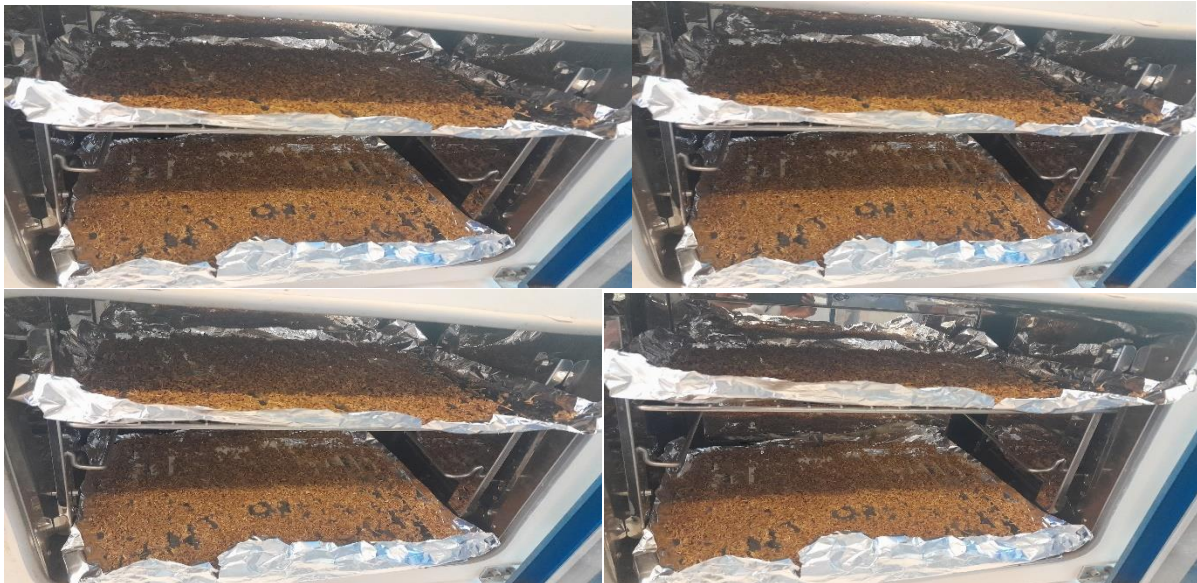


Figure 9: Oven Drying of Fenugreek Seed



Figure 10 Germinated Fenugreek Seed



Figure 11: Soaking fenugreek seed