Mekelle University

College of Dryland Agriculture and Natural Resources (CoDANR)

Department of Food Science and Postharvest Technology (FSPT)

Effect of Blending Hull-Less Barley Varieties and Refined Wheat Flour on Nutritional and Sensory Attributes of Bread

By

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A Thesis Submitted to the Department of Food Science and Post-Harvest Technology

In Partial Fulfillment of the Requirements for the Master of Science Degree in Food Processing Technology

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October

Declaration

I, **the undersigned student,** hereby present my thesis entitled **“Effect of blending hull-less barley varieties and wheat flour on nutritional and sensory attributes of bread”** 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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Name of student Signature Date

APPROVAL

As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Brtukan Gdey Hshe **ID. No. CDANR/pr080/21** entitled **"Effect of blending hull-less barley varieties and wheat flour on nutritional and sensory attributes of bread"** for consideration by the Food Science and Post-harvest Technology. We recommend that it be accepted as fulfilling the thesis requirement.

Hagos Hailu Kassegn (Assist. Prof.) ______________ 28/11/2025
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As a member of the board of examiners of the M.Sc. thesis opens a defense examination, we certify that we have read and evaluated the thesis done by Brtukan Gdey Hshe and examined the candidate. We recommended the thesis be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Food Processing Technology.

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Dedication

This work is dedicated to my beloved family for their unwavering support throughout every path of my life.

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Statement of the author

First, I declare that this thesis is my work and all sources and material 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 and 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 Dry land Agriculture and Natural Resource 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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Biography

The author, Brtukan Gdey Hshe, was born on June 06, 1997, G.C, in Mekelle. She completed her elementary school at Hayelom Primary School, and she extended her secondary education at Adiha Secondary School, and her preparatory education at Ayder. After she completed her preparatory school, she joined Mekelle University College of Dryland Agriculture and Natural Resources, Department of Food Science and Post-harvest Technology, in 2015/2016. From 2015/2016 to 2019, she studied for her BSc. Degree in Food Science and Post-harvest Technology. She was awarded a B.Sc. degree in Food Science and Postharvest Technology in July 2019. Soon after graduation, she became a staff member of the food science and post-harvest technology department at Mekelle University. In 2021, she joined the post graduate studies at Mekelle University to pursue her studies leading to a Master of Science Degree in Food Processing Technology in the regular M.Sc. program.

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Abbreviations

AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
BHBF	Blue hull-less barley flour
BLHBF	Black hull-less barley flour
CHO	Carbohydrate
CRD	Completely Randomized Design
CV	Coefficient of variation
EA	Emulsion Activity
ES	Emulsion Stability
FAO	Food and Agriculture Organization
g	gram
GP	Germination Percentage
hr	Hour
LSD	Least Significant Difference
MC	Moisture Content
OAC	Oil Absorption Capacity
RWF	Refined wheat flour
WAC	Water Absorption Capacity
WHBF	White hull-less barley flour
TKW	Thousand-Kernel Weight

ABSTRACT

Wheat grains, most of their nutrients are concentrated in the outer bran layers, which are removed during milling, leaving wheat flour mainly with starch. However, interests have been towards improvement in the nutritional profiles of wheat bread by supplementing it with large amounts of cereal, such as hull-less barley flour. The main objective of this study was to investigate the nutritional profile and sensory quality of hull-less barley and refined wheat flour blended bread. The experiment was planned, as a factorial arrangement with three factors (blending ratios, varieties and sourdough starter culture ratios) and different levels: - into the blending ratio of hull-less barley flour to wheat flour (25, 35, and 45percent of three different hull-less barley varieties (white, blue and black) and the germinated sour dough starter culture (15 g, 30 g and 45 g) laid out in completely randomized design (CRD) with three replications. The result of this study revealed that the proximate composition (percent) of the blended bread on a dry basis was significantly different ($P < 0.05$). The black barley variety consistently showed the highest nutritional enhancement, significantly increasing the protein, fiber, ash, and mineral content compared to the control wheat bread. Blending ratio was directly proportional to nutritional improvement but inversely related to sensory acceptability. The effect of germinated sourdough starter culture on all proximate composition parameters showed a significant difference ($P<0.05$). The germinated sourdough starter further boosted the nutritional profile; however, higher levels slightly reduced sensory acceptability. Sensory evaluation showed that refined wheat bread (control) scored highest in overall acceptability, while increasing barley content reduced the preference, mainly at a 45percent substitution level. However, blends up to 35% hull-less barley (especially white and blue varieties) remained satisfactory to the panelists. The black hull-less barley variety showed the highest nutritional benefits, while the white barley blends were more sensorially preferred next to 100 percent refined wheat bread. These findings suggest that blending hull-less barley with wheat flour enhances the nutritional profile of bread, although optimization of processing methods is necessary to maintain sensory quality. A 25-35% substitution level is recommended to achieve a significant nutritional enrichment while maintaining sensory acceptability.

Keywords: *hull-less barley, blending ratio, sourdough, proximate composition, mineral composition, sensory acceptability*

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Bread is well known as the principal food worldwide (Minervini *et al*., 2014), and wheat is the most significant cereal for bread-making. The increase in functional bakery products could offer a remarkable prospect for presenting several new uses of barley (Baik & Ullrich, 2008). In recent years, research on hull-less barley as a functional food has gained interest because of its health promoting phytochemicals like β-glucan and polyphenol, which can be blended with wheat flour to improve its nutritional quality (Yu *et al*., 2021).

Hull-less barley (*Hordeum vulgare*) Varieties are named *Demhay* (ደምሃይ) in Tigrigna, in the northern part of Ethiopia, where it is marginally harvested. White, blue, and black are the three varieties of hull-less barley in Ethiopia based on their color. Due to the diversity of genotypes, the differences in these colored barley phenols and their relationship with antioxidant activity are not well known (Ge *et al*., 2021). Compared to ordinary barley, colored barley varieties are more suitable for producing higher quantities of anthocyanin and antioxidants (Kim *et al*., 2007). The variations in the colored hull-less barley phenols and their relation to antioxidant activity are due to genotype variety (Ge *et al*., 2021). The composition of any plant product depends upon the environmental conditions under which the plant grows, agricultural practices, and soil conditions (Bellaloui *et al*., 2015). Due to this, comparing and evaluating hull-less barley's phenolic components, quantities, and antioxidant activities among the different varieties is essential (Ge et al., 2021). The black contains the most abundant species, while the white shows the strongest antioxidant capacity, and the content of phenolic compounds varies depending on the color of the hull-less barley. However, to the best of the authors the content of blue hull-less barley is unknown because no research has been done on it (Xiangzhen *et al*., 2021). Farmers admire the processing and perception assets of the three types. White is assumed to be the best for bread, *Tihni*, and *Injera* making. Black is preferable for making *Kollo* and *Siwa*. Preferences for blue use were not reported (Grando & Macpherson, 2005). However, it is preferable for *Kollo* in our community, around Mekelle, Tigray, and the northern part of Ethiopia.

Hull-less barley has a higher nutritional value because of its essential amino acids compared to covered barley (Blake, 2011). Besides, it has a higher content of vitamins and minerals; it is easy for the flour process; there is a reduction in costs because of the lack of a bran removal step; there is less production of broken grains during transportation and processing; and there is better digestion due to the lower content of phytic acids and other inhibitors (Moza & Gujral, 2016). The consumption of hull-less barley bread is suitable for diabetic patients and those with an allergy to wheat due to the lower levels of glucose and gluten in the hull-less barley compared to wheat (Moriartey, 2009). The research aims to investigate the effect of hull-less barley flour varieties on the nutritional and sensory quality of bread. It will help to determine the overall acceptability of the bread quality and to evaluate its sensory quality and proximate composition. In addition, to understand the differences in the hull-less barley varieties, to recommend the best variety for bread making, and to create awareness of improved bread processing among consumers.

1.2 Statement of the problem

Wheat is the primary food for most of the world (Shiferaw *et al*., 2013). Wheat grains, most of their nutrients are concentrated in the outer bran layer and germ. Unfortunately, these nutrients are removed during milling, retaining wheat flour mainly with the starch. Hence, the nutritional value of wheat-based products is low due to the refining, which reduces the nutritional value of wheat flour (Ahmed *et al*., 2023). Bread is one of the several wheat-based products largely consumed worldwide. It is a convenient source of energy and other nutrients, though; the content of nutrients and their bioavailability is low in traditional breads (Swieca *et al*., 2017). However, it can be further enhanced by blending it with the flours of other nutritious grains (Iqbal *et al*., 2022). Hull-less barley, with its high nutritional composition, easy processing nature, and acceptable palatability, is not widely applicable (Blake *et al*., 2011), which makes it a neglected grain in Tigray, Ethiopia. The need for healthy food products and domesticating neglected grains with high nutritional value and health benefits is undeniable and timely. Hence, the blending of hull-less barley varieties could improve the nutritional quality of bread. This could help select the best hull-less barley varieties and create awareness of their applicability.

1.3 Significance of the study

The significance of the study was to enhance the nutritional profile of bread by blending refined wheat flour with hull-less barley varieties and using sourdough starter cultures. This provides a high level of dietary fiber, which is beneficial for digestion and the prevention of non-communicable diseases. It also provides higher levels of essential minerals, such as iron, zinc, and calcium. These benefits are predominantly valued by vulnerable populations and in states like Tigray, where access to diverse nutritious foods is limited due to various reasons such as war, drought, and economic constraints. The findings encourage the use of hull-less barley, a neglected and underutilized crop in Ethiopia's Tigray region, by demonstrating its high nutritional value. This study can motivate local farmers to cultivate and conserve these indigenous barley varieties. These findings could lead to improved livelihoods for smallholder farmers and enhanced regional food sovereignty. This study was significant in providing information related to the consumption trend and sensory acceptability of bread made from indigenous hull-less barley.

The study provides practical evidence on the effects of different barley varieties and blending ratios, filling a knowledge gap concerning hull-less barley. It also emphasizes the trade-offs between nutritional enhancement and sensory acceptability, which is a fundamental concern in product development theory.

1.4 Objectives

1.4.1 General objective

The general objective was to assess the effect of blending hull-less barley varieties and wheat flour on the nutritional and sensory attributes of bread

1.4.2 Specific objectives

- To determine the proximate composition, mineral composition, and functional properties of hull-less barley varieties and wheat blended flour
- To assess the effect of sourdough starter culture on the proximate and mineral composition of hull-less barley varieties and wheat flour blended bread
- To evaluate the sensory acceptability of hull-less barley and wheat flour blended breads

1.5 Hypothesis

1.5.1 Null hypothesis

There is no significant difference between the proximate composition and sensory acceptability of refined wheat bread and hull-less barley blended bread.

CHAPTER 2: LITERATURE REVIEW

2.1 Hull-less Barley

Barley (*Hordeum vulgare L.*) ranks fourth among the largest cereal grains produced in the world after wheat, rice, and corn. It is one of the most genetically diverse cereals (Goudar *et al*., 2018). Barley is a nutritious cereal, but only about 2percent of this grain is used as human food (Baik & Ullrich, 2008). However, due to its potential health benefits, it has attracted the attention of researchers and food processors in the past 15 years. It is also recognized as a functional grain because it contains high levels of β-glucan and phytochemicals (Sharma & Kotari, 2017a). Barley can be classified into spring and winter types, two-row and six-row, hulled and hull-less varieties, and brewing, food, or feed, based on its end-use.The difference between two-row and six-row barley refers to the arrangement of the kernels on the barley head, also known as the spike. In two-row barley, there are two rows of kernels on each spike, whereas in six-row barley, there are six rows (Lukinac & Jukić, 2022). It is categorized into hulled, covered, and hull-less barley based on the presence or lack of the husk on the grain (Lukinac & Jukić, 2022). Highland areas of the world, such as the Central Siberian Highlands in Russia, the Qinghai-Tibet Plateau in China, the northwest Indian Himalayas, Nepal, and Ethiopia, are frequent places to cultivate barley. Canada and Germany are two more highland areas. The Tibetan Plateau has been the home of hull-less barley (*Hordeum vulgare L. var. nudum Hook. f..)* production for 3,500 years, mainly in Bhutan, Nepal, and China. Tibet is one of the domestication and diversity centers for cultivated barley (Dai *et al*., 2012). Outstanding qualities include high-stress resistance, quick development, excellent adaptability, cold tolerance, and consistent yield (Zhu *et al*., 2015). With around 70percent of all croplands still devoted to it, it remains the most common crop in Tibet (Zenga *et al*., 2015). Because the entire grain can be used to produce a meal or processed into flour, it is very suitable for human use (Kaur *et al*., 2019). It is not only used as a human staple food in the form of noodles, steamed bread, nutrition powder, etc., but also as animal feed (Ge *et al*., 2021). Barley yields poorly due to poor management and a lack of resistance to biotic stress. Furthermore, farmers cultivating barley have no access to information and improved cropping system technologies. Upgraded sustainable production of barley can play a significant role in improving food security (Grando & Macpherson, 2005). Customer disrespect for hull-less barley was common in the 20th century.

However, nowadays it's becoming more well-liked as a healthy food in North America, Europe, and other nations where barley isn't normally grown (Dickin *et al*., 2012). The functional components of hull-less barley have drawn a lot of interest in the past few years because of its high protein and vitamin content, low-fat level, and plenty of phenolic compounds like ferulic acid, flavonols, and flavones (Siebenhandl-Ehn *et al*., 2011). Compared to genotypes of hulled barley, hull-less barley grain has a much greater amount of arabinoxylan and β-glucan, making it a good source of soluble and insoluble dietary fiber (Kinner *et al*., 2011). Hull-less barley is of medium height with a tan stem that prefers clay soil and a high content of organic fertilizers, with the unique characteristic that it is covered with only very thin glumes; therefore, it is sometimes called "naked barley" (Grando & Macpherson, 2005). Hull-less barley, compared to hulled barley, is caused by a recessive gene called nude that stops the production of hulls (Meints & Hayes, 2019). In the food industry, the ability to prevent the growth of caryopsis and barley husk is recognized as advantageous because it reduces the space required for storage and transportation. Furthermore, threshing preserves vitamins and minerals (Yangcheng *et al*., 2016). In structure and appearance, hull-less barley is comparable to wheat; however, its composition is different in terms of bioactive compounds, which are gaining acceptance because of the potential health benefits of dietary fiber, beta-glucan, antioxidants, and phenolic components (Moza & Gujral, 2016). Hull-less barley is rich in fatty acids, carbohydrates, protein, fiber, and β-glucan. It is extensively used in the food sector for pasta, baked goods, and alcoholic beverages (Abdel-Aal & Choo, 2014).

According to Vasan *et al*. (2014) and Kaur *et al*. (2019), hull-less barley is a multi-purpose cereal crop grown for food, malting, and general purposes (feeding) throughout the world. Hull-less barley, with its high level of digestible energy for feeding or higher levels of malt extract for fermenting, has continually captivated end users of barley. Especially with the increased use of mash filters and centrifuges in the lautering step of brewing, there has been growing interest in using hull-less barley varieties for malting.

Mash filters do not require the hull for filtration purposes during wort separation, making them an ideal technology for naked barley (Krstanovi´c *et al*., 2016). Hull-less barley has potential importance in increasing malt extract due to the lack of hull.

2.2 Hull-less barley in Ethiopia, Tigray

In Ethiopia, barley (*Hordeum vulgare L.)* is the most important cereal crop after wheat, *teff*, maize, and sorghum (Melkamu *et al*., 2019). Ethiopia is a barley producer in Africa, following Morocco, accounting for nearly 25percent of the total barley production in the region (Rashid *et al*., 2019). Ethiopian barley germ-plasm has been significantly used globally as a source of useful genes for traits such as disease resistance (Daba, 2015). According to Kaso and Guben (2015), barley is a staple crop for many Ethiopians, particularly highlanders, as it grows at all elevations but performs best at higher altitudes in the country's northern and central regions. It is the most dependable cereal under severe marginal conditions, being far more successful than other crops at withstanding drought, frost, low soil fertility, and even on severely damaged mountain slopes. Depending on the storage environment, barley grains can be kept for five to twenty-five years, with cold and dry locations being ideal for long-term storage (Befikadu, 2014). In developing nations like Ethiopia, barley is basically grown as a food crop, compared to industrialized nations where it is predominantly used for brewing, malting, and animal feed. Due to its wide range of uses, barley is considered the “king of grains” in much of the country, with low farm input supplies such as fertilizer and improved seed (Kaso and Guben, 2015). Farmers in barley farming are exercising different traditional cultural practices, following the diverse agroecologies of the country (Ali, 2018). Ethiopian farmers cultivate two varieties of barley: food barley and malt barley. The majority of barley farmers prefer to grow food barley, which is the main ingredient for several staple dishes such as *injera*, porridge, and bread. Barely an inexpensive cereal compared to maize, wheat, and *teff*, it is frequently substituted by lower-income families (Meints *et al*., 2016). In terms of beverages, a variety of alcoholic and non-alcoholic regional drinks, such as *tella*, *shamet*, and *korefe*, are prepared locally using barley grains for everyday consumption or special occasions (Mohammed *et al.*, 2016). *Tella* is the most widely consumed alcoholic beverage in Ethiopia (Lee *et al*., 2015). Recently, there has been an increasing demand for farmers to grow malted barley, which accounts for 10 percent of the total barley production (Lakew *et al*., 2016).

Ethiopia's primary barley-producing areas are Shewa, Arsi, Gojam, Gonder, Welo, and Bale zones, and the Tigray region, accounting for almost 85 percent of the nation's total production (Embaye, 2014).

Tigray, in the Ethiopian northern highlands, is one of the most significant barley-producing regions, with a long history of grain cultivation and a variety of topographical, climatic, and sociocultural variables (Dido *et al*., 2020). Hull-less barley is called *Demhay* (ደምሃይ) in Tigrigna, the northern part of Ethiopia, where the area is slightly cultivated.

Hull-less barley is cultivated at the same time as barley with a hull (Akhmedova, 2020). Because hull-less barley can be used directly, the interviewed farmwomen in Tigray believed it was more suitable for meal preparation than hulled barley. Because of its superior popping features, farmers say hull-less barley is best for making *kolo* and, due to its greater flavor and fermentation capabilities, for making *siwa* (*tella*). Some classic tunes and sayings that express this include "*siwaken le siwa demhay eyu lihameme lehiyu*" which translates to: your local beer prepared from hull-less barley; it is so good that it can cure the sick. Locals sing this during festivals and marriage ceremonies to express how exceptional the hull-less malt in local beverage preparation is (Fetien *et al*., 2008). However, its cultivation is decreasing because its yield is relatively low and requires better soil and more water than other varieties (Yirga Haile, 2018). However, grain yield and quality traits of hull-less barley genotypes differ widely with genotypes, years, and growing conditions (Bleidere *et al*., 2013).

Hull-less barley types have a lower prevalence and a restricted distribution than the hulled types (Cavallero *et al*., 2004). However, Zenga *et al*. (2015), described that Tibetan hull-less barley occupies ~70percent of croplands in Tibet and has the highest field production since it is very adaptable to extreme environmental conditions at high altitudes. Different formal agricultural research has given little attention to hull-less barley, even though it is richer in proteins and lysine than hulled barley (Edney, 2010). Because scientific information on drought resistance in naked barley germplasm is scarce, it is more challenging to obtain cultivars that yield well under drought-prone conditions (Ahmed *et al*., 2016). Therefore, programs in Ethiopia must seek local knowledge and practices for managing the diversity of naked barley to conserve it and breed more drought-resistant cultivars (Fetien *et al*., 2008).

2.3 β-glucan

β-glucan is a large, linear, non-starch polysaccharide built on mixed linkage β(1→3)/(1→4)-D-glucose units localized in large amounts in the endosperm cell wall of oats (*Avena sativa*) and barley (*Hordeum vulgare*) (Lazaridou *et al*., 2007). Barley beta-glucan is a type of soluble fiber found in barley grains. Total β-glucan contents of barley grain range from 2.5 to 11.3percent by weight of the kernel, but they usually fall between 4 and 7percent, though β-glucan levels as high as 13 to 17percent have been reported for some barley genotypes (De Arcangelis *et al*., 2019). The β-glucan content of cereal grains is influenced by genotype and environmental factors. However, the genetic background of oats and barley is considered to be far more significant than environmental conditions (Tomasi *et al*., 2019). The major environmental factor that influences β-glucan level is the availability of water during grain maturation (Dickin *et al*., 2011). Dry conditions before harvest result in high β-glucan levels, while moist conditions lower the level (Lazaridou *et al*., 2007). β-glucan content had significant positive correlations with grain yield, growth time, and seed size, as well as significant negative correlations with protein content and hull content (Šimić *et al*., 2019). Health claims have been approved by several government agencies besides the United States Food and Drug Administration (USFDA) and the European Food Safety Authority (EFSA) about the consumption of β-glucan, a significant component of barley (Sharma & Kotari, 2017b). The U.S. Food and Drug Administration (FDA) concluded in 2005 that there is a cause-and-effect relationship between β-glucan consumption and its ability to lessen the risk of coronary heart disease. Assuming that barley β-glucans and oat β-glucans have similar effects, the European Food Safety Authority (EFSA) has stated that it will permit the claim that "regular consumption of β-glucans contributes to the maintenance of normal blood cholesterol concentrations" in Europe (Panel & Chain, 2009).

β-glucan is predicted to become a significant supplement in human nutrition for its proven cholesterol-lowering effect (Singla *et al*., 2024), and to have positive effects on diabetes (Bozbulut & Sanlier, 2019), several immune diseases (Han *et al*., 2022), and suppression of cancer development (Xu *et al*., 2016). Indeed, in the forthcoming EU Health Claims Regulation, several articles filed an approval procedure for registering β-glucan-containing products as health-beneficial products. Food must include β-glucans from oats, barley, oat bran, or mixes of minimally or non-processed beta-glucans in one or more servings, at least three g/day to bear the claim (Tekin-Cakmak *et al*., 2024). Hull-less barley has also drawn more attention recently for food product development with value-added products and multiple food applications (Das *et al*., 2023).

2.4 Some Ethiopian barley-based foods

Foods containing barley may be classified as functional foods, particularly since they received certification in 2006 from the U.S. Food and Drug Administration (Baik & Ullrich, 2008). Any fresh or processed meal that has qualities to advance health or prevent disease, in addition to providing a basic source of nutrients, is considered a functional food (Helkar *et al*., 2016).

Barley-based meals have been categorized as functional foods because they can change blood glucose levels to treat and prevent diabetes and lower the risk of cardiovascular disease (Lazaridou & Biliaderis, 2007). Scientific judgments recently published by the European Food Safety Authority (EFSA) state that the health requirements for β-glucans from oats and barley are justified in terms of reducing postprandial glycemic response, maintaining normal blood low density lipoprotein (LDL) cholesterol levels, and improving satiety, leading to lower energy intake (Zaremba, 2018). The food value of barley as a source of energy is greatly acknowledged by the farmers (Newton *et al*., 2011). For main, side, ceremonial, and recuperating dishes, the food and beverage products are prepared from ground/milled barley flour, whole or cracked grains, and roasted or boiled grains (Shewayrga & Sopade, 2011). Most of the main meal foods in Ethiopia, prepared from barley flour, are *injera*, *kita*, and *dabo* (bread). *Kolo* is the most widely consumed side dish prepared from roasted whole or de-hulled barley grain. *Genfo*, *beso*, and *chuko* are among the ceremonial dishes prepared from barley. *Genfo* is one of the most widely consumed foods in Ethiopia. It is preferred as a main meal for breakfast (Tilahun *et al*., 2021) but is most commonly consumed during special celebrations such as birthdays and weddings. *Beso* and *chuko* are basically prepared from the same type of flour, prepared from intensely roasted barley (Mohammed *et al*., 2016).

To breastfeeding mothers, some barley dishes are served with the belief that they enhance breast milk production. Some dishes are claimed to be a remedy for gastritis, while others are reported to be a good substitute for breast milk and are good for healing broken bones and fractures (Gubatz *et al*., 2010). For foods prepared from flour, the milling of barley is done either by a special stone mill (traditional hand-grinding grains using a stone grinder) or a motorized mill. Containers made of clay (pots) or mud, and/or animal skin are used for storing flour (Shewayrga & Sopade, 2011).

2.5 The use of hull-less barley flour as a blend in wheat bread

Wheat is the most important cereal for making bread due to its large amounts of gluten and superior baking functionality, compared to other cereals (Newton *et al.*, 2011). Thus, adding other cereal flours, such as barley, is regarded as one way of maximizing the nutritional value of bread (Farag *et al*., 2022). Among the grains, barley and hull-less barley are more important due to some properties such as vitamin E, beta-glucan, and soluble fibers.

In leavened bread, substituting barley flour or fractions of ground barley for wheat flour increases soluble fiber and reduces gluten, weakening the bread's cellular structure. Barley flour is less able to form a gluten complex after hydration and mixing because gliadin is replaced by hordenines (Arendt & Zannini, 2013).

In bread prepared by Knuckles *et al*. (1997), β-glucan fractions (obtained by aqueous extraction) and barley flour were added. In percentages of 20 percent and 40 percent, dry-milled and sieved barley flour was substituted for wheat flour. All bread quality metrics were low and unacceptable when 40 percent barley flour was used. Despite having a deeper color and lower volume, consumers found the bread with 20 percent dry-milled barley flour to be satisfactory in terms of appearance, color, texture, taste, and odor.

Several studies have demonstrated that up to 45 percent of additional barley flour can be used in the preparation of leavened bread without compromising its quality (volume, texture, color, or porosity) (Lukinac & Jukić 2022). A higher percentage of barley flour produces darker, less gluten- and protein-containing loaves with increased crumb density, ash content, and hardness. However, the nutritional qualities of bread are improved when barley flour is used in place of wheat flour during its making. Dhingra & Jood (2004) stated that at the 5percent level, all of the enhanced breads had an overall acceptability score that was comparable to the control. Up to 15percent substitution, breads manufactured with wheat and barley flour were considered acceptable; however, at 20percent substitution, the total acceptability score was significantly reduced compared to the control group (100 percent wheat flour). When barley flour partially replaces wheat flour, the nutritional and organoleptic qualities are preserved while producing a beneficial and healthy product enhanced with minerals, β-glucan, and dietary fiber (Dhingra & Jood, 2002).

2.6 Nutritional composition of hull-less barley

2.6.1 Vitamin and mineral contents of hull-less barley

Hull-less barley is a principal source of multiple vitamins such as niacin, thiamine, and selenium, as well as minerals like iron, magnesium, zinc, phosphorus, and copper (Kaur *et al*., 2019). The mineral content of barley kernels varies from 2–3percent, depending on the genotype, whereas the minerals present in the seed are mainly present in the aleurone, embryo, and pericarp tissues (Gubatz *et al*., 2010). Phosphorus plays a vital role in forming the mineral matrix of bone, in addition to forming several life-critical compounds comprising adenosine triphosphate (ATP, which is the energy currency of the body) (Kolodiazhnyi, 2021). Moreover, phosphorus is the main component of nucleic acids, which are the building blocks of the genetic code. Zinc helps to heal injuries and works wonders for the skin, whereas selenium plays a primary role in lowering the chances of colon cancer. Selenium also plays a key role in several metabolic pathways, like antioxidant defense systems, immune function, and thyroid hormone metabolism (Kaur *et al*., 2019).

2.6.2 Biochemical constituents of hull-less barley

As stated by Kaur *et al*. (2019), hull-less barley has an overall higher content of biochemical constituents compared to hulled barley (Table1). In particular, the energy, protein, fat, β-glucan, and calcium constituents of hull-less barley are higher than hulled barley. Whereas the carbohydrate and crude fiber content were lower than hulled barley, since husks include a large percentage of kernel crude fiber content. β-glucan is more desirable when barley is grown for food purposes, and based on a nutritional point of view, hull-less barley is superior compared to hulled barley (Farag *et al*., 2022).

Oscarsson *et al*. (1996) reported that the ash and dietary fiber content of hull-less barley is lower than that of covered barley (Table2). While the starch, protein, fat, sugars, and β-glucan content are superior to covered barley, this fact shows that hull-less barley is nutritious and can provide people with better nutritional values than covered barley.

Table 1: Biochemical constituents of hull-less and hulled barley

No.	Bio-chemical Constituents	Hull-less barley	Hulled barley
1	Energy (Cal)	370	350
2	Protein (percent)	13	10
3	Carbohydrate (percent)	73.9	78
4	Fat (percent)	2.2	1
5	β-glucan (percent)	4.1	3.9
6	Crude fiber (percent)	1.4	5
7	Calcium (percent)	5	2

Source: Kaur *et al*., 2019

Table 2: Chemical composition of covered and hull-less barley grain (dry matter basis)

No.	Chemical Composition (percent)	Covered Barley	Hull-less Barley
1	Starch	57.7	60.7
2	Protein	12.2	15.1
3	Fat	2.5	2.7
4	Sugars	1.2	1.5
5	Ash	2.1	1.6
6	Dietary Fiber	20.6	16.6
7	β-glucan	4.8	5.7

Source: Oscarsson *et al*., 1996

2.6.3 Health benefits

Hull-less barley has several health benefits. It possesses cholesterol-lowering properties, and physicians recommend eating barley for heart patients as it creates hypercholesterolemia effects inside the body (Škrbić *et al*., 2009a). The soluble dietary fiber β-glucan helps to absorb and eliminate bile acids formed from the cholesterol present in the liver (Kaur *et al*., 2019). They are associated with lowering plasma cholesterol, reducing the glycemic index, and reducing the risk of colon cancer (Shaveta *et al*., 2019). Hull-less is an excellent wellspring of fiber that keeps the human body toxin-free. Barley is high in dietary fiber, which acts as an energy source for the beneficial bacteria that are found in our large intestine. The fiber in barley is fermented by these bacteria, which leads to the production of butyric acid, which acts as a principal fuel for the intestinal cells. This maintains the intestine's health, which helps to diminish the movement time of feces and keeps the stomach hygienic. Therefore, it lowers the risk of colon malignancy and hemorrhoids (Kaur *et al*., 2019).

Soluble dietary fibers (SDFs) are found in hull-less barley, which is responsible for reducing the activity of intestinal enzymes and lowering glucose levels and glycemic response. Since SDFs are highly susceptible to fermentation, they also induce increased production of short-chain fatty acids (SCFAs), which are known to reduce the risk of cardiovascular diseases. Hull-less barley efficiently enables women to abstain from developing gallstones. It contains a higher content of insoluble fiber, which encourages people to decrease bile acid discharge, increase insulin sensitivity, and lower their triglyceride levels. An article in the American Journal of Gastroenterology states that intake of a fibrous diet by women has a 17percent lower possibility of gallstone development when compared with other women (Kaur *et al*., 2019).

Recently, the importance of using barley as a “functional food” in human nutrition was recognized, especially after the approval of health claims for barley and barley β-glucan by the European Food Safety Authority (EFSA) and the US Food and Drug Administration (FDA) (Nakov *et al*., 2022). β-glucan is a significant component that forms the cell wall in barley endosperm and accounts for 75percent of the endosperm cell wall mass. β-glucans are found to help in weight control programs, decrease cholesterol levels in plasma, enhance lipid metabolism, lower the glycemic index, and reduce the threat of colon cancer (Murphy *et al*., 2020).

CHAPTER 3: MATERIALS AND METHODS

3.1 Description of study area

Laboratory analysis of physical and functional properties, nutritional compositions, mineral content, and sensory acceptability of the hull-less barley varieties and refined wheat blended flour, and bread was conducted in Mekelle University, Food Science and Postharvest Technology, and chemistry department laboratories.

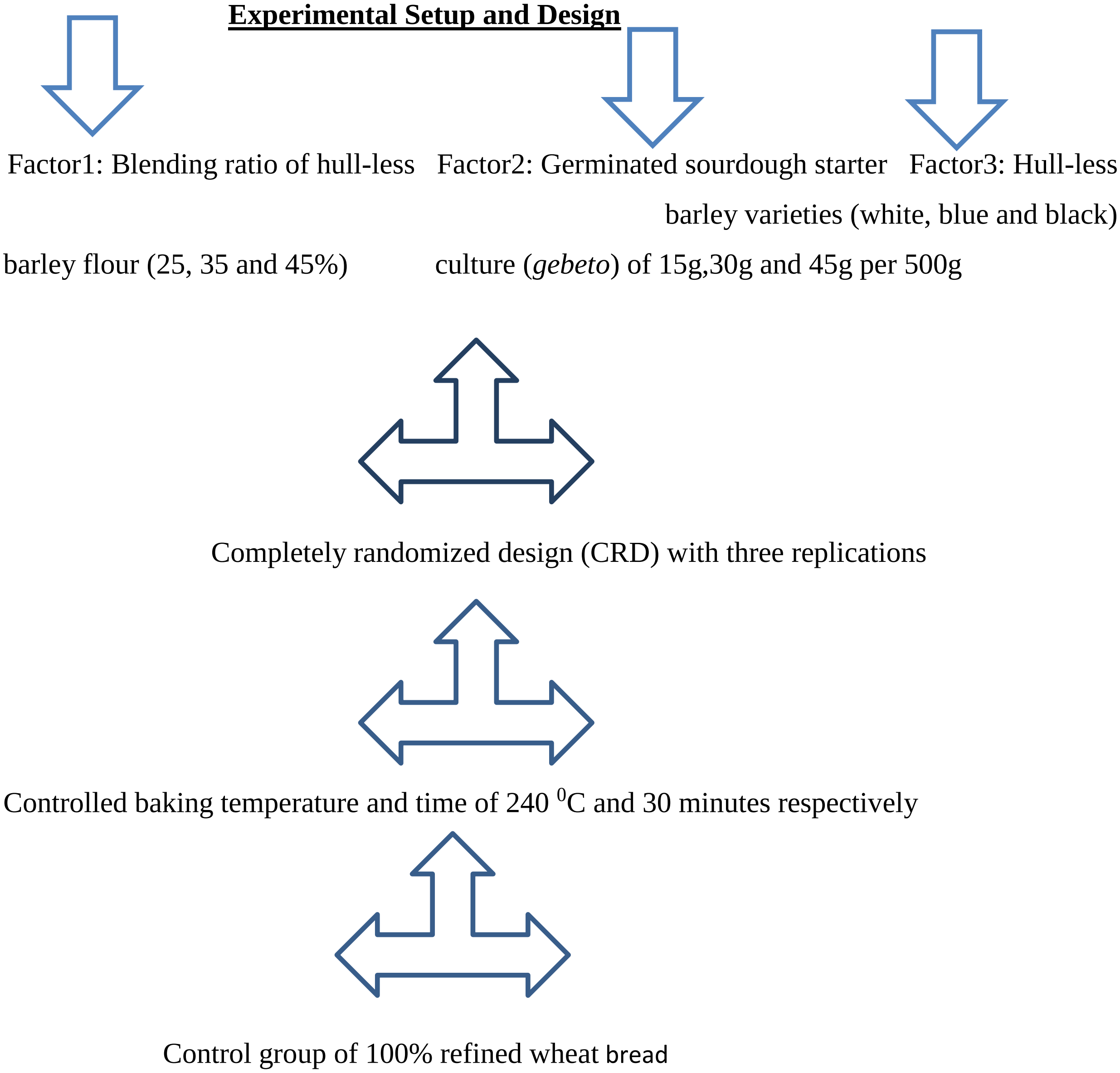
3.2 Raw Material Collection

A 10 kg sample of refined wheat flour, commercial yeast, and salt were purchased from the Mekelle local market. Three hull-less barley varieties grain (15 kg each) were also purchased from Atsbi Wonberta Zahraro Woreda eastern zone which is a major barley growing zone with a profound habit of using barley based foods. All samples were transported and kept in the food science and post-harvest technology laboratories until further analysis.

3.3 Experimental Setup and Design

The experiment was planned, as a factorial arrangement with three factor and different levels: the blending ratio of hull-less barley flour to wheat flour (25, 35, and 45percent hull-less barley flour), the three varieties of hull-less barley (white, blue and black) and the moist germinated sourdough starter culture (*gebeto*) (15g, 30g and 45g) laid out in completely randomized design (CRD) with three replications (Fig-1). Flour blends were prepared by mixing hull-less barley flour with refined wheat flour in the proportions of 25%:75%, 35%:65% and 45%:55%, respectively, for white, blue and black hull-less barley variety. All sour dough starter cultures were prepared in a 1:1:1 w/w ratio of germinated hull-less barley flour, refined wheat flour, and water, and fermented for 16 hours at room temperature (25-30^{0}C) (Perri *et al.*, 2023b). The baking temperature of the bread was controlled at 240 ^{0}C, with a baking time of 30 minutes. Bread prepared using 100percent refined wheat flour (RWF) was used as the control group. The effect of the blending ratio of the three hull-less barley varieties (white, blue and black) and the germinated sourdough starter cultures on the nutritional quality and sensory acceptability of the composite bread was studied. The bread was evaluated for sensory attributes using descriptive methods (colour, aroma, texture, taste, and mouth feel) using a 7-point hedonic scale (Granato *et al.*, 2010).

Experimental Setup and Design



Factor1: Blending ratio of hull-less barley flour (25, 35 and 45%)

Factor2: Germinated sourdough starter culture (*gebeto*) of 15g,30g and 45g per 500g

Factor3: Hull-less barley varieties (white, blue and black)

Completely randomized design (CRD) with three replications

Controlled baking temperature and time of 240 ^{0}C and 30 minutes respectively

Control group of 100% refined wheat bread

Figure 1: Experimental design

3.4 Sample Preparation Methods

3.4.1 Processing of hull-less barley into flour

Hull-less barley grain was manually cleaned, sorted, sieved in a fine local sieve to remove impurities and broken seeds, and stored in plastic bags. From the three hull-less barley grain varieties (5 Kg for each) was stored in plastic bags for physical properties and germination, while the remaining was milled to whole flour using a grinder named RRH miller to pass a 0.4 mm screen. Then, the flour was kept in an airtight sealed plastic bucket at room temperature until used for proximate composition analysis, mineral composition analysis, functional properties, and bread making. Moist germinated-sourdough starter culture (*gebeto*) of white, blue and black hull-less barley varieties of 15, 30, and 45g was used for each blend.

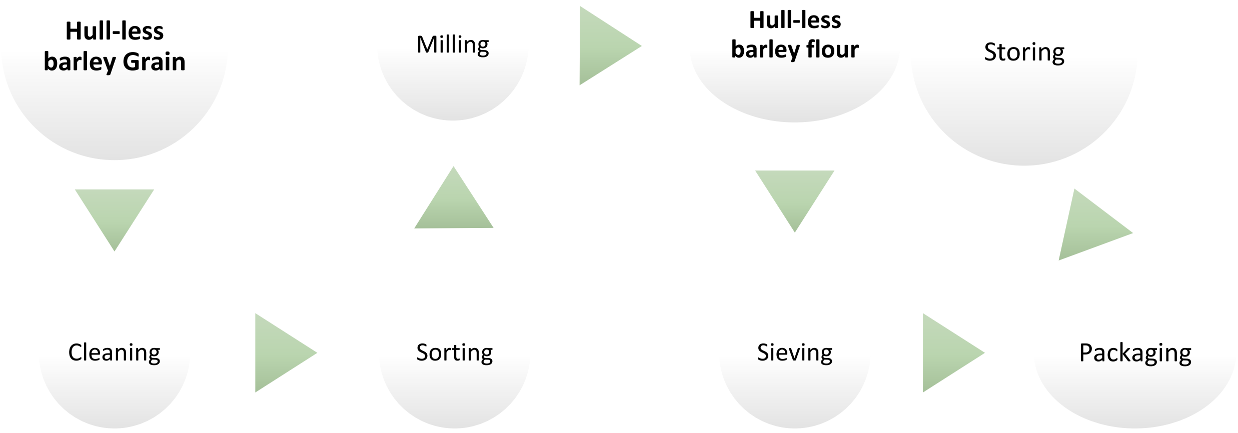


Figure 2: Flow diagram for the preparation of hull-less barley flour

3.4.2 Germination of white, blue and black hull-less barley varieties

The cleaned hull-less barley varieties (white, blue, and black) were washed repeatedly to remove soil, broken seeds, and other impurities. The kernels were then soaked in distilled water for 24 hours at a room temperature of 21.45^{0}C. Next, the water was drained using a local sieve and the grain transferred and spread on muslin cloths to allow oxygen to enter, at the same time lowering contamination. The grain germinated for 72 hrs. at an average of 23.5°C and 79.66 percent relative humidity by spraying distilled water daily to maintain an adequate hydration level. The germinated seeds were dried at 55°C for 24 hrs (Perri *et al*., 2023a); and ground in a RRH multi-functional grinder to obtain fine whole grain flour, packed in airtight plastic bags then stored in a refrigerator.

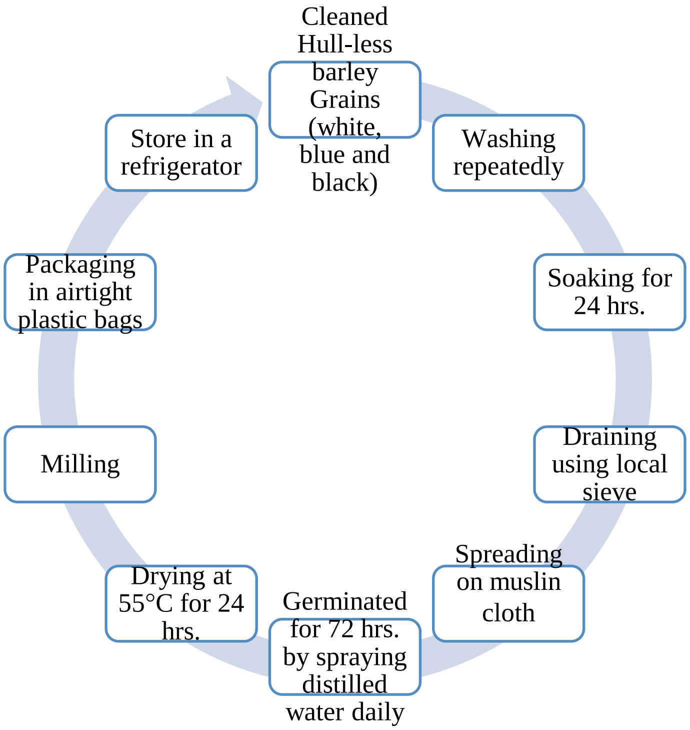


Figure 3: Germination Process flow diagram of white, blue and black hull-less barley varieties

3.5 Sample analysis methods

3.5.1 Nutritional analysis

The refined wheat flour obtained from the local market was used as a control. All samples of refined wheat flour and hull-less barley blended flour, bread made from the blends at different ratio and bread made using 15g, 30g, and 45g of sourdough starter cultures were analyzed for proximate composition (moisture content, crude fat content, crude protein content, crude fiber content, total ash, and total carbohydrate content) according to the standards of AOAC and AACC (2000).

3.5.1.1 Moisture Content

The moisture content of the samples was determined according to AOAC (2000) using the official method. Trays made of aluminum were dried in an oven at about 105 ^{0}C for 1 hr. and then cooled in a desiccator. The weight of the dry trays was measured (M1).

About five (5) g of the sample was measured and placed in dry trays (M2). The samples were dried in an oven at about 105 ^{0}C for 3 hours after repeated heating and cooling until a constant weight was obtained. The trays containing dried samples were cooled in a desiccator and weighed again (M3).

$$\mathrm{M.C}(\%) = \frac{\mathrm{M2-M3}}{\mathrm{M2-M1}} * 100$$

Where:

M.C= Moisture content of the sample (percent)
M1= Mass of the weighing tray (g)
M2= Mass of sample and weighing tray before drying (g)
M3 Mass of sample and weighing tray after drying (g)

3.5.1.2 Crude Fat

Crude fat analysis was determined using the Soxhlet extraction method, according to (AACC, 2000). Ground sample (5 g) was weighed and added to a thimble. The thimble with the sample was placed in a 50 ml beaker and dried in an oven for 2 hr. at 100^{0}C. A 150-200ml 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 is complete, the extracted fat is placed in a fume hood to evaporate the solvent in a steam bath (~100^{0}C) until no odor of the solvent is detectable. Then, the beaker with contents was dried in an oven for 30 minutes at 100^{0}C. Finally, the beaker's contents were removed, cooled in a desiccator, and weighed (mf). The amount of fat in the flour was calculated using the following formula:

$$\mathrm{Fat(percent)} = \left(\frac{\mathrm{mf} - \mathrm{mi}}{\mathrm{m}}\right) * 100$$

Where:

- mf = dried mass of fat with beaker (g)
- mi = mass of beaker (g) and
- m = sample mass (g)

3.5.1.3 Crude Protein

The protein content of all flour samples was determined according to the (AACC, 2000)**.** Ground samples were analyzed using the Kjeldahl method. A sample weight of 5g was added to the Kjeldahl digestion flask. A catalyst mixture (Na_2SO_4) mixed with anhydrous $CuSO_4$ in the ratio of 50:5 for 5 g was added. After the addition of 5 mL of concentrated H_2SO, the digestion flask was placed in the digester, and the temperature was brought to 350^0C and allowed to digest for overnight until digestion is completed. The flask was removed from the digester and allowed to cool. Once it is cooled, the contents of the flask are diluted by 150 ml of distilled water. Then, 150 ml (40percent NaOH) was added to the digestion flask to neutralize the acid and make the solution slightly alkaline. The contents were distilled immediately by inserting the digestion tube line into the receiver flask that contains 125 ml of 4percent boric acid solution, and about 750 ml of distillate was collected. Finally, the distillate was titrated with a standard 0.1N HCl, and the percentage of nitrogen was converted to the percentage of protein by using the appropriate conversion factor (protein = 5.83*%N). Urea was used as a control in the analysis.

$$N(\%) = (\frac{\mathrm{V\ HCl} * \mathrm{N\ HCl} * 14}{\mathrm{m} * 1000}) * 100$$

$$Protein(\%) = \mathrm{N}(\%) * C$$

Where;

- V HCl = Volume of HCl in liters consumed to the endpoint of the titration
- N HCl =Normality of HCl (often used is 0.1 N),
- m = The sample weight on a dry matter basis (g),
- 14= Molecular Weight of Nitrogen (g/mol)
- N= Nitrogen (percent)
- C= Conversion factor is 5.83 for barley
- P= Protein (percent)

3.5.1.4 Crude Fiber Content

The crude fiber was analyzed according to the AACC Method 32-10.01 (2000). A ground sample (5 g) was accurately weighed (m1) and placed in a 600 mL beaker. This was digested with 500 mL of boiling 1.25% sulfuric acid for 30 minutes under reflux. The mixture was immediately filtered and washed with boiling water. The residue was then digested with 500 mL of boiling 1.25% sodium hydroxide for 30 minutes under reflux. The final mixture was filtered through a pre-weighed crucible, and the residue was washed with boiling water and ethanol. The crucible and residue were dried at 110^0C to a constant weight, cooled in a desiccator, and weighed (m2). The dried residue was ashed at 550°C until white, cooled in a desiccator, and weighed again (m3). The total crude fiber of the flour was expressed as a percentage as follows:

$$\%\ \mathrm{F} = \left(\frac{\mathrm{m2} - \mathrm{m3}}{\mathrm{m1}}\right) * 100 percent$$

Where:

F= the total crude fiber (percent)

m_1 = mass of sample (g in wet basis)

m_2 = mass of the sample before ashing (g)

m_3 = mass of the sample after ashing (g)

3.5.1.5 Total Ash

The total ash content of the samples was determined according to AOAC's (2000) official method. Porcelain dishes for the analysis were washed with diluted hydrochloric acid in boiling, distilled, and demineralized water subsequently. The clean porcelain dish was ignited at 550 ^{0}C in a muffle furnace for 3 hours. The dish was removed from the furnace and cooled in a desiccator. The mass of the dish was measured as M1. About 5 g of the hull-less barley flour sample was weighed into the porcelain dish (M2). The samples were charged at 120 ^{0}C for 1 hr. and placed in a muffle furnace at 550 ^{0}C until ashing was complete. The sample was removed from the muffle furnace and placed in desiccators. Finally, the residues were weighed as M3.

$$\text{Ash content (percent)} = \left(\frac{\mathrm{m3} - \mathrm{m1}}{\mathrm{m2} - \mathrm{m1}}\right) * 100$$

Where:

m_1 = mass of empty crucible (g)

m_2 = mass of crucible + sample after ashing (g)

m_3 mass of the final sample with crucible (g)

3.5.1.6 Carbohydrate content

The total carbohydrate content of the samples was determined according to (Pearson, 1976) by subtracting the sum of the percentages of moisture, crude protein, crude fat, crude fiber, and ash content.

$$\begin{aligned}\text{percent of carbohydrate} &\\ &= 100 - (\text{percent Moisture content} + \text{percent crude protein} + \text{percent fiber} \\ &+ \text{percentcrude fat} + \text{percent ash})\end{aligned}$$

3.5.2 Mineral analysis

3.5.2.1 Iron Determination

Iron content was analyzed using the UV-VIS spectrophotometer method (AACC, 2000). A sample of 5 g was weighed into the ash vessel that had been pre-ignited at 550 ^{0}C and cooled in the desiccator. The sample was carbonized over the blue flame of the Bunsen burner and put in the muffle furnace at 550 ^{0}C until ashing was completed. The ash was then dissolved in 10 ml of diluted 3 M HCl, and the solution was evaporated nearly to dryness in a steam bath. The residue was re-dissolved quantitatively in 20 ml of 1 M HCl and filtered through coarse porosity filter paper into a 100 ml volumetric flask. A standard solution (10g Fe/ml) was prepared from analytical-grade iron wire by dissolving 0.01 g in 20 ml HCl and 50 ml distilled water and then diluted to 1 L. Finally, 100 ml of this solution was diluted to 1 liter. A sample (10 ml) was taken into a 25-ml volumetric flask, and 1 ml of 1-phenanthroline was added. A series of standard solutions (0.2–4.0 g Fe/ml) was made. After 30 minutes, the absorbance of the sample, standard, and blank was read with a UV-VIS spectrophotometer at 510 nm. Iron content was calculated using the formula:

$$Iron(mg/100g) = \frac{\text{C} * \text{DF} * 10}{\text{sample mass (g)(db)}}$$

Where

C= Concentration of sample in ppm

DF=dilution factor (if any used)

10 is a conversion factor since 10 ml will be analyzed from 100 ml

3.5.2.2 Calcium Determination

The calcium content of the samples was determined by the atomic absorption spectrophotometer (AACC, 2000) official method. A sample of 5 g was transferred into an ash 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 placed 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 on a steam bath. The residue was re-dissolved quantitatively in 20 mL of 2M HCl and filtered through coarse porosity filter paper into a 100 mL volumetric flask. The residue on the paper was washed thoroughly with water and diluted to the 100 mL mark. A standard solution (25 µg Ca/mL) was prepared from analytical grade $CaCO_3$ by dissolving 0.0625 g in 30 mL to prevent the $CaCO_3$ from fizzing and spitting violently when we add the solution in the next step, then add 50 mL of distilled water and diluting to 1 L. Finally, calcium was measured by adding enough La stock solution to make the final dilution 1percent La (i.e., 5 mL La solution to 50 mL flask) and was added to the sample and final standard solutions (0, 2, 2.5, 3, 3.5, 4, and 4.5 ppm). The absorbance of the sample was read with an atomic absorption spectrophotometer at 422.7 nm. The calcium content was calculated using the following formula:

$$\text{Calcium mg/100g} = \frac{(Cs - Cb)\ V \times D}{S}$$

Where: Cs and Cb = a concentration in µg/ml of analytic and blank, respectively;

V = original volume (50 mL); D dilution factor (if original solution is diluted) = dilution volume (mL/original aliquot volume (mL) used for dilution; S = a sample mass in g (db)

3.5.2.3 Zinc Determination

Zinc content was determined by an atomic absorption spectrophotometer (AACC, 2000). A sample (5 g) was taken into the ashing vessel (that will be pre-ignited) at 550^0C and cooled in desiccators. Ashing was done at 500^0C, and the ash was dissolved in a volume of HCl-H_2O (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 213.8 nm using air-acetylene as a source of flame for atomization with atomic absorption. A standard solution (10 g Zn/ml) was prepared from analytical grade ZnO by dissolving 0.01 g into 10 ml of 6M HCl and diluting to 100 ml, and 5 ml of the solution was taken and diluted to 500 ml with distilled water, and a series of standard solutions was prepared to construct the calibration curve. Zinc content was calculated using the following formula:

$$Zinc(ppm) \ = \frac{\left(\frac{\mu g}{ml}\right) * 100}{\text{sample mass (db)}}$$

Where µg/ml is the absorbance reading concentration.

3.5.3 Physical properties

3.5.3.1 Germination percentage

One hundred seeds were acquired in three replications from each hull-less barley variety, white, blue, and black, and germinated. The number of normal seedlings was recorded at 72 hours after germination began. The germination percentage was calculated by dividing the germinated grains by the total grains.

$$\text{Germination percentage (percent)} = \frac{\text{Number of grains germinated}}{\text{Total number of grains}} * 100$$

3.5.3.2 Thousand kernel weight

A thousand grains in triplicate were selected randomly and weighed on an electric weighing balance.

3.5.3.3 Bulk density

For estimating bulk density, the barley grains were filled in a measuring cylinder up to a certain level from a constant height, followed by weighing. The bulk density was determined by using the formula as mentioned below:

$$Bulk\ density = weight(g)/volume(ml)$$

3.5.4 Functional properties

3.5.4.1 Water and oil absorption capacity

Water and oil absorption capacity were determined by the method described by Adebowale *et al*. (2005). Five grams of flour sample was mixed with 50 ml of water or oil in the centrifuge tube, which was allowed to stand at ambient temperature for 30 minutes. The suspension was centrifuged at 3,000 rpm for 30 minutes; thereafter, the supernatant was collected in a measuring cylinder, and the amount of water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant. The same procedure was applied for determining the oil absorption capacity, wherein oil was used instead of water. The water/oil retained by the flour was calculated as water/oil absorbed, i.e., ml of water/oil absorbed per gram of sample.

$$\begin{aligned}&\text{Water absorption capacity (WAC)}\\&\qquad = \text{Volume of water absorbed (ml)}/\ \text{weight of sample (hull} - \text{less barley flour) (g)}\end{aligned}$$

$$\begin{aligned}&\text{Oil absorption capacity (OAC)}\\&\qquad = \text{Volume of oil absorbed (ml)}/\ \text{weight of sample (hull} - \text{less barley flour) (g)}\end{aligned}$$

3.5.4.2 Emulsion Activity (EA) and Emulsion Stability (ES)

Emulsion activity (EA) and emulsion stability (ES) were determined by the method explained by Yasumatsu *et al*. (1972). An emulsion of 5 g of flour sample, 50 ml of water, and 50 ml of olive oil was prepared in a calibrated centrifuge tube, which was centrifuged at 2000 × rpm for 10 minutes. The ratio of the height of the emulsion layer to the total height of the mixture was calculated as a percentage.

$$Emulsifying\ activity\ (EApercent) = \frac{\text{Height of emulsified layer}}{\text{Height of total contents in the tube}} * 100$$

Emulsion stability was determined with the same method, except that the emulsion in the centrifuge tube was initially heated in a water bath (100^0C) for 30 minutes and subsequently cooled for 15 minutes under tap water before centrifugation. Emulsion stability was calculated as:

$$Emulsifying\ stability\ (ESpercent) = \frac{\text{Height of emulsified layer}}{\text{Height of total contents in the tube}} * 100$$

3.6 Bread preparation methods

The bread was prepared using two methods, one using commercial yeast in which blended flours were added directly, and the other using sourdough starter culture (figure2). The bread recipe was as follows: 500 g refined wheat flour, 15 g yeast, 7.5 g salt, and 300 ml water (Makowska *et al*., 2023). In the test samples, refined wheat flour was mixed with 25 percent, 35 percent, and 45 percent concentrations of hull-less barley flour. The amount of yeast, salt and water was unchanged. The dough was prepared using the straight dough method.

All of the compounds were mixed for 5 minutes. Next, the dough was removed from the bowl and placed in optimal fermentation conditions with a fermentation time of 240 min (Borsuk *et al*., 2021). The dough was left for 60 minutes at room temperature in the fermentation chamber. The dough was punched, divided into pieces of equal weight (200 g), molded, and placed in a fermentation chamber for proofing (~20 min). The bread was baked in a baker's oven at 240°C for 30 minutes (Pejcz *et al*., 2016).

In the other method, sourdough starter culture was prepared using 15, 30, and 45 g of germinated hull-less barley flour. The starting point of 15g for sourdough preparation was taken from Makowska *et al*. (2023). All sourdough starter cultures (g*ebeto's*) were prepared in a 1:1:1 ratio of germinated hull-less barley flour, refined wheat flour, and water, and fermented for 16 hours at room temperature (25-30^{0}C) (Perri *et al*., 2023b). After that, the bread was made using the above-mentioned recipe of Makowska *et al*. (2023), except replacement of yeast with sourdough starter culture. Afterward, the obtained pieces of bread were left at room temperature for 2 hours to cool down to a subsequent sensory evaluation.

Bread preparation methods

Using commercial yeast

Mix all the compounds for 5 Min.

Ferment for 240 min

Using sourdough starter culture

Sour dough preparation in1:1:1 ratio of germinated hull-less barley flour, refined wheat flour, and water

Fermented for 16 hours at room temperature

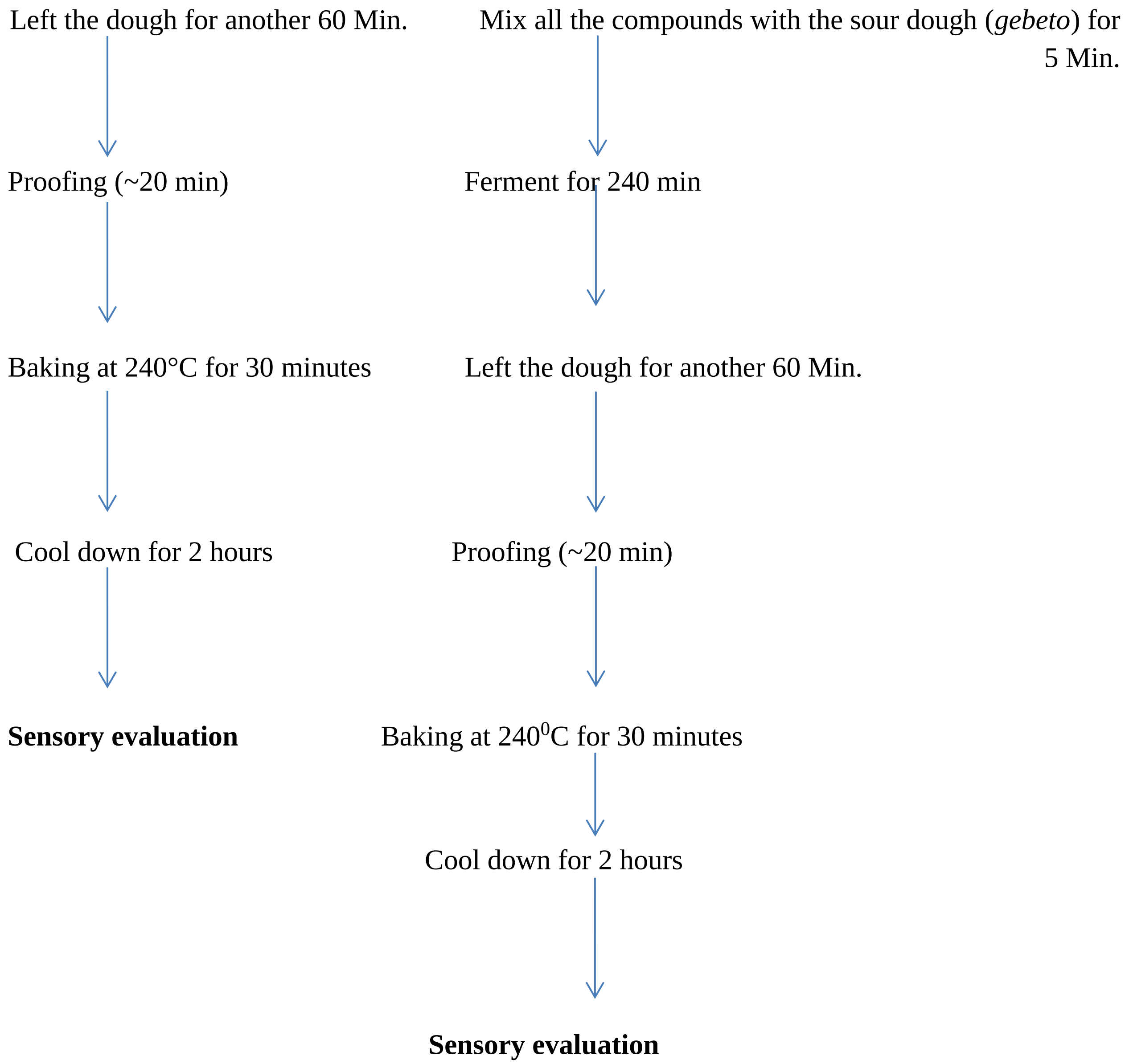


Figure 4: Bread preparation using commercial yeast and sourdough starter culture (*gebeto* or wild yeast)

3.7 Sensory Analysis

The bread was evaluated for sensory attributes using descriptive methods (color, aroma, texture, taste, and mouth feel) using a 7-point hedonic scale as follows: 1 = strongly disliked; 2 = moderately disliked; 3 = slightly disliked; 4 = neither like nor dislike; 5 = slightly liked; 6 = moderately liked; and 7 = strongly liked (Granato *et al*., 2010). The sensory quality was evaluated by 30 semi-trained panelists. The average score for all the sensory characteristics of the bread was expressed as an overall acceptability.

3.8 Statistical Analysis

The data of physical and functional properties, nutritional profile, and sensory acceptability were exposed to the analysis of variance (ANOVA) using Minitab version 21. Means of results were separated by Tukey's least significant difference (LSD), with the p-value less than 0.05 considered statistically significant. All measurements were performed in triplicate, and the data were subjected to statistical analysis. Then the results were expressed as mean ± standard deviation. In the study, descriptive statistics, including tables and figures, were used to report the analyzed data.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Physical properties of white, blue, and black hull-less barley varieties

4.1.1. Germination percentage

The germination percentage (GP) was determined from the ratio of germinated grains to the total grains across three different varieties of hull-less barley: white, blue, and black, at 72 hours, 23.5^0C and presented in Table 3 and figure 3. After 72 hours of germination, GP of Blue (96 percent) was significantly ($P < 0.05$) higher compared to white (89 percent) and black (85 percent). The results obtained in this study are higher than those reported by Tokhetova *et al. (2020)*, who revealed an average germination percentage of 76.5 to 70.2 percent. The variation in germination percentage can be due to the effect of moisture stress and temperature (Ghazi *et al*., 2007); lower water absorption capacity during steeping (Habschied *et al., 2021)*; genetic variation among barley varieties (Mohammed and Baldwin, 2023; Pavlína *et al., 2006)*; and kernel size (Najah and Ali, 2017). Therefore, the above-mentioned reasons can affect the germination percentage of the three hull-less barley varieties.

Table 3: Germination percentage of the three different hull-less barley varieties

Varieties	Germination percentage	CV (percent)
White	89 ± 1.00^b	1.12
Blue	96 ± 1.00^a	1.04
Black	85 ± 1.00^c	1.18

Where, CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, Means with different letters in the same column are significantly different at $P < 0.05$.

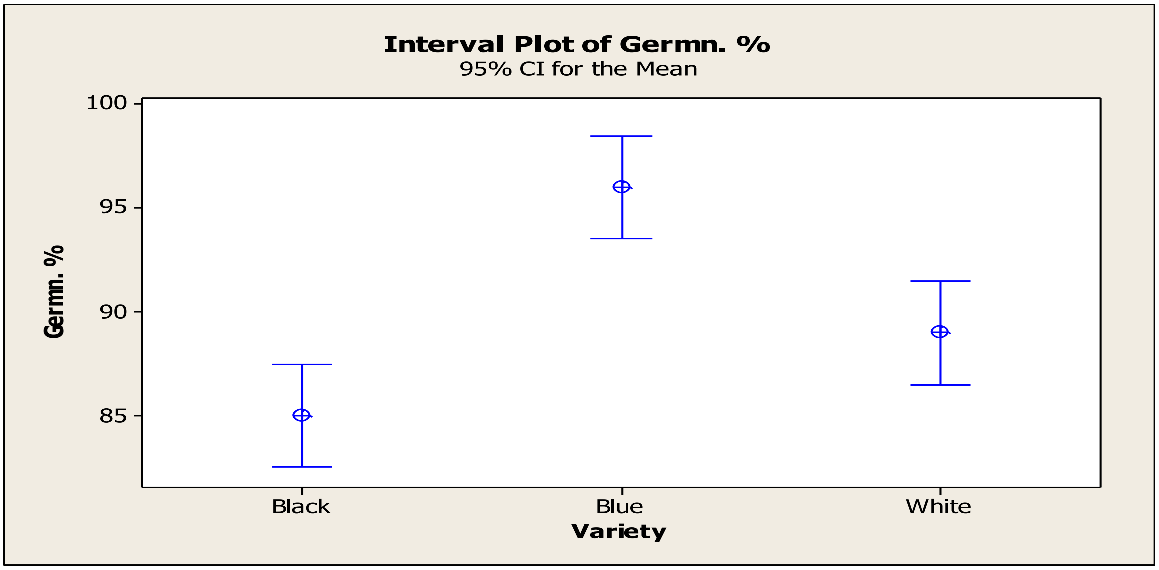


Figure 5: Interval plot of germination percentage

4.1.2. Bulk density and thousand-kernel weight

The bulk density of the germinated and non-germinated hull-less barley varieties shows a significant difference. The bulk density of white, blue, and black non-germinated hull-less barley varieties possesses the values of 0.79, 0.62, and 0.78 g/ml, respectively, as presented in Table 4. The white hull-less barley scored the maximum value, followed by black, while the blue hull-less barley scored the least value in both germinated and non-germinated. The bulk density was found to be higher than samples reported by Rani *et al. (2020)* (0.75 to 0.71 g/ml) except for blue varieties (0.62 g/ml). The variations in results might be due to genetic differences. Besides, Ahmed *et al. (2023)* reported that the bulk density depends on the particle size and moisture content of flour. When the hull-less barley varieties are germinated, the bulk density of the white, blue, and black varieties decreases to 0.58, 0.48, and 0.49 g/ml, respectively. The results show that germination influences the bulk density of the three varieties. However, the results are similar to the bulk density of non-germinated hull-less barley varieties reported by Ahmed *et al. (2023)* (0.49 to 0.48 g/ml). Though it contradicts the values of 0.72 g/ml reported by Girma and Abera (2020), which was germinated for three days.

The thousand-kernel weight is crucial for assessing the quality of grains and determines the growth and strength of seedlings. The thousand-kernel weight of the non-germinated and germinated hull-less barley varieties was found to vary between 49.51 g and 41.96 g and 44.99 g and 36.78 g, respectively.

The white variety scored the maximum value (49.51 g and 44.99 g); the black showed the intermediate value of 48.87 g and 38.1 g, whereas the blue showed the minimum value of 41.96 g and 36.87 g in both non-germinated and germinated thousand-kernel weight analyses, respectively. The thousand-kernel weight of the non-germinated hull-less barley varieties in this study is higher than reported by Kumari *et al*. (2023) (46.83 g), Tokhetova *et al*. (2020) (44.5 to 40.9 g), Kalnina *et al*. (2013)(43.6 to 40.6 g), and Rani *et al*. (2020) (38.99 to 30.75 g). Girma Tura & Abera (2020) reported the thousand-kernel weight of germinated barley (36.43 to 33.75 g), which is in contrast to the current finding obtained. As stated by Rani *et al*. (2020), the thousand-kernel weight is influenced by cultivar, environmental conditions, and agricultural practices.

Table 4: Grouping Information of bulk density and thousand-kernel weight

Variety and treatment	**Bulk density**	**1000 KWt**
White-Non-germinated	0.799±0.0001^a	49.513±0.015^a
Black-Non-germinated	0.787±0.0001^b	48.876±0.013^b
Blue-Non-germinated	0.625±0.0001^c	41.96±0.02^c
White-Germinated	0.585±0.0015^d	44.99±0.01^d
Black-Germinated	0.489±0.0015^e	38.1±0.1^e
Blue-Germinated	0.484±0.001^f	36.787±0.185^f
CV (percent)	4.34	3.66

Where, CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different (P<0.05).

4.2. Functional properties of hull-less barley varieties, and refined wheat blended flour

4.2.1 Water absorption capacity (WAC) and Oil absorption capacity (OAC)

Sharma & Kotari. (2017a) stated that WAC influences the baking time, shelf life, and textural properties of the final product and the higher water retention is interrelated with the lower staling rate of bread. The water absorption capacity for hull-less barley varieties blended with refined wheat flour ranges from 2.5267 to 1.9533 ml/g, as presented in Table 5. This result opposes the study reported by Rani *et al*. (2020) (2.7 to 2.4 ml/g) and by Ahmed *et al*. (2023) (1.73 to 1.55 ml/g). The reason could be due to the diverse concentration of proteins and their interaction with water and conformational properties of the varieties. All hull-less barley and refined wheat flour blends recorded higher values than refined wheat flour, which is 1.63ml/g. The addition of barley flour (from hull-less and hulled barley varieties) to wheat flour enhanced its ability to absorb water and oil. This could be due to fiber and protein content differences as stated by Ahmed *et al*. (2023). Besides, Punia *et al*. (2022) states that the increment in WAC and OAC may be attributed due to the increase in the β-glucan level.

Table 5: Water absorption capacity and oil absorption capacity of the three hull-less barley varieties

Variety	Treatments	WAC (ml/g)	OAC (ml/g)
White	25% of WHBF and 75% of RWF	2.526 ± 0.0153^{a}	2.24 ± 0.03^{a}
	35% of WHBF and 65% of RWF	2.325 ± 0.0153^{b}	1.826 ± 0.0153^{e}
	45% of WHBF and 55% of RWF	2.103 ± 0.0153^{d}	1.742 ± 0.0115^{f}
Blue	25% of BHBF and 75% of RWF	2.35 ± 0.01^{b}	1.97 ± 0.02^{c}
	35% of BHBF and 65% of RWF	2.31 ± 0.01^{b}	1.836 ± 0.00577^{e}
	45% of BHBF and 55% of RWF	2.18 ± 0.02^{c}	1.78 ± 0.02^{ef}
Black	25% of BLHBF and 75% of RWF	2.112 ± 0.0153^{d}	2.127 ± 0.0115^{b}
	35% of BLHBF and 65% of RWF	2.02 ± 0.01^{e}	2.023 ± 0.0153^{c}
	45% of BLHBF and 55% of RWF	1.953 ± 0.0252^{f}	1.91 ± 0.02^{d}
RWF	100percent of RWF	1.633±0.0153^{g}	1.331 ±0.0361^{g}
CV (percent)		**6.25**	***5.8***

Where, WHBF, BHBF and BLHBF represents white, blue, and black hull-less barley flour respectively RWF= refined wheat flour CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different (P<0.05).

The oil absorption capacity is significant in determining the mouth-feel, texture, and flavor maintenance capability of food products (Rani *et al*., 2020). The oil absorption capacity was found to be higher in hull-less barley varieties blended with refined wheat flour at 25% and lower in bread made from 100% of refined wheat flour (2.24 to 1.74 ml/g respectively). The results obtained in this study are higher than the outcomes reported by Rani *et al*. (2020) (1.6 to 1.4 ml/g) and Punia *et al*. (2022) (1.94 to 1.07 ml/g). The refined wheat flour scored the lowest water and oil absorption capacity. The highest water absorption capacity was found in flour blended with 25% of white hull-less barley flour, which was significantly different (P<0.05) from the remaining blends and refined wheat flour. The score of the blends of 35% of WHBF, 25% of BHBF and 35% of BHBF was non-significantly (p>0.05) different to each other.

4.2.2. Emulsion activity and emulsion stability

The emulsion activity and emulsion stability of hull-less barley and refined wheat flour of three different varieties are presented in Table 6. The table revealed that the emulsion activity was found to be highest in 45% of BHBF and 55% of RWF (59.9 percent), followed by 45% of BLHBF and 55% of RWF (58.31percent). The least emulsion activity was observed in 25% of BLHBF and 75% of RWF (50.58). The result was higher than reported by Rani *et al.* (2020) (51.35 to 41.85 percent) and by Punia *et al.* (2022) (38.5 to 33.3percent). The highest emulsion stability was found in 45% of BLHBF and 55% of RWF (48.67percent) and the lowest in 100% RWF (35.87percent). Rani *et al.* (2020) reported the emulsion stability to range between 36.0 and 35.3 percent. The result of this study scored higher and ranged from 48.67 to 42.96 percent. The greater adherence of protein fraction on the granules had a significant role in the emulsion stability of hull-less barley varieties, as stated by Rani *et al.* (2020).

Table 6: Emulsion activity and emulsion stability of hull-less barley varieties and refined wheat flour blends

Variety	Treatments	EA (percent)	ES (percent)
White	25% of WHBF and 75% of RWF	52.95 ± 0.04^{cd}	43.44 ±0.01^{de}
	35% of WHBF and 65% of RWF	53.42 ± 0.1^{c}	45.66 ±0.05^{c}
	45% of WHBF and 55% of RWF	53.59 ± 0.05^{c}	46.69 ±0.03^{bc}
Blue	25% of BHBF and 75% of RWF	52.2 ± 0.5^{d}	42.96 ±0.01^{e}
	35% of BHBF and 65% of RWF	53.26 ± 0.131^{cd}	44.29 ±0.493^{d}
	45% of BHBF and 55% of RWF	59.9 ± 0.7^{a}	46.92 ±0.1^{b}
Black	25% of BLHBF and 75% of RWF	50.58 ± 0.05^{e}	44.42 ± 0.42^{d}
	35% of BLHBF and 65% of RWF	52.2 ±0.5^{d}	45.66 ± 0.01^{c}
	45% of BLHBF and 55% of RWF	58.31 ±0.05^{b}	48.67 ± 0.02^{a}
RWF	100% RWF	53.42 ± 0.673^{c}	35.87 ± 0.955^{f}
CV (percent)		**5.16**	**7.57**

Where, WHBF, BHBF, and BLHBF represents white, blue and black hull-less barley respectively RWF= refined wheat flour CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different (P<0.05).

4.3 Effect of blending ratio of hull-less barley varieties and refined wheat flour on proximate composition of the blended flour

The different parameters, such as total ash, crude fat, crude fiber, moisture content, crude protein, and carbohydrate of the 100percent refined wheat flour and blended flour, are shown in Table 7. The ash content has an important role in determining the amount of essential minerals or inorganic compounds (Rani *et al*., 2020). The result revealed that the percent total ash content of hull-less barley and refined wheat flour blends, and 100percent of refined wheat flour, ranged from 1.566 to 1.11percent. Among the three varieties, black hull-less barley flour blended with refined wheat flour attained a maximum value of 1.566 percent while 100percent of refined wheat flour scored the least percent, which is 1.11. The ash content obtained in this study was lower than the results reported by Ahmed *et al*., (2023) (1.97 to 0.89 percent), and Perri *et al*., (2023) (2.44 to 0.49 percent), and higher than results reported by Choi *et al*., (2015) (0.74 to 0.91 percent). Similar to this study the above studies stated that the lowest value was observed in refined wheat flour as most of the minerals are removed during milling since they are attached with the bran. The crude fat was found to be higher in the black variety blended with refined wheat flour (3.6833percent), followed by the blue variety, which is 3.6333 percent. The least value was observed in the 100 percent refined wheat flour (2.85 percent). The crude fat content of refined wheat flour as reported by Rani *et al*. (2020) was in the range of 3.24 to 2.69 percent which is higher than the results of the current study. The crude fat content of refined wheat flour as reported by Ahmed *et al*. (2023) was in the range 2.25 to 1.3percent which is lower than the current study. This significant variation might be due to the basic genetic make-up of the crop. The black variety was found to contain the highest crude fiber (3.41 to 2.99 percent), followed by the blue variety (2.36 to 2.23 percent), and the white variety (2.26 to 2.1 percent). The least value was observed in refined wheat flour (1.893 percent).

Table 7: Effect of blending ratio of hull-less barley varieties and refined wheat flour on proximate composition of the blended flour in percentage (dry weight basis)

Treatments	**Total ash (percent)**	**Crude fat (percent)**	**Crude fiber (percent)**	**M.C. (percent)**	**Crude protein (percent)**	**CHO (percent)**
25% of WHBF and 75% of RWF	1.22333 ± 0.0152^{f}	3.39 ± 0.0153^{f}	2.106 ± 0.0115^{g}	11.393 ± 0.0115^{d}	13.903 ± 0.0306^{g}	67.98 ±0.02^{b}
35% of WHBF and 65% of RWF	1.34000 ± 0.01^{d}	3.47 ± 0.0153^{e}	2.156 ± 0.0115^{f}	11.5 ± 0.01^{d}	14.007 ± 0.0451^{f}	67.523 ±0.0551^{c}
45% of WHBF and 55% of RWF	1.42000 ± 0.01^{c}	3.54 ± 0.0153^{cd}	2.266 ± 0.0115^{e}	11.85 ± 0.02^{c}	14.153 ± 0.0252^{de}	66.767 ±0.0058^{de}
25% of BHBF and 75% of RWF	1.303 ± 0.0208^{e}	3.403 ± 0.005^{f}	2.23 ± 0.02^{e}	11.377 ± 0.02^{d}	14.247 ± 0.032^{d}	67.603 ±0.056^{c}
35% of BHBF and 65% of RWF	1.413 ± 0.005^{c}	3.506 ± 0.015^{de}	2.316± 0.0153^{d}	11.413 ± 0.015^{d}	14.42 ± 0.036^{c}	66.953 ±0.028^{d}
45% of BHBF and 55% of RWF	1.477 ± 0.005^{b}	3.63 ± 0.013^{b}	2.36 ± 0.01^{d}	11.87 ± 0.02^{c}	14.57± 0.02^{b}	66.55 ±0.01^{e}
25% of BLHBF and 75% of RWF	1.342 ± 0.005^{d}	3.426 ± 0.011^{f}	2.99 ± 0.02^{c}	12.023 ± 0.157^{b}	14.05 ± 0.015^{ef}	66.16 ±0.2^{f}
35% of BLHBF and 65% of RWF	1.486 ± 0.01^{b}	3.57 ± 0.015^{c}	3.286 ± 0.015^{b}	12.3 ± 0.01^{a}	14.66 ± 0.0656^{b}	64.773 ±0.074^{g}
45% of BLHBF and 55% of RWF	1.566 ± 0.005 ^{a}	3.68 ± 0.007^{a}	3.416 ± 0.02^{a}	12.36 ± 0.005^{a}	14.85± 0.03^{a}	64.246 ±0.065^{h}
RWF	1.11 ±0.01^{g}	2.85 ± 0.02^{g}	1.893 ± 0.015^{h}	11.213 ± 0.015^{e}	12.021 ± 0.03^{h}	70.263 ±0.06^{a}
CV (percent)	**9.52**	**6.45**	**12.38**	**4.67**	**5.43**	**2.44**

Where, WHBF, BHBF and BLHBF= white, blue and black hull-less barley flour respectively RWF= refined wheat flour CV= coefficient of variation, All the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different ($P<0.05$).

Moisture content is an important component playing a major role in the shelf-life of cereals, and the difference in moisture content amongst different barley varieties could be due to their genotypic variations, the maturity of samples, and agro-climatic climatic (Rani *et al*., 2020). The level of moisture content varied from 12.3 to 11.21percent in this study. The results are higher compared to Ahmed *et al*. (2023) (11.41 to 10.79percent) and Rani *et al*. (2020) (8.97 to 7.91percent), and lower than the results reported by Kalnina *et al*. (2013) (15.2 to 14.3percent). The crude protein content was found to be highest in the black variety (14.85percent), and lowest in 100percent refined wheat flour (12.02percent). The value of protein content was similar to the result reported by Kalnina *et al*. (2013) (14.1 to 12.3percent). The result was different from Ahmed *et al*. (2023) (11.38 to 9.36 percent) and Gordana *et al*. (2021)(16.93 to 12.77percent). The carbohydrate content is significant in the energy value of the human diet; the carbohydrate content in this study was found to be in the range of 70.263 to 64.246 percent. The results in this study scored lower than results reported by Rani *et al.*, (2020) (73.75 to 71.38percent) and higher than Perri *et al*. (2023) (63.35percent). Most of their nutrients are concentrated in the outer bran layer of wheat grains, and these nutrients are removed during milling, leaving wheat flour mainly with the starch. Henceforth, the nutritional quality of wheat-based products is low because of the poor nutritional value of wheat flour (Ahmed *et al*., 2023). In this study, the difference in their proximate composition could be due to the variation in their genetic composition and growing environmental conditions, as stated by Rani *et al.* (2020). Škrbić *et al*. (2009b) stated that the nutritional value of food supplemented with barley depends on the level of supplementation and the type of barley used.

4.4 Effect of blending ratio of hull-less barley varieties and refined wheat flour on proximate composition of the blended bread

The different parameters, such as total ash, crude fat, crude fiber, moisture content, crude protein, and carbohydrate, of the 100 percent refined wheat bread and blended bread are shown in Table 8. The result revealed that the percent total ash content of hull-less barley and refined wheat flour blended bread, and 100 percent of refined wheat bread, ranged from 1.643 to 1.033 percent. Ash contents of the bread samples significantly increased with increasing hull-less barley supplementation levels. The highest result was obtained in 45 percent of black hull less barley blended with refined wheat bread, and the lowest result was obtained in bread made with 100 percent of refined wheat bread. 35% of White, blue and black hull-less barley blended bread showed non-significant differences, while the remaining blends are significantly different. The results obtained in this study are different from those of Škrbić *et al*. (2009b) (2.09 to 1.34percent), Robles-Ramírez *et al*. (2020) (2.13 to 1.57percent).

The crude fat content ranged from 3.447 to 2.77 percent, and the 45percent black hull-less barley blended with refined wheat flour scored the highest results, while the refined wheat bread scored the lowest. In this study, the result of crude fat content is higher than the value reported by Liu *et* *al*. (2020) (2.33 to 1.09percent) and Robles-Ramírez *et al*. (2020) (2.25 to 1.62percent), though lower than Škrbić *et al*. (2009b) (3.7 to 2.82 percent).

There was a significant difference (P<0.05) among the bread treatments in crude fiber content. The increase in hull-less barley ratio increases the crude fiber content in all treatments. The highest result observed was in 45percent black hull-less barley blended with refined wheat, which was 3.117percent. The lowest result obtained in refined wheat bread was due to the removal of bran during milling, which was 1.803percent. The result obtained in this study was higher than the findings of Škrbić *et al*. (2009b) (2.23 to 0.36percent) and Alu'datt *et al*. (2012) (2.55 to 0.85 percent). As stated by Supriya M. (2015), the leading role of fiber is to keep the digestive system healthy, and its negative impact is that it reduces product volume, texture, and the ability of gluten protein to aggregate during dough making.

The moisture content ranged from 11.613 to 10.457percent and 45percent of black hull-less barley blended with refined wheat flour scored the maximum result, while the least result was observed in 100percent refined wheat bread. The result obtained in this study was higher than those reported by Liu *et al*. (2020), which ranged from 10.82 to 9.08percent. As described by Robles-Ramírez *et al*. (2020), the dough absorbs more water due to the high fiber content of barley, and, as a result, the bread made with barley flour has higher moisture content than wheat bread.

The crude protein content of the treatments ranges from 14.863 to 12.053percent. The black variety showed the highest effect in all proximate composition analysis of the bread, except for carbohydrate. This could be due to their genetic composition. In contrast, the bread made with 100percent refined wheat bread scored minimum results in all except for carbohydrate, and this could be due to the refined wheat flour used, as it primarily contains the endosperm part.

The results obtained in this study are higher than those reported by Robles-Ramírez *et al*. (2020) (13.12 to 11.32percent) and by Alu'datt *et al*. (2012) (11.55 to 10.6percent), and lower than the results reported by Škrbić *et al*. (2009a) (16.88 to 14.28percent).

The carbohydrate content of bread made from 100percent refined wheat flour scored the highest result (71.3percent), followed by 25 percent of white hull-less barley blended to refined wheat flour (69.27percent), and 45percent of black hull-less barley blended bread scored the lowest value

(65.69 percent). This result was in contrast with results reported by Alu’datt *et al*. (2012) (85.65 to 81.55 percent), and the variation could be due to the difference in blending ratio.

Table 8: Effect of blending ratio of hull-less barley varieties and refined wheat flour on proximate composition of the blended bread

Treatments	**Total ash (%)**	**Crude fat (%)**	**Crude fiber (%)**	**M.C. (%)**	**Crude protein (%)**	**CHO (%)**
25% of WHBF and 75% of RWF blended bread	1.316±0.005^{g}	3.09±0.01 ^{g}	1.903±0.004 ^{i}	11.02±0.01 ^{g}	13.863±0.011^{i}	69.270±0.036^{b}
35% of WHBF and 65% of RWF blended bread	1.437±0.005^{e}	3.1633±0.001 ^{e}	2.013±0.003 ^{h}	11.043±0.005 ^{fg}	14±0.01 ^{h}	68.743±0.025^{d}
45% of WHBF and 55% of RWF blended bread	1.49±0.01^{d}	3.2267±0.005^{d}	2.17±0.01 ^{f}	11.143±0.011 ^{e}	14.15±0.00 ^{f}	68.150±0.00^{e}
25% of BHBF and 75% of RWF blended bread	1.393±0.011^{f}	3.1367±0.007 ^{f}	2.113±0.007 ^{g}	11.067±0.005 ^{f}	14.263±0.0057^{e}	68.073±0.011 ^{f}
35% of BHBF and 65% of RWF blended bread	1.456±0.015^{e}	3.2100±0.01^{d}	2.246±0.0057^{e}	11.247±0.025 ^{d}	14.413±0.005 ^{d}	67.327±0.028^{g}
45% of BHBF and 55% of RWF blended bread	1.55±0.000^{c}	3.2733±0.007 ^{c}	2.31±0.01 ^{d}	11.31±0.01 ^{b}	14.583±0.01 ^{c}	66.673±0.015 ^{h}
25% of BLHBF and 75% of RWF blended bread	1.443±0.005^{e}	3.226±0.011^{d}	2.766±0.005 ^{c}	11.457±0.0115 ^{c}	14.063±0.005 ^{g}	69.043±0.016^{c}
35% of BLHBF and 65% of RWF blended bread	1.59 ±0.01^{b}	3.3633±0.00^{b}	2.957±0.0054 ^{b}	11.543±0.0115 ^{b}	14.637±0.011^{b}	66.387±0.014 ^{i}
45% of BLHBF and 55% of RWF blended bread	1.643±0.0057^{a}	3.445±0.0056 ^{a}	3.117±0.0057 ^{a}	11.613±0.0057 ^{a}	14.863±0.0153 ^{a}	65.69±0.01 ^{j}
RWB	1.033±0.0057^{h}	2.77±0.01 ^{h}	1.803±0.01 ^{j}	10.457±0.0112 ^{h}	12.053±0.0055 ^{j}	71.3±0.03 ^{a}
CV (percent)	**5.46**	**3.47**	**7.67**	**3.44**	**4.35**	**2.33**

Where, WHBF, BHBF and BLHBF= white, blue and black hull-less barley flour respectively, RWF= refined wheat flour, RWB= refined wheat bread, CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different (P<0.05).

4.5 Effect of blending ratio of hull-less barley varieties and refined wheat flour on mineral concentration

Mineral concentration of hull-less barley varieties and refined wheat flour blends is presented in Table 9.

Table 9: Effect of blending ratio of hull-less barley varieties and refined wheat flour on mineral concentration of flour in mg/100g (dry weight basis)

Treatments	**Variable**		
	Iron (mg/100g)	**Zinc (mg/100g)**	**Calcium (mg/100g)**
25% of WHBF and 75% of RWF	2.37 ± 0.0057^{e}	1.763± 0.011^{i}	32.983 ± 0.126^{g}
35% of WHBF and 65% of RWF	2.416 ± 0.01^{d}	1.806± 0.015^{h}	33.9 ± 0.361^{f}
45% of WHBF and 55% of RWF	2.466 ± 0.007^{bc}	1.886± 0.011^{g}	35.697 ± 0.041^{d}
25% of BHBF and 75% of RWF	2.392 ± 0.005^{de}	1.964± 0.013^{f}	33.667 ± 0.153^{f}
35% of BHBF and 65% of RWF	2.46 ± 0.003^{c}	2.006± 0.012^{e}	34.75 ± 0.218^{e}
45% of BHBF and 55% of RWF	2.493 ± 0.015^{b}	2.143± 0.005^{d}	36.247 ± 0.045^{c}
25% of BLHBF and 75% of RWF	2.416 ± 0.012^{d}	2.212± 0.015^{c}	35.473 ± 0.025^{d}
35% of BLHBF and 65% of RWF	2.483± 0.01b^{c}	2.283± 0.015^{b}	37.803 ± 0.045^{b}
45% of BLHBF and 55% of RWF	2.533 ± 0.013^{a}	2.351± 0.011^{a}	40.033 ± 0.076^{a}
RWF	2.112 ± 0.015^{f}	0.913± 0.005^{j}	28.347 ± 0.025^{h}
CV (percent)	**4.69**	**7.48**	**5.59**

Where, WHBF, BHBF and BLBF are white, blue and black hull-less barley varieties respectively RWF= refined wheat flour CV= coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different ($P<0.05$).

Effect of blending ratio of hull-less barley varieties and refined wheat flour on the concentration of zinc, and calcium content showed significant ($p < 0.05$) difference. Besides, the iron concentration showed significant ($p < 0.05$) difference on most of the treatments. Some of the treatments share letters that shows they are not significantly different ($p > 0.05$) from each other. An increment rate of 2.112 to 2.533 mg/100g for iron, 0.913 to 2.351 mg/100g for zinc, and 28.347 to 40.033 mg/100g for calcium as refined wheat flour substituted with hull-less barley flour was observed. The blend that showed better mineral concentration for iron, zinc and calcium was a blend of 45% BLHBF and 55% RWF. The results obtained in this study are lower than reported by Kaur *et al.*, (2024) (6.6 to 4.33 mg/100g for iron, 2.66 to 2.25 mg/100g for zinc, and 36.8 to 31.2 mg/100g for calcium) and by El-Taib *et al.*, (2018) (4.27 to 2.1 mg/100g for iron, 3.26 to 1.89 mg/100g for zinc and 38.1 to 36.6 mg/100g for calcium) except for 45% of BLHBF treatment which scored 40.033mg/100g. The results obtained in this study scored higher for iron, zinc, and calcium concentration than results reported by Kumari *et al*. (2023) (2.2 to 1.46 mg/100g for iron, 1.89 to 0.58 mg/100g for zinc, and 24.04 to 5.39 mg/100g for calcium).

4.6 Effect of blending ratio of hull-less barley varieties and refined wheat flour on Mineral content of the blended bread

Mineral concentration of blended bread with its blending ratio is presented in Table 10.

Table 10: Effect of blending ratio of hull-less barley varieties and refined wheat flour on Mineral content of the blended bread

Variety	**Treatments**	**Iron (mg/100g)**	**Zinc (mg/100g)**	**Calcium (mg/100g)**
White	25% of WHBF and 75% of RWF blended bread	2.346 ± 0.005^{f}	1.763 ± 0.005^{i}	33.017 ±0.0764^{i}
	35% of WHBF and 65% of RWF blended bread	2.416 ± 0.015^{d}	1.796 ± 0.005^{h}	34.033 ±0.153^{g}
	45% of WHBF and 55% of RWF blended bread	2.453 ± 0.005^{c}	1.876 ± 0.007^{g}	35.73 ± 0.01^{d}
Blue	25% of BHBF and 75% of RWF blended bread	2.386 ± 0.005^{e}	1.953 ± 0.005^{f}	33.59±0.01^{h}
	35% of BHBF and 65% of RWF blended bread	2.45 ± 0.01^{c}	1.997 ± 0.0057^{e}	34.877 ±0.011^{f}
	45% of BHBF and 55% of RWF blended bread	2.486 ± 0.005^{b}	2.14 ± 0.01^{d}	36.137 ±0.015^{c}
Black	25% of BLHBF and 75% of RWF blended bread	2.42 ±0.01^{d}	2.207 ± 0.0057^{c}	35.467 ± 0.0057^{e}
	35% of BLHBF and 65% of RWF blended bread	2.476 ± 0.007^{bc}	2.286 ± 0.0057^{b}	37.823 ± 0.0057^{b}
	45% of BLHBF and 55% of RWF blended bread	2.533 ±0.015^{a}	2.357 ± 0.0057^{a}	40.003 ± 0.0208^{a}
Wheat	RWB	2.103 ± 0.005^{g}	0.91 ± 0.01^{j}	28.337 ± 0.01^{j}
CV (percent)		**4.78**	**12.59**	**8.56**

Where, WHBF, BHBF and BLHBF= white, blue and black hull-less barley flour respectively, RWB= refined wheat bread, CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different (P<0.05).

The effect of blending ratio on the iron, zinc, and calcium content showed a significant (P< 0.05) difference. The maximum and minimum iron (2.533 to 2.103mg/100g), zinc (2.375 to 0.91mg/100g), and calcium content (40.003 to 28.337 mg/100g) were observed in 45percent of black hull-less barley blended with refined wheat flour, and 100percent of refined wheat bread, respectively. Škrbić *et al*. (2009a) stated that the bread made with supplemented barley had two times higher zinc content than wheat bread. Hence, the hull-less barley-supplemented breads scored higher results compared to the bread made from 100percent refined wheat flour in this study.

In general, increasing the blending ratio of hull-less barley varieties increased the mineral content of the breads as observed in this study, and there is significant variation in the mineral composition among the hull-less barley varieties blended with refined wheat flour.

4.7 Effect of germinated sourdough starter cultures on the proximate composition of the blended bread

The proximate composition of the blended breads with the germinated sourdough starter cultures is presented in Table 11. The effect of germinated sour dough starter culture on all proximate composition parameters (total ash, crude fat, crude fiber, moisture content, crude protein, and carbohydrate) show significant ($P<0.05$) difference. The mean total ash, crude fat, crude fiber, moisture content, and crude protein of the blended bread significantly increased with increasing the sourdough starter culture. The highest value in all proximate compositions except for carbohydrate was observed in black hull-less barley composite bread made using 45g of sour dough starter cultures (1.96, 3.8, 3.23, 11.8, 18.4, and 60.49 percent of total ash, crude fat, crude fiber, moisture content, crude protein, and carbohydrate content, respectively).

The observed reduction in total carbohydrate (CHO) content could be associated with an increase in perceived sweetness in the sourdough bread could be attributed to the complex biochemical processes of fermentation. The sourdough fermentation process transforms them by breaking down complex, starches into simpler, sweeter sugars, while also reducing the total amount of digestible carbohydrate overall. Refined wheat bread made from commercial yeast showed the least value in all proximate composition parameters except for carbohydrate, which was the highest score (72.83). The results obtained in this study are in agreement with those of Franco *et al.*, (2021), except that ash decreased from 2.97 to 2.87percent. The protein content increased from 9.61 to 9.82, fat from 0.58 to 0.62, and carbohydrate decreased from 58.8 to 58.2 percent.

Table 11: Effect of germinated sourdough starter cultures on the proximate composition of the blended bread

Treatments	**Total ash (percent)**	**Crude fat (percent)**	**Crude fiber (percent)**	**M.C. (percent)**	**Crude protein (percent)**	**CHO (percent)**
Bread made using 25% of WHBF and 15g of sourdough	1.423±0.011^{m}	3.206±0.005^{l}	1.956± 0.011^{n}	11.063±0.01^{o}	16.11±0.01^{t}	66.24±0.0^{b}
Bread made using 25% of WHBF and 30g of sourdough	1.463±0.015^{l}	3.32±0.01^{ijk}	2.026±0.015^{mn}	11.113±0.012^{mno}	16.263±0.011^{r}	65.817±0.01^{c}
Bread made using 25% of WHBF and 45g of sourdough	1.552±0.005^{i}	3.442±0.005^{g}	2.133±0.015^{kl}	11.167±0.011^{lm}	17±0.015^{n}	64.733±0.066^{e}
Bread made using 35% of WHBF and 15g of sourdough	1.476±0.005^{kl}	3.226±0.005^{l}	2.053±0.012^{lmn}	11.077±0.015^{no}	17.017±0.005^{n}	65.15±0.02^{d}
Bread made using 35% of WHBF and 30g of sourdough	1.54±0.01^{ij}	3.316±0.004^{ijk}	2.112±0.01^{klm}	11.14±0.012^{lm}	17.347±0.01^{kl}	64.543±0.035^{e}
Bread made using 35% of WHBF and 45g of sourdough	1.7±0.0115^{ef}	3.44±0.01^{gh}	2.2±0.112^{jk}	11.227±0.012^{k}	17.913±0.005^{f}	63.517±0.075^{ijk}
Bread made using 45% of WHBF and 15g of sourdough	1.503±0.005^{jk}	3.34±0.01^{ijk}	2.13±0.01^{kl}	11.283±0.005^{ij}	17.317±0.015^{l}	64.427±0.142^{efg}
Bread made using 45% of WHBF and 30g of sourdough	1.637±0.011^{gh}	3.446±0.02^{g}	2.103±0.02^{klm}	11.36±0.005^{h}	17.473±0.011^{ij}	63.98±0.02^{h}
Bread made using 45% of WHBF and 45g of sourdough	1.793±0.005^{c}	3.543±0.005^{ef}	2.19±0.11^{jk}	11.417±0.015^{g}	18.04±0.01^{d}	63.017±0.032^{lmn}
Bread made using 25% of BHBF and 15g of sourdough	1.473±0.005^{kl}	3.223±0.01^{l}	2.113±0.012^{klm}	11.12±0.005^{lmn}	16.22±0.01^{s}	65.85±0.0^{c}
Bread made using 25% of BHBF and 30g of sourdough	1.553±0.015^{i}	3.3±0.02^{k}	2.2±0.01^{jk}	11.17±0.01^{l}	16.543±0.011^{p}	65.233±0.025^{d}
Bread made using 25% of BHBF and 45g of sourdough	1.667±0.005^{fg}	3.354±0.01^{i}	2.312±0.015^{hi}	11.25±0.11^{jk}	17.227±0.005^{m}	64.19±0.01^{fgh}
Bread made using 35% of BHBF and 15g of sourdough	1.656±0.01^{gh}	3.35±0.01^{ij}	2.256±0.011^{ij}	11.273±0.012^{jk}	17.337±0.01^{kl}	64.127±0.028^{gh}
Bread made using 35% of BHBF and 30g of sourdough	1.743±0.01^{d}	3.425±0.015^{gh}	2.32±0.01^{ghi}	11.357±0.005^{h}	17.857±0.017^{g}	63.3±0.034^{jkl}
Bread made using 35% of BHBF and 45g of sourdough	1.85±0.02^{b}	3.532±0.011^{f}	2.403±0.005^{gh}	11.433±0.01^{g}	18.257±0.015^{b}	62.523±0.041^{op}
Bread made using 45% of BHBF and 15g of Sourdough	1.736±0.005^{de}	3.604±0.015^{cd}	2.34±0.01^{ghi}	11.33±0.012^{hi}	17.37±0.01^{k}	63.617±0.005^{ij}

Bread made using 45% of BHBF and 30g of sourdough	1.846±0.01^{b}	3.701±0.015^{b}	2.416±0.015^{g}	11.437±0.005^{g}	17.54±0.01^{h}	63.223±0.335^{klm}
Bread made using 45% of BHBF and 45g of sourdough	1.94±0.01^{a}	3.84±0.012^{a}	2.523±0.015^{f}	11.557±0.02^{ef}	18.167±0.005^{c}	61.973±0.046^{q}
Bread made using 25% of BLHBF and 15g of sourdough	1.533±0.03^{ij}	3.31±0.01^{jk}	2.785±0.015^{e}	11.457±0.01^{g}	16.4±0.011^{q}	64.513±0.02^{ef}
Bread made using 25% of BLHBF and 30g of sourdough	1.623±0.02^{h}	3.402±0.015^{h}	2.864±0.011^{de}	11.533±0.001^{f}	16.71±0.005^{o}	63.867±0.05^{hi}
Bread made using 25% of BLHBF and 45g of sourdough	1.712±0.01^{de}	3.543±0.02^{ef}	2.949±0.005^{cd}	11.613±0.012^{cd}	17.353±0.005^{k}	62.831±0.03^{no}
Bread made using 35% of BLHBF and 15g of sourdough	1.636±0.005^{gh}	3.423±0.013^{gh}	3.006±0.01^{c}	11.603±0.02^{de}	17.443±0.015^{j}	62.883±0.03^{mn}
Bread made using 35% of BLHBF and 30g of sourdough	1.713±0.007^{de}	3.533±0.012^{f}	3.107±0.015^{b}	11.667±0.01^{c}	18.17±0.01^{c}	61.81±0.026^{qr}
Bread made using 35% of BLHBF and 45g of sourdough	1.841±0.01^{b}	3.647±0.01^{c}	3.176±0.012^{ab}	11.667±0.034^{c}	18.393±0.005^{a}	61.273±0.063^{s}
Bread made using 45% of BLHBF and 15g of sourdough	1.732±0.01^{de}	3.583±0.015^{de}	3.15±0.01^{ab}	11.66±0.005^{c}	17.497±0.011 ^{i}	62.377±0.032^{p}
Bread made using 45% of BLHBF and 30g of sourdough	1.85±0.02^{b}	3.7±0.01^{b}	3.19±0.01^{ab}	11.743±0.005^{b}	17.963±0.005 ^{e}	61.553±0.023^{rs}
Bread made using 45% of BLHBF and 45g of sourdough	1.96±0.01^{a}	3.805±0.015^{a}	3.239±0.1^{a}	11.803±0.01^{a}	18.4±0.005^{a}	60.493±0.419^{t}
RWB	1.046±0.02^{n}	1.816±0.015^{m}	1.8±0.01^{o}	10.453±0.11^{p}	12.053±0.011^{u}	72.83±0.026^{a}
CV (percent)	**3.36**	**5.3**	**5.75**	**2.44**	**4.94**	**3.5**

Where, WHBF, BHBF and BLHBF= white, blue and black hull-less barley flour respectively, RWB= refined wheat bread, CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different (P<0.05).

4.8 Effect of germinated sourdough starter culture on the mineral content of the blended bread

As shown in Table 12, the effect of germinated sour dough starter culture on the mineral content of the blended bread showed a significant difference (P<0.05). The highest iron content was observed in bread made using 45% of BLHBF, BHBF and WHBF and 45g of sourdough starter culture (2.706, 2.705, and 2.703mg/100g, respectively), while the lowest was observed in refined wheat bread (2.113).

Table 12: Effect of germinated sourdough starter culture on mineral content of blended breads

Treatments	**Iron (mg/100g)**	**Zinc (mg/100g)**	**Calcium (mg/100g)**
Bread made using 25% of WHBF and 15g of sourdough	2.375 ±0.005^{l}	1.826 ±0.005^{o}	32.983 ±0.01^{m}
Bread made using 25% of WHBF and 30g of sourdough	2.426 ±0.0047^{k}	1.897 ±0.007^{n}	33.233 ±0.0153^{l}
Bread made using 25% of WHBF and 45g of sourdough	2.500 ±0.000^{hi}	2.013 ±0.01^{jk}	33.400 ±0.0057^{k}
Bread made using 35% of WHBF and 15g of sourdough	2.456 ±0.011^{j}	1.827 ±0.011^{o}	34.033 ±0.0115^{i}
Bread made using 35% of WHBF and 30g of sourdough	2.50±0.01^{hi}	1.896 ±0.005^{n}	34.433 ±0.005^{h}
Bread made using 35% of WHBF and 45g of sourdough	2.6 ±0.01^{e}	1.974 ±0.01^{1}	34.710 ±0.004^{g}
Bread made using 45% of WHBF and 15g of sourdough	2.553 ±0.005^{f}	1.936 ±0.005^{m}	35.725 ±0.01^{d}
Bread made using 45% of WHBF and 30g of sourdough	2.653 ±0.005^{cd}	1.983 ±0.007^{l}	35.737 ±0.003^{d}
Bread made using 45% of WHBF and 45g of sourdough	2.705 ±0.011^{a}	2.035 ±0.004^{ij}	35.75 ±0.005^{d}
Bread made using 25% of BHBF and 15g of sourdough	2.413 ±0.015^{k}	1.986 ±0.005^{kl}	33.59 ±0.002^{j}
Bread made using 25% of BHBF and 30g of sourdough	2.543 ±0.012^{fg}	2.047 ±0.006^{i}	33.653 ±0.01^{j}
Bread made using 25% of BHBF and 45g of sourdough	2.616 ±0.011^{e}	2.100 ±0.001^{h}	33.683 ±0.007^{j}
Bread made using 35% of BHBF and 15g of sourdough	2.480 ±0.000^{ij}	2.013 ±0.011^{jk}	34.877 ±0.005^{f}
Bread made using 35% of BHBF and 30g of sourdough	2.567 ±0.005^{f}	2.097 ±0.01^{h}	34.917 ±0.005^{f}
Bread made using 35% of BHBF and 45g of sourdough	2.676 ±0.004^{bc}	2.196 ±0.01^{f}	34.943 ±0.01^{f}
Bread made using 45% of BHBF and 15g of sourdough	2.513 ±0.005^{h}	2.144 ±0.005^{g}	36.137 ±0.02^{c}
Bread made using 45% of BHBF and 30g of sourdough	2.6±0.01^{e}	2.215 ±0.004^{f}	36.187 ±0.005^{c}
Bread made using 45% of BHBF and 45g of sourdough	2.703 ±0.004^{ab}	2.303 ±0.012^{e}	36.253 ±0.015^{c}
Bread made using 25% of BLHBF and 15g of sourdough	2.420 ±0.01^{k}	2.206 ±0.003^{f}	35.467 ±0.005^{e}
Bread made using 25% of BLHBF and 30g of sourdough	2.523 ±0.005^{gh}	2.293 ±0.005^{e}	35.523 ±0.0115^{e}
Bread made using 25% of BLHBF and 45g of sourdough	2.615 ±0.007^{e}	2.380 ±0.000^{d}	35.605 ±0.007^{de}

Bread made using 35% of BLHBF and 15g of sourdough	2.513 ±0.005 ^{h}	2.323 ±0.011^{e}	37.823 ±0.005^{b}
Bread made using 35% of BLHBF and 30g of sourdough	2.61 ±0.017 ^{e}	2.4±0.013^{d}	37.877 ±0.004^{b}
Bread made using 35% of BLHBF and 45g of sourdough	2.703 ±0.005 ^{ab}	2.5±0.01^{b}	37.910 ±0.01^{b}
Bread made using 45% of BLHBF and 15g of sourdough	2.55 ±0.01 ^{fg}	2.393 ±0.005^{d}	40.003 ±0.02^{a}
Bread made using 45% of BLHBF and 30g of sourdough	2.626 ±0.0047 ^{de}	2.452 ±0.006^{c}	40.073 ±0.005^{a}
Bread made using 45% of BLHBF and 45g of sourdough	2.706 ±0.005^{a}	2.54 ±0.012^{a}	40.113 ±0.012^{a}
RWB	2.113 ±0.015^{m}	0.910 ±0.01^{p}	28.337 ±0.01^{n}
CV (percent)	**2.96**	**4.69**	**5.85**

Where, WHBF, BHBF, and BLHBF represents white, blue and black hull-less barley flour respectively, RWB is 100percent of refined wheat bread, CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different (P<0.05).

The highest zinc content was observed in bread made using 45% of BLHBF and 45g of sourdough starter culture, followed by bread made using 35% of BLHBF and 45g of sourdough starter culture (2.54 and 2.5mg/100g, respectively). Here, it was also observed that the increase in germinated sourdough starter culture has a positive impact on the zinc content.

The lowest was observed in refined wheat bread (0.91mg/100g). The black hull-less barley variety was dominant in the mineral contents, which could be genetically rich in minerals. In this study, the increase in germinated sourdough starter culture increased the iron and zinc content. This was similar to the report of Naji-Tabasi *et al*. (2022) in which the amount of iron and zinc was observed to be 3.15 to 1.21mg/100g and 2.29 to 1.27mg/100g, respectively.

The effect of germinated sour dough starter culture ratios has shown no significant difference in the calcium content (p>0.05), yet a little variation was observed between treatments. Bread made using 45% of BLHBF and 45g,30g and 15g of sourdough starter culture have shown dominant values of 40.113, 40.073, and 40.003 mg/100g, respectively, while RWB scored the least value (28.337mg/100g).

4.9 Effect of the blending ratios and germinated sourdough starter cultures on the sensory acceptability of bread

4.9.1 Effect of the blending ratio of hull-less barley varieties and wheat flour on the sensory acceptability of bread

The effect of the blending ratio of hull-less barley varieties with wheat flour on the sensory acceptability of bread prepared using commercial yeast is presented in Table 13. The control group generally scored the highest on most attributes (crust color, texture, appearance, and overall acceptability) on a scale of seven, indicating it was the most preferred. However, in the aroma bread made from 25% of blue and white hull-less barley flour scored higher respectively (5.6 and 5.55) than the control group (5.05). The best-performing treatments after the control were bread made from 25% and 35% of white hull-less barley flour in all the sensory attributes except for aroma, where 25% of blue hull-less barley flour scored the highest result. All treatments had higher scores for taste which shows the acceptance of the blended breads by the panelists. Bread made from 45% of blue hull-less barley flour and 25%, and 35% of black hull-less barley flour had lower scores, particularly in crust color, appearance, and overall acceptability. While bread made from 45% of black hull-less barley flour consistently scored the lowest result in all categories. The increase in the hull-less barley varieties' blending ratio negatively affects the texture and color of the composite bread. This could be due to the dilution of gluten proteins, as stated by Koksel & Cetiner (2024). However, most of the treatments were acceptable by the panelists' judgment. Research reveals that bread made with a blend of wheat and barley flour has appropriate texture qualities (Lin et al., 2012). According to Liu et al. (2020), wheat flour (WF) enriched with 80percent hull-less barley flour (HLBF) results in reduced quality but is still acceptable. Compared to hull-less barley (HLB) breads, which had a specific volume of 2.71 to 1.62 ml/g, wheat bread had a specific volume of 4.97 ml/g, which was much greater. However, there was no noticeable difference in the crust color between the wheat and HLB breads. This implies that, since the color of HLB bread is similar to that of wheat bread, consumers may find it interesting.

Table 13: Effect of the blending ratio of hull-less barley varieties to wheat flour on the sensory acceptability of bread

Treatment code	**Crust Color**	**Texture**	**Taste**	**Appearance**	**Aroma**	**Overall acceptability**
25% of WHBF and 75% of RWF blended bread	5.85 ± 0.443^{ab}	5.85 ± 0.489^{b}	6 ± 0.649^{abc}	6.2 ± 0.553^{b}	5.55 ± 0.510 ^{ab}	6.15 ± 0.51^{b}
35% of WHBF and 65% of RWF blended bread	5.73 ± 0.470^{b}	5.79 ± 0.470^{b}	6.6 ± 0.503^{a}	5.9 ± 0.523^{b}	4.95 ± 0.486^{cd}	6 ±0.307^{bc}
45% of BHBF and 55% of RWF blended bread	4.75 ± 0.571^{de}	5.65 ± 0.759^{bc}	6.15 ± 0.671^{abc}	5.05 ± 0.489^{cd}	4.45 ± 0.489^{d}	5.15 ±0.223^{de}
25% of BHBF and 75% of RWF blended bread	5.04 ± 0.513^{bc}	5.55 ± 0.510^{bc}	5.75 ± 0.671^{cde}	5.9 ± 0.523^{b}	5.6 ± 0.513^{a}	5.9 ±0.616^{bc}
35% of BHBF and 65% of RWF blended bread	4.87 ± 0.649^{cd}	5.15 ± 0.605^{cde}	5.95 ± 0.745^{bcd}	5 ± 0.553^{cd}	5 ± 0.447^{c}	5.55 ±0.51^{cd}
45% of BHBF and 55% of RWF blended bread	4.48 ± 0.598^{e}	5.30 ± 0.410^{bcd}	5.85 ± 0.443^{cd}	4.90 ± 0.523^{cd}	4.65 ± 0.615^{cd}	5.10 ±0.324^{de}
25% of BLHBF and 75% of RWF blended bread	4.62 ± 0.605^{cd}	5.10 ± 0.649^{cde}	5.45 ± 0.489^{def}	5.35 ± 0.639^{c}	5.05 ± 0.605^{bc}	5.00 ±0.641^{e}
35% of BLHBF and 65% of RWF blended bread	4.40 ± 0.688^{de}	4.75 ± 0.489^{de}	5.20 ± 0.718^{ef}	4.55 ± 0.510^{d}	4.65 ± 0.511^{cd}	4.65 ±0.51^{e}
45% of BLHBF and 55% of RWF blended bread	3.95 ± 0.605^{f}	4.65 ± 0.510^{e}	5± 0.553^{f}	4.65 ± 0.510^{d}	4.45 ± 0.489^{d}	4.9 ± 0.414^{e}
100% RWB	6.3 ± 0.657^{a}	6.5 ± 0.607^{a}	6.45 ± 0.510^{ab}	6.75 ± 0.4443^{a}	5.05 ± 0.512^{abc}	6.7 ± 0.598^{a}
CV (%)	**7.88**	**4.46**	**3.67**	**6.92**	**3.20**	**4.62**

Where, WHBF,BHBF, and BLHBF represents white, blue and black hull-less barley varieties respectively, RWB= refined wheat bread, CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different (P<0.05).

4.9.2 Combined effect of germinated sour dough starter cultures, blending ratios and hull-less barley varieties on the sensory acceptability of the blended bread

This sensory test compares twenty-seven treatments against a control group made from 100 percent refined wheat bread using commercial yeast across six sensory attributes: crust color, texture, taste, appearance, aroma, and overall acceptability as presented in Table 14. The control group dominates in all sensory attributes except for the aroma bread made using 25% of WHBF and 45g of sour dough starter culture scored the highest result (5.55), followed by bread made using 35% of WHBF and 45g of sour dough starter culture (5.25). Bread made from 25% of WHBF and 30g of sour dough starter culture showed a higher crust color (6.00) next to the control group (6.30), while bread made from 45% of black hull-less barley and 45g of sour dough starter culture showed the least acceptability (2.45). The lowest scores of appearance, color, and texture in the breads could be due to high fiber content and more colored substances in the seed coat of hull-less barley, which led to poor structure and color of the blended breads, as stated by Yu *et al*. (2021). In general, the bread made using 30g of sourdough starter culture showed better texture, followed by bread made using 45g of sourdough starter culture, while the least texture characteristics were observed in bread made using 15g of sour dough starter culture. Surprisingly, bread made from 45% of black hull-less barley and 15g of sourdough starter culture showed a higher taste value (5.00), following the control group. Bread made from 25%, 35% and 45% of WHBF using 30g of sourdough starter cultures showed non-significant (P>0.05) differences in taste preference. The breads made using 45g of germinated sourdough starter culture characterized by the lowest test preference due to the excessive sweet taste, which leads to arise a question from the panelists whether sugar was added as an ingredient or not. As reported by Perri *et al*. (2023b), the reason could be due to the excessive α-amylase activity of the sprouted flours, which led to the release of maltodextrins that are responsible for the highest sweet taste and the darkest crust color as a consequence of the Millard reaction. Compared to the control group, bread made from 35% of WHBF using 30g of sourdough starter cultures, 35% of WHBF using 15g of sourdough starter culture, and 45% of WHBF using 30g of sourdough starter cultures, showed the best appearance (4.65, 4.5, and 4.45) while bread made from 45% of BLHBF using 45g of sourdough starter culture scored the least appearance (2.5). The highest aroma preference was observed in bread made from 25% of WHBF using 45g of sourdough starter culture (5.55), followed by bread made from 35% of WHBF using 45g of sourdough starter culture (5.25) and the control group (5.05), while the least was observed in bread made from 45% of BLHBF using 45g of sourdough starter culture (3.40). The control group scored the highest overall acceptability (6.60), followed by bread made from 25% of WHBF and 30g of germinated sourdough (5.15), bread made from 35% of WHBF and 30g of germinated sourdough, and bread made from 25% of WHBF and 15g of germinated sourdough (4.60). Bread made from

45% of BLHBF using 45g of sourdough starter culture scored the minimum overall acceptability (2.45).

Table 14: Effect of the different ratios of germinated sour dough starter cultures on the sensory acceptability of the blended breads

Treatment code	**Crust Color**	**Texture**	**Taste**	**Appearance**	**Aroma**	**Overall acceptability**
Bread made from 25% of WHBF and 15g of germinated sourdough	4.95 ± 0.223^{cde}	3.6 ± 0.681^{cdefgh}	4.35 ±0.671^{bcdef}	4.35 ± 0.671^{bcd}	4.5 ±0.513^{cdefgh}	4.6 ±0.503^{bc}
Bread made from 35% of WHBF and 15g of germinated sourdough	4.8 ± 0.523^{cdef}	3.4 ± 0.754^{efghi}	4.55 ±0.686^{bcde}	4.5 ± 0.503^{bc}	4.45 ±0.510^{cdefghi}	4.45 ±0.510^{cdef}
Bread made from 45% of WHBF and 15g of germinated sourdough	4.45 ± 0.510^{efgh}	3.30 ± 0.801^{fghi}	4.7 ±0.470^{bcd}	4.05 ± 0.759^{bcdef}	4.5 ±0.513^{cdefgh}	4.25 ±0.639^{cdefgh}
Bread made from 25% of BHBF and 15g of germinated sourdough	4.55 ± 0.510^{defgh}	3.80 ± 0.523^{bcdefg}	3.50 ±0.827^{hijk}	3.6 ± 0.681^{efghi}	4.35 ±0.489^{defghij}	4.35 ±0.489^{cdefg}
Bread made from 35% of BHBF and 15g of germinated sourdough	4.6 ± 0.598^{defg}	3.6 ± 0.503^{cdefgh}	4.25 ±0.639^{cdefg}	4.05 ± 0.605^{bcdef}	4.20±0.696^{fghijk}	4.05 ±0.605^{cdefghi}
Bread made from 45% of BHBF and 15g of germinated sourdough	3.9 ± 0.641^{hi}	3.35 ± 0.489^{fghi}	4.50 ±0.513^{bcde}	3.85 ± 0.671^{cdefgh}	4.3 ±0.47^{efghij}	3.7 ±0.47^{hij}
Bread made from 25% of BLHBF and 15g of germinated sourdough	3.95 ± 0.686^{ghi}	3.5 ± 0.607^{defgh}	3.8 ±0.523^{fghi}	3.4 ± 0.503^{fghij}	4.00 ±0.562^{ghijkl}	3.9 ±0.447^{efghij}
Bread made from 35% of BLHBF and 15g of germinated sourdough	4 ± 0.725^{ghi}	3.5 ± 0.513^{defgh}	4.05 ±0.605^{defgh}	3.9 ± 0.447^{cdefgh}	4.2 ±0.696^{fghijk}	3.95 ± 0.51^{defghi}
Bread made from 45% of BLHBF and 15g of germinated sourdough	3.5 ± 0.513^{ij}	3.15 ± 0.366^{hij}	5 ±0.562^{b}	3.7 ± 0.47^{defgh}	3.85 ±0.489^{ijkl}	3.7 ±0.47^{hij}
Bread made from 25% of WHBF and 30g of germinated sourdough	6.00 ± 0.649^{ab}	4.3 ± 0.470^{b}	4.8 ±0.4104^{bc}	4.45 ± 0.51^{bc}	4.95 ±0.51^{abcd}	5.15 ±0.366^{b}

Bread made from 35% of WHBF and 30g of germinated sourdough	4.9±0.447^{cde}	4.2 ± 0.410^{bc}	4.9 ±0.3078^{bc}	4.65 ± 0.489^{b}	4.85 ±0.366^{bcde}	4.6 ±0.503^{bc}
Bread made from 45% of WHBF and 30g of germinated sourdough	4.6±0.503^{defg}	4.05 ± 0.605^{bcd}	4.85 ±0.3663^{bc}	4.45 ± 0.605^{bc}	4.7±0.47^{bcdef}	4.5 ±0.513^{cde}
Bread made from 25% of BHBF and 30g of germinated sourdough	5.45 ±0.510^{bc}	3.9 ± 0.447^{bcdef}	4.1 ±0.641^{defgh}	4 ± 0.459^{bcdefg}	4.5±0.513^{cdefgh}	4.55 ±0.605^{bcd}
Bread made from 35% of BHBF and 30g of germinated sourdough	4.4±0.754^{efgh}	4 ± 0.324^{bcde}	4.7 ±0.47^{bcd}	4.3 ± 0.657^{bcd}	4.5±0.513^{cdefgh}	4.05 ±0.686^{cdefghi}
Bread made from 45% of BHBF and 30g of germinated sourdough	4.15 ±0.587^{fghi}	3.8 ± 0.523^{bcdefg}	4.55 ±0.51^{bcde}	3.85 ± 0.671^{cdefgh}	4.35 ±0.587^{defghij}	3.95 ±0.51^{defghi}
Bread made from 25% of BLHBF and 30g of germinated sourdough	4.15 ±0.745^{fghi}	3.55 ± 0.51^{defgh}	3.65 ±0.489^{ghij}	3.55 ± 0.51^{efghi}	4.25 ±0.443^{efghijk}	3.85 ±0.587^{fghij}
Bread made from 35% of BLHBF and 30g of germinated sourdough	4.05 ±0.51^{ghi}	3.4 ± 0.598^{efghi}	4.4 ±0.503^{bcdef}	3.55 ± 0.887^{efghi}	3.9±0.788^{hijkl}	3.45 ±0.686^{ijk}
Bread made from 45% of BLHBF and 30g of germinated sourdough	3.65 ±0.489^{i}	3.45 ± 0.51^{defghi}	3.9 ±0.447^{efgh}	3.25 ± 0.716^{hijk}	3.65 ±0.489^{kl}	3.5 ±0.513^{ijk}
Bread made from 25% of WHBF and 45g of germinated sourdough	5.15 ±0.671^{cd}	3.65 ± 0.489^{cdefgh}	3.15 ±0.745^{ijkl}	3.45 ± 0.51^{fghij}	5.55 ±0.51^{a}	3.55 ±0.605^{ijk}
Bread made from 35% of WHBF and 45g of germinated sourdough	4±0.649^{ghi}	3.2 ± 0.616^{ghi}	4.05 ±0.686^{defgh}	4.15 ± 0.587^{bcde}	5.25 ± 0.444^{ab}	3.75±0.55^{ghij}
Bread made from 45% of WHBF and 45g of germinated sourdough	3.55 ±0.605^{i}	3.3 ± 0.733^{fghi}	3.95 ±0.686^{efgh}	3.35 ± 0.745^{ghij}	4.35 ±0.489^{defghij}	3.65 ±0.587^{hij}
Bread made from 25% of BHBF and 45g of germinated sourdough	4.45 ±0.510^{efgh}	3.05 ± 0.605^{hijk}	3.00 ±0.459^{jkl}	3.00 ± 0.459^{ijkl}	4.7±0.571^{bcdef}	3 ±0.562^{klm}

Bread made from 35% of BHBF and 45g of germinated sourdough	3.5±0.513^{ij}	2.85 ± 0.366^{ijkl}	3.55 ±0.510^{hij}	3.40 ± 0.598^{fghij}	4.6 ±0.503^{cdefg}	3.3 ±0.571^{jkl}
Bread made from 45% of BHBF and 45g of germinated sourdough	2.85 ±0.587^{jk}	2.45 ± 0.510^{klm}	2.85 ±0.671^{klm}	2.8 ± 0.523^{jkl}	3.8±0.523^{jkl}	2.7 ±0.571^{lmn}
Bread made from 25% of BLHBF and 45g of germinated sourdough	3.65 ±0.489^{i}	2.55 ± 0.510^{jklm}	2.30 ±0.470^{m}	2.62 ± 0.503^{l}	3.90 ±0.641^{hijkl}	2.50 ±0.513^{mn}
Bread made from 35% of BLHBF and 45g of germinated sourdough	2.65 ±0.489^{k}	2.25 ± 0.444^{lm}	2.8 ±0.523^{lm}	2.6 ± 0.503^{kl}	3.75 ±0.55^{jkl}	2.65 ±0.489^{mn}
Bread made from 45% of BLHBF and 45g of germinated sourdough	2.45 ±0.489^{k}	2.25 ± 0.366^{m}	2.5 ±0.513^{lm}	2.5 ± 0.513^{l}	3.4 ±0.503^{l}	2.45 ±0.47^{n}
100% RWB	6.30 ±0.657^{a}	6.5 ± 0. 607^{a}	6.6 ±0.503^{a}	6.75 ± 0.443^{a}	5.05 ±0.510^{abc}	6.6 ±0.598^{a}
CV(PERCENT)	**5.8**	**4.24**	**3.92**	**3.76**	**2.38**	**4.06**

Where, WHBF, BHBF, and BLHBF are white, blue, and black hull-less barley flour respectively. RWB= Refined wheat bread CV = coefficient of variation, all the values are means of triplicate analysis ± standard deviation, means that do not share a letter are significantly different (P<0.05).

5. CONCLUSION

The study reveals that blending refined wheat flour with hull-less barley improves the nutritional quality of bread, particularly crude protein, crude fiber, ash, and minerals like iron, zinc, and calcium. There is a significant difference in the proximate composition, mineral concentration, and sensory quality among hull-less barley varieties, blending ratios, and bread made from different sourdough starter cultures. The black hull-less barley variety exhibited the highest nutritional benefits, while the white variety had better sensory acceptability.

The incorporation of germinated sourdough starter culture further improved the nutritional profile, though higher levels (45g) slightly reduced sensory scores due to increased sweetness and altered texture. Refined wheat bread scored higher in all sensory parameters, but blends with up to 35percent hull-less barley were still acceptable.

Hull-less barley can be a valuable ingredient for producing nutritionally enriched bread, particularly for populations requiring higher dietary fiber and micronutrient intake. However, careful consideration of blending ratios and processing techniques is necessary to balance nutritional enhancement with consumer acceptability.

6. RECOMMENDATION

Hull-less barley utilization in Tigray lacks attention; further work is needed to extend its utilization for food, and requires each individual's contribution. Based on the results of the study, the following are recommended:

- Studies on enhancing processing techniques (e.g., fermentation time, baking conditions) to improve sensory attributes of high-barley-content bread are needed.
- Black hull-less barley was found to be the best in terms of nutritional profile, while scoring the lowest value in overall acceptability. However, a 25–35percent substitution of hull-less barley flour (particularly white or blue varieties) is recommended to maintain acceptable sensory properties while improving nutritional value. Moreover, it should be promoted for functional food applications, though blending with wheat flour is advised to improve palatability.
- The use of 30g of germinated sourdough starter culture can enhance nutritional quality of bread without significantly compromising taste and texture.
- Food industries and responsible bodies should encourage the use of hull-less barley in bakery products to enhance nutritional security.

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Appendix

Appendix-1 Sensory evaluation sheet

Sensory Evaluation Sheet for hull-less barley, and wheat flour composite bread

Use the scales listed below to indicate your attitude by checking at 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

Like Moderately----------------------6

Like Slightly --------------------------5

Neither like nor dislike----------------4

Dislike Slightly------------------------3

Dislike Moderately---------------------2

Dislike Extremely-----------------------1

Code	**Sensory Attributes**					
	Crust Color	**Texture** (Softness, Chewiness, Crumble)	**Taste** (Saltiness, Sweetness, sourness)	**Appearance** (uniformity, color, crust quality)	**Aroma**	**Overall Acceptability**
01						
02						
03						
04						
05						
06						
07						
08						
09						
10						

Code	**Sensory Attributes**					
	Crust Color	**Texture** (Softness, Chewiness, Crumble)	**Taste** (Saltiness, Sweetness, sourness)	**Appearance** (uniformity, color, crust quality)	**Aroma**	**Overall Acceptability**
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Additional Comments --

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Appendix 2: Some laboratory images

Germination initiation of white, blue and black hull-less barley varieties respect

Different blends of Bread and Sensory evaluation of the breads